

Receptor activator of nuclear factor- κ B ligand and osteoprotegerin: maintaining the balance to prevent bone loss

Anne-Priscille Trouvin
Vincent Goëb

Department of Rheumatology, Rouen
University Hospital, Rouen, France

Abstract: Bone remodeling requires a precise balance between resorption and formation. It is a complex process that involves numerous factors: hormones, growth factors, vitamins, and cytokines, and notably osteoprotegerin (OPG) and receptor activator for nuclear factor- κ B (RANK) ligand. The signaling pathway OPG/RANK/RANKL is key to regulation for maintaining the balance between the activity of osteoblasts and osteoclasts in order to prevent bone loss and ensure a normal bone turnover. In this review, the RANK/RANKL/OPG pathway is described. The multiple interactions of various factors (hormones, cytokines, growth factors, and vitamins) with the OPG/RANK/RANKL pathway are also commented on. Finally, the effects of denosumab, a human monoclonal antibody that binds to RANKL and thereby inhibits the activation of osteoclasts, and of strontium ranelate are also described. Indeed, these two new drugs afford appreciable assistance in daily care practice, helping to prevent bone loss in patients with osteoporosis.

Keywords: osteoprotegerin, OPG, RANK, RANKL, denosumab, strontium ranelate, osteoporosis

Introduction

Bone remodeling is a phenomenon that first allows growth and then participates in the mineral homeostasis of calcium and phosphorus. Bone remodeling is a complex process involving numerous cells and cytokines. Recently, a major signaling pathway has been discovered that gives a new orientation for osteoporosis treatment. Bone remodeling is a balance between formation and resorption through controlling the activity of osteoblasts and osteoclasts. In numerous bone diseases, this becomes unbalanced in favor of resorption, creating bone loss. Throughout this review, the variation of this balance according to the patient's gender or age will be described, as well as the new treatment options based on the newly discovered signaling pathway OPG/RANK/RANKL. For this review, we conducted a PubMed search from 1995 to 2010 using the key words "osteoprotegerin (OPG)", "receptor activator of nuclear factor- κ B ligand (RANKL)", "RANK", "aging", "postmenopausal", "denosumab", and "strontium ranelate".

The OPG/RANKL/RANK signaling pathway Normal bone turnover

The cells most involved in bone turnover are osteoclasts and osteoblasts. These cells have counter effects on bone. The former are the resorption cells; the latter are the formation cells. The balance between activation and apoptosis of cells is the key to

Correspondence: Vincent Goëb
Service de Rhumatologie, CHU-Hôpitaux
de Rouen, 76031 Rouen Cedex, France
Tel +33 232889019
Fax +33 232889110
Email goebvince@yahoo.fr

maintaining bone mass. Formation of new bone goes through four steps: osteoclast activation, bone resorption, reversal with osteoclast inhibition and osteoblast activation, and finally bone formation. Therefore, everything starts by osteoclastogenesis. Osteoclasts are derived from the hematopoietic lineage and differentiate in order to degrade bone.¹ The osteoclast precursor matures into a multinucleated cell and attaches itself to the bone surface, where it is attracted by different factors such as cytokines, hormones, and growth factor, and differentiates into an activated osteoclast. Once activated, the osteoclast starts degrading the bone surface, forming a lacuna. The third phase of the cycle is apoptosis of the osteoclast once the resorption phase is achieved, to allow the formation of new bone by the preosteoblasts that have matured in order to constitute new bone and regulate its mineralization. Once bone formation is achieved, osteoblast apoptosis leads them either to osteocytes or to transform to bone surface lining cells.

Throughout aging, bone turnover unbalances in favor of bone resorption. Osteoporosis is a chronic bone disease characterized by a decreased bone mass leading to fragile bone and an increased risk for fractures, notably hip, vertebral, and forearm fractures, which are the source of a loss of autonomy and increased mortality in the elderly.²

Suppression or control of bone resorption is therefore a major therapeutic strategy to prevent or diminish bone loss. A critical signaling pathway with three major proteins, OPG, RANK, and RANKL, was discovered, thereby enlightening the cellular regulation of bone formation. OPG was the first protein of the pathway to be identified in 1997.³ It was discovered in mice by sequencing random clones, and the full-length gene was proven to encode for a new tumor necrosis factor (TNF) receptor. OPG knockout mice revealed severe osteoporosis, whereas OPG overexpressing mice showed osteopetrosis.⁴ OPG is a member of the TNF receptor family but is atypical, as it is a secreted protein with no transmembrane domain. It contains four homologous domains for binding its target, RANKL. OPG is produced by many types of tissue, including osteoblasts, endothelial cells, vascular smooth muscle, and lymphoid cells, and other cell types, raising the question of the specificity of this protein in the bone mass regulation.⁵

It has been established that osteoclastogenesis requires osteoclast activation by two newly identified molecules: macrophage colony-stimulating factor (M-CSF) and RANKL. Both of them are necessary to activate gene transcription allowing osteoclast differentiation.⁶ RANKL is another new member of the TNF ligand family. It is produced by

osteoblast lineage cells and activated T-cells. M-CSF and RANKL have complementary activities. M-CSF increases the pool of osteoclast precursors, whereas RANKL binds to its receptor RANK expressed on osteoclast precursors and mature osteoclasts, enhances osteoclast differentiation, and promotes its activation while inhibiting its apoptosis.^{7,8}

RANK, the receptor of RANKL, as with OPG, is a receptor from the TNF family. Its essential role for the transduction of the RANKL signal was established in the late 1990s.⁹ In transgenic mice, knockout of either RANK or RANKL led to the same phenotypes, concluding that RANK and RANKL had very few roles apart from their interactions.⁴

The bone turnover pathway is a triad: RANKL binds to its receptor RANK in order to induce osteoclast differentiation, activation, and survival, whereas OPG acts as a decoy receptor to RANKL and therefore inhibits osteoclast activation and bone resorption.¹⁰ The balance of RANKL/RANK and OPG is thus essential to modulate osteoclastogenesis and bone remodeling, and the regulators involved in this tight control are numerous (Figure 1).

Expression of the OPG/RANKL pathway

Multiple factors (hormones, cytokines, growth factors, and vitamins) have been described as interacting with the OPG/RANKL pathway. The first of these regulation factors, estrogen, is one of the most important known hormones to participate in bone turnover. The results of the different work concerning estrogen and its interaction with the pathway are not conflicting because estrogen has been shown to stimulate OPG secretion^{11,12} and also to downregulate the expression of RANKL.¹³

Studies on the effects of androgens have yielded conflicting results on their effects on OPG. Androgens upregulated OPG expression in cultured mice osteoblasts,¹⁴ whereas in human osteoblastic cells it was shown that OPG mRNA levels and protein serum levels were significantly decreased by 5- α -dihydrotestosterone.¹⁵ RANKL expression is not affected by androgens in both studies.^{14,15} However, Proell et al in 2009 studied orchietomized (ORX) rats and measured free serum RANKL in bone marrow supernatants. In ORX rats, this level was threefold higher than controls; once treated with testosterone, RANKL returned to control levels.¹⁶ These results were in the same trend as in another study in which an inverse correlation was found between RANKL in bone marrow cell extracts and testosterone in ORX rats.¹⁷

Parathyroid hormone (PTH) is a proresorptive hormone as it has been described from the bone stimulation observed in patients with hyperparathyroidism. PTH treatment on cultured

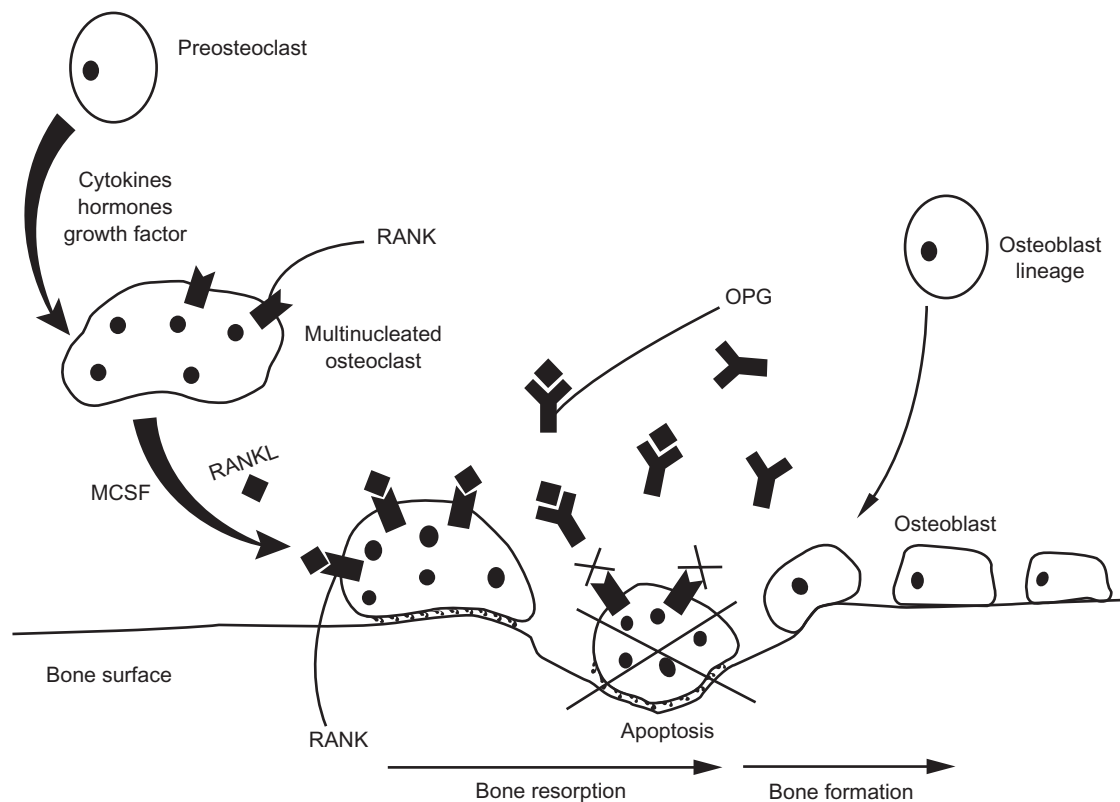


Figure 1 Bone turnover through the OPG/RANK/RANKL pathway. The osteoclast precursor matures into a multinucleated cell under the influence of numerous factors such as cytokines, hormones, and growth factors. The multinucleated cell differentiates into an activated osteoclast in the presence of MCSF and RANKL. Once activated, the osteoclast starts degrading the bone surface, forming a lacuna. OPG, the decoy receptor of RANKL, inhibits the continuous binding of RANKL on RANK, therefore leading the osteoclast to its apoptosis. Thereafter, bone formation starts with preosteoblasts that have matured into osteoblasts in order to constitute new bone.

bone marrow cells and osteoblasts in mice showed a decreased expression of mRNA levels of OPG and a reciprocal enhanced regulation of RANKL.¹⁸ The same results were found in a study on parathyroidectomized infusion with continuous PTH. A dose-dependent OPG decrease with a reciprocal RANKL increase was shown.¹⁹ Seck et al found conflicting results while analyzing the expression of OPG and RANKL in human bone tissue *in vivo* in women having surgery for breast cancer. Their conclusion stated that menopausal status failed to show any change in the expression of OPG and RANKL. However, higher levels of PTH were correlated to low levels of both OPG and RANKL mRNA transcript.²⁰ A cohort study of women of all ages also showed that serum OPG negatively correlated to PTH.²¹

Among other regulators of the bone turnover signaling pathway, numerous cytokines can promote or, on the contrary, decrease bone resorption. IL-1 is a proresorptive cytokine that upregulates RANKL and RANK and has yielded conflicting results on its action among OPG. Interleukin 7 (IL-7),²² IL-17, and TNF- α upregulate RANKL and are therefore considered proresorptive agents, whereas IL-4, IL-13, and interferon- γ are considered antiresorptive agents by suppressing osteoclastogenesis.⁴

Growth factors are another category of the OPG/RANKL pathway regulators. Insulin-like growth factor (IGF)-1 is already known as an important growth factor for bone turnover because bone responsiveness to IGF-1 decreases with age.²³ Zhao et al studied the interaction between IGF-1 and the OPG/RANKL pathway in healthy Chinese women. Their results showed a negative correlation between IGF-1 and OPG as well as a negative correlation with the OPG/RANKL ratio. However, IGF-1 positively correlated to RANKL. The authors' conclusion was that the effects of IGF-1 on bone may be mediated by the OPG/RANKL pathway.²⁴ The major limitations of this study were the use of serum levels that did not reflect the exact bone microenvironment RANKL levels and also the use of serum levels of RANKL, which are very hard to define given that the assays are not very sensitive.

Another study on mice²³ showed that IGF-1 can stimulate the expression of RANKL and M-CSF in young but not old animals; therefore, the regulatory role of this growth factor is lost with aging. The authors also determined that the alteration of the IGF-1 role was due to impaired receptor activation even though it did not account for the entire alterations of the IGF-1 role in bone turnover.

Another growth factor was looked into. Pigment epithelium-derived factor (PEDF) is a potent growth factor for inhibiting angiogenesis. PEDF expression in bone has been demonstrated, as well as its positive action on differentiation of osteoblastic cells. Akiyama et al explored the link between bone turnover, and particularly osteoclast function, and PEDF.²⁵ PEDF was shown to stimulate OPG expression in osteoblasts and in osteoclast precursor cells; on the other hand, RANKL expression was suppressed by PEDF. Moreover, PEDF was shown to inhibit osteoclast differentiation. The authors' conclusion was that PEDF could be a bone resorption inhibitor via the upregulation of OPG.

Genetic variations and mutations have been identified in the OPG/RANKL pathway, and the contribution of their polymorphism to bone mass density (BMD) has been studied. In a large cohort of Chinese patients with either an extremely high hip BMD or an extremely low hip BMD, men with TC/CC genotypes of the polymorphism rs9594782 of the *RANKL* gene (*TNFRSF11A*) had a significantly higher risk of extremely low hip BMD and lower whole-body BMD. In the same cohort, the *A163G* polymorphism of the promoter region of the OPG gene (*TNFRSF11B*) was also linked to hip BMD with a significant association between the GG genotype and a reduced risk of low hip BMD.²⁶ In a recent and large European cohort of men, Roshandel et al identified multiple single nucleotide polymorphisms in *OPG*, *RANKL*, and *RANK* genes associated with bone turnover markers and BMD.²⁷ Genetic variations of this essential pathway seemed to influence bone turnover, but these results have still to be confirmed in other populations.

The OPG/RANK/RANKL pathway through aging

Although rodent studies in aged mice had shown that RANKL mRNA levels increased with age, OPG mRNA levels decreased in aged mice.^{28,29} Studies in humans have drawn the consensus that OPG increases with age in both men and women.^{21,30-32} This increase has been shown in a healthy aged population as well as in patients who have osteoporosis, but it can also be seen in other diseases, such as Paget's disease of the bone and rheumatoid arthritis.⁴ On the other hand, RANKL evolution through aging has yielded conflicting results in several studies regarding analysis in women and in men of the RANKL/OPG ratio.

In women

Menopause is a crucial aging step for women, characterized by an estrogen deficiency leading to bone loss. In vitro studies of human osteoblast revealed that estrogen induces OPG

production.^{11,33,34} Studies involving postmenopausal women to determine a relationship between the menopause and an increase in OPG put forth different conclusions. Oh et al in a study on healthy Korean women of all ages, found that OPG levels were significantly higher in postmenopausal women compared with premenopausal women,³⁵ and in a large cohort of Austrian women aged from 19 to 96 years, OPG serum level was found to be negatively correlated with serum estradiol.²¹ However, it has also been shown that OPG serum levels were positively correlated to age but not significantly to the menopausal status.^{20,36}

In women treated for menopause, Han et al showed a significant decrease in OPG serum levels after 1 year of hormone replacement therapy for estrogen alone or combined with progesterone.³⁷ Dehydroepiandrosterone (DHEA) is another hormone linked to aging, and it is known to decrease with aging. An in vitro study of osteoblasts cultured with DHEA showed that the expression of the ratio of OPG/RANKL mRNA was increased, leading the authors to conclude that DHEA could inhibit bone resorption through the OPG/RANKL pathway.³⁸

As the results of the relationship between OPG and menopause are conflicting, the link between bone resorption markers and OPG is also conflicting. One study reported a weak negative correlation between OPG serum level and bone turnover markers in a cohort of postmenopausal women.³⁹ In several other cohorts, no correlation was found between OPG and bone resorption markers in women whatever their menopause status was.^{36,40} In contrast, a positive relationship between bone turnover markers and OPG was found in postmenopausal women⁴¹ and in both men and women in a study by Indridason et al.⁴²

Finally, the link between OPG levels, BMD, and vertebral fracture has also yielded conflicting results. In some studies, no association was found between BMD and OPG,^{21,39,42,43} whereas other works demonstrated a significant inverse correlation between OPG and BMD,⁴⁴ notably in postmenopausal women without any hormone replacement therapy.⁴⁵ A positive correlation was found in two small studies of postmenopausal women^{36,46} and corroborated by a larger study with a follow-up of 5–10 years.⁴⁷

In addition to the conflicting relationship between BMD and OPG, the link between the prevalence of vertebral fractures and OPG serum levels has still not reached a consensus. Indeed, low^{36,41,46} and high⁴⁸ OPG levels have been associated with vertebral fractures (Table 1).

The study of RANKL in postmenopausal women is even more complicated than OPG because the assay that

Table 1 Correlation between osteoprotegerin and different markers in women

	Women		
	Correlation		
	Positive	Absence	Negative
Aging	Kudlacek et al, ²¹ Mazziotti et al, ³⁰ Pulsatelli et al, ³¹ Nabipour et al, ³² Oh et al, ³⁵ Mezquita-Raya et al, ³⁶ Khosla et al, ⁴⁰ Fahrleitner-Pammer et al, ⁴¹ Indridason et al, ⁴² Liu et al, ⁴³ Uemura et al ⁵⁰		
Estradiol	Rogers et al ³⁹	Khosla et al ⁴⁰	Kudlacek et al ²¹
Testosterone		Khosla et al ⁴⁰	
PTH			Kudlacek et al ²¹
BTMs	Mezquita-Raya et al ³⁶ (tALP, TRAP), Fahrleitner-Pammer et al, ⁴¹ Indridason et al ⁴² (OC), Liu et al ⁴³ (urinary NTx)	Nabipour et al, ³² Han et al, ³⁷ Khosla et al ⁴⁰	Rogers et al, ³⁹ Indridason et al ⁴² (TRAP)
BMD	Nabipour et al, ³² Mezquita-Raya et al, ³⁶ Chiba et al, ⁴⁶ Stern et al ⁴⁷ (women with HRT)	Han et al, ³⁷ Rogers et al, ³⁹ Khosla et al, ⁴⁰ Indridason et al, ⁴² Liu et al, ⁴³ Uemura et al ⁵⁰	Jorgensen et al ⁴⁵

Abbreviations: PTH, parathyroid hormone; BTM, bone turnover markers; tALP, total alkaline phosphatase; TRAP, tartrate-resistant acid phosphatase; OC, osteocalcin; NTx, cross-linked N-telopeptide of type I collagen; BMD, bone mass density; HRT, hormone replacement therapy.

measures RANKL serum levels is not as sensitive as the one used for OPG. In an animal study, serum RANKL of OPG knockout mice was increased by ovariectomy.⁴⁹ In a few studies that were conducted to determine the relationship between aging and RANKL in postmenopausal women, an increase of RANKL expression was described in postmenopausal women compared with premenopausal women and women using estrogen treatment.¹³ Conflicting results can again be found. Some studies did not demonstrate any significant difference in RANKL and menopausal status,²⁰ whereas some others found a decrease of RANKL in elderly subjects.³¹ Similar to OPG, the relationship between RANKL and BMD does not reach any consensus. Some works found no association;^{36,43} others found an inverse correlation.^{45,50}

In men

All studies performed in men found that the OPG serum level increases with age.^{21,30,35,40,42,44,45,51–53} As in women, the correlation between OPG and sex steroids in men is very conflicting in the different publications. Some articles showed a positive correlation between free testosterone²¹ and free estradiol levels and OPG.⁵² In contrast, some studies concluded that there was a negative correlation between OPG and sex steroids in men: bioavailable testosterone and estradiol.⁴⁰ Khosla et al went further and compared the role of testosterone with estrogen in elderly males. The results showed that in hypogonadal conditions a testosterone

supplementation treatment decreased OPG serum levels.¹² Furthermore, a negative association between OPG and PTH was found in men.^{21,52}

Regarding the possible association between OPG levels and BMD, numerous studies observed very different results. Stern et al found that higher OPG levels were associated with higher BMD of the lumbar spine.⁴⁷ In the same trend, another study found a borderline positive correlation between OPG and whole-body BMD.⁴² Mostly, it seems to be either a negative correlation or no correlation at all that is found. Khosla et al and Oh et al both concluded that there was a negative correlation between OPG serum levels and BMD.^{40,44} Furthermore, in a recent article, Jorgensen et al also found a negative association between OPG and BMD according to a multiple linear regression. In the same study, however, they did not find any correlation between OPG and bone loss after a 6-year follow-up.⁴⁵ This absence of correlation between OPG serum levels and BMD was also the conclusion of Szulc et al in a study of men aged 19–85 years.⁵²

When looking at the correlation between OPG and bone turnover markers, the results once again are very conflicting in men as well as in women. Some authors report a positive association between OPG and osteocalcin, whereas a negative correlation to bone resorption markers was observed.⁴² This negative correlation with bone resorption is also noticed by Szulc et al.⁵² On the other hand, some studies showed a negative association to osteocalcin⁴⁴ and a positive one between OPG and bone resorption markers⁴⁰ (Table 2).

Table 2 Correlation between osteoprotegerin and different markers in men

	Men	
	Correlation	
	Positive	Negative
Aging	Kudlacek et al, ²¹ Mazziotti et al, ³⁰ Pulsatelli et al, ³¹ Khosla et al, ⁴⁰ Indridason et al, ⁴² Szulc et al, ⁵² Gannage-Yared et al ⁵³	
Estradiol	Kudlacek et al, ²¹ Szulc et al ⁵²	Khosla et al, ⁴⁰ Oh et al ⁴⁴
Testosterone	Oh et al, ⁴⁴ Szulc et al ⁵²	Khosla et al ⁴⁰
PTH		Kudlacek et al, ²¹ Szulc et al ⁵²
BTM	Khosla et al, ⁴⁰ Indridason et al ⁴² (OC)	Oh et al, ⁴⁴ (OC) Szulc et al ⁵²
BMD	Indridason et al, ⁴² Stern et al, ⁴⁷ Szulc et al ⁵²	Khosla et al, ⁴⁰ Oh et al, ⁴⁴ Jorgensen et al ⁴⁵

Abbreviations: PTH, parathyroid hormone; OC, osteocalcin; BMD, bone mass density.

Conflicting results are also found in men regarding the evolution of serum RANKL. A study that was conducted to determine the relationship between RANKL serum levels and mRNA in the bone microenvironment showed that serum RANKL was negatively correlated to age, whereas mRNA was positively correlated to the age of the subjects. Levels of mRNA were also positively associated to bone turnover markers such as osteocalcin, alkaline phosphatase, and urinary deoxypyridinoline.⁵¹ When looking at the ratio RANKL/OPG, a positive correlation was found with osteocalcin and estradiol levels, whereas no correlation was found with serum total testosterone. A trend for a significant positive correlation between RANKL and osteocalcin was noticed.⁴⁴ In a large recent study, Jorgensen et al found no correlation between RANKL and age, and no correlation either to BMD or bone loss after 6 years of follow-up. Moreover, the ratio RANKL/OPG was not correlated to bone loss.⁴⁵ On the other hand, Stern et al in the Rancho Bernardo Study, found that the serum RANKL concentrations were negatively correlated to BMD in men.⁴⁷

Limits and difficulties of the studies

As previously shown, studying OPG and RANKL in humans is very difficult, as it appears that the results are conflicting from one publication to another. All these data have to be balanced by some major problems highlighted in a few

publications. First of all, the assays used to quantify RANKL serum level are not very sensitive. Indeed, in a recent study, only 63% of the patients had a detectable RANKL.⁴⁵ Secondly, only a few studies looked at the specific bone environment regarding the expression of OPG and RANKL, mostly due to the difficulty of obtaining bone samples. Moreover, OPG and RANKL are locally active molecules, and tested serum is removed from the site of disease; therefore, serum levels might only partially reflect the actual local mechanism. Finally, no reference ranges have been established regarding OPG and RANKL serum level. They vary from one study to another depending on the assays used. Currently, standard levels for OPG and RANKL are not well established.

Preventing bone loss: treatment through the RANKL/OPG pathway

As shown in the rodent studies, inhibiting RANKL or RANK led to the concept of inhibiting their pathway as a treatment for osteoporosis. After testing recombinant molecules of either OPG or RANK and finding all exhibiting the ability to bind RANKL and suppress bone resorption, all these molecules were abandoned. Recently, two new drugs emerged: 1) denosumab, directly from translation of the basic science of RANKL inhibition, and 2) strontium ranelate, which has proven effects on the RANKL/OPG pathway that emerged secondarily.

Denosumab

Denosumab is a human monoclonal IgG2 antibody with a very high affinity and specificity to RANKL. By binding to RANKL, as OPG does in physiological conditions, denosumab blocks its binding to RANK and thereby inhibits the osteoclast activation. In preclinical studies, denosumab did not suppress bone resorption in normal mice because of its specificity to human RANKL. In knockin mice expressing chimeric (human/murine) RANKL, denosumab reduced bone resorption and increased cortical and cancellous bone mass.⁵⁴

In a phase I study comparing denosumab with placebo in healthy postmenopausal women, a single subcutaneous injection resulted in a rapid, profound, and sustained decrease in bone resorption marker.⁵⁵ In a phase II study, McClung et al compared denosumab with placebo and alendronate in postmenopausal women with low BMD. Denosumab was administrated at a dose of 6, 14, or 30 mg every 3 months or 14, 60, 100, 210 mg every 6 months over a 12-month period. Results showed an increase in BMD at lumbar spine, total hip, and distal third of the radius. The results on BMD were

superior to placebo and similar to or better than for weekly alendronate. Again, a decrease of bone resorption marker was observed rapidly after denosumab injection, and the suppression of bone turnover appeared to be dose dependent.⁵⁶ In a trial extension, these results were sustained over 24 months.⁵⁷ In phase I and II studies, the dose of 60 mg every 6 months seemed to be the more effective dose and was thus investigated in further phase III studies.

Bone et al conducted a randomized controlled versus placebo study on denosumab over 2 years in postmenopausal osteopenic women. They observed a significant increase in BMD at all sites ($P < 0.0001$ versus placebo) after 24 months of treatment. At the same time, bone turnover markers (serum C-telopeptide [CTX1], tartrate-resistant acid phosphatase 5b, and intact N-terminal propeptide of type I procollagen [P1 NP]) were significantly suppressed.⁵⁸ A large phase III study, the FREEDOM trial, was conducted by Cummings et al in a population of 7868 postmenopausal osteoporotic women. They received either 60 mg of denosumab every 6 months or a placebo over a period of 36 months. The occurrence of any new vertebral, nonvertebral, and hip fractures was studied, and the authors observed that denosumab significantly reduced the risk of new fracture with a relative decrease of 68%, 40%, and 20% of vertebral, hip, and nonvertebral fractures, respectively.⁵⁹

The unique effect of denosumab as a RANKL inhibitor creates a new category of antiresorptive agent that is very different from bisphosphonates. Under denosumab, the bone turnover markers decreased quicker and in a more pronounced way than in the alendronate group.⁵⁶ Moreover, once the denosumab treatment is stopped, its effects on bone remodeling seem to be completely reversible.⁶⁰ Furthermore, compared with alendronate and placebo, denosumab was shown to be the more efficient treatment in improving bone mechanical properties of the femoral neck.⁶¹

A noninferiority trial was conducted in a head-to-head comparison of denosumab and alendronate whereby 1189 postmenopausal women with a T-score of -2.0 or less at lumbar spine or total hip were included and randomized. They received either 60 mg subcutaneous injection of denosumab every 6 months and weekly oral placebo or 70 mg oral alendronate and subcutaneous placebo injections every 6 months. The change from baseline BMD (in the total hip, femoral neck, trochanter, lumbar spine, and one-third radius) at 6 and 12 months was studied, as well as the bone turnover markers at months 1, 3, 6, 9, and 12. At 12 months, 94% of the patients had completed the study. The results showed a significant increase in BMD in the denosumab treatment group

compared with alendronate. Increase in BMD at the total hip in denosumab group was 3.5% at 12 months versus 2.6% in the alendronate group ($P < 0.0001$). A significant increase was also observed at all measured sites. The bone turnover markers studied were serum CTX1 and intact N-terminal P1 NP. They were significantly decreased in the denosumab group compared with alendronate. A maximal reduction of P1 NP was observed at 3 months in the denosumab group compared with a maximal decrease at 9 months in the alendronate group. Nevertheless, the median decreases in CTX1 were similar for both treatment groups at 12 months.⁶²

In terms of safety and tolerability, phase I and II studies reported similar rates of all adverse and severe adverse events in placebo, denosumab, and alendronate groups. However, a small, transient, non-dose-dependent and asymptomatic increase of serum PTH associated with a decrease of serum calcium was observed⁵⁶ under denosumab. In phase III studies, the same security profile was observed with no significant difference between placebo and denosumab. Although patients undergoing treatment with bisphosphonates, notably those receiving IV bisphosphonate therapy with chemo-, radiation-, or corticosteroid therapy, may develop jaw osteonecrosis, no such disabling adverse event was reported under denosumab.⁶³

Strontium ranelate

Strontium ranelate is an oral daily drug formed of two atoms of stable strontium and an organic moiety (ranelic acid). Strontium ranelate combines the antiresorptive effect on bone with an additional anabolic action. In vitro studies have shown different effects of strontium ranelate on osteoblasts and osteoclasts. When studying in vitro osteoblasts, strontium ranelate induced a concentration-dependent increase in OPG mRNA expression as well as OPG secretion. On the other hand, RANKL mRNA and concentration were markedly decreased. The proposed mediator of these effects by Brennan et al was the calcium-sensing receptor. Indeed, knocking down the receptor abolished the effects of strontium ranelate on OPG and RANKL.⁶⁴ These findings were corroborated in another in vitro study in which increasing concentrations of strontium ranelate downregulated osteoclastic differentiation. Moreover, the authors showed, as in the previous publication, that this downregulation was mediated by calcium-sensing receptor and by the inhibition of the RANKL-induced nuclear translocation.⁶⁵ Atkins et al observed the same increase of the OPG/RANKL by strontium ranelate in osteoblasts.⁶⁶ The increase in BMD is not obligatorily correlated to a reduction of the risk of fracture, as the 'quality' of bone formation, and

notably of the network of trabeculae, is as important as its 'quantity'. To date, no studies have demonstrated that the in vitro effects of strontium ranelate on OPG/RANKL are fully relevant to its risk reduction of fracture.

In rodents,⁶⁷ in vivo studies showed that strontium ranelate decreased bone resorption and increased bone formation. In a phase II study, strontium ranelate was compared with placebo in postmenopausal osteoporotic women with at least one vertebral fracture. An increase in lumbar spine BMD was significantly higher in the strontium ranelate group than in the placebo group. Moreover, urinary excretion of cross-linked N-telopeptide significantly decreased in the treatment group compared with placebo, showing a decrease of bone resorption. In this study, the dose of 2 g/day was considered to offer the best balance between efficacy and safety.⁶⁸ In the phase III study, a dose of 2 g/day of strontium ranelate was compared with placebo over 3 years. After 3 years of treatment, the increase in BMD at all measured sites was significant in the strontium group compared with the placebo group. The increase in BMD at lumbar spine was notably of 12.7% in the strontium group. However, it is well known that this BMD increase is not fully correlated to a risk reduction of new fracture, as strontium ranelate produces a BMD measurement artifact; therefore, its action is hardly comparable with denosumab. After 3 months of therapy, bone formation markers were significantly higher in the strontium ranelate group than in the placebo group. Moreover, the authors observed a risk reduction of 41% of new vertebral fractures during the 3-year study period in the treated group compared with placebo. No significant difference in the incidence of serious adverse events was observed between the groups. However, diarrhea was the most frequent adverse event in the strontium ranelate group, with a small but significantly higher rate compared with the placebo group ($P = 0.02$). This effect disappeared after the first 3 months of treatment.⁶⁹

Conclusion

Normal bone turnover and stable bone mass depend on the balance between OPG and RANKL. The catabolic effects of RANKL are controlled by OPG, which prevents activation of RANKL and therefore limits the formation, activity, and survival of osteoclasts. The OPG/RANK/RANKL signaling pathway is complex and requires numerous factors that interact together. Clinical trials of denosumab and strontium ranelate demonstrate evidence of their ability to prevent bone loss in patients with osteoporosis. These treatments may also play a significant role in future therapeutic strategies by

preventing structural damage in rheumatoid arthritis and by reducing bone resorption in metastatic bone diseases, notably in breast and prostate cancer.

Acknowledgment

The authors thank Dr Claire Wenham (Unit of Musculoskeletal Disease, Leeds Teaching Hospital, Leeds, UK) for her comments and second reading of the manuscript.

Disclosure

The authors disclose no conflict of interest.

References

- Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature*. 2003;423(6937):337–342.
- Compston J. Osteoporosis: social and economic impact. *Radiol Clin North Am*. 2010;48(3):477–482.
- Rogers A, Eastell R. Circulating osteoprotegerin and receptor activator for nuclear factor kappaB ligand: clinical utility in metabolic bone disease assessment. *J Clin Endocrinol Metab*. 2005;90(11):6323–6331.
- Kearns AE, Khosla S, Kostenuik PJ. Receptor activator of nuclear factor kappaB ligand and osteoprotegerin regulation of bone remodeling in health and disease. *Endocr Rev*. 2008;29(2):155–192.
- Khosla S. Minireview: the OPG/RANKL/RANK system. *Endocrinology*. 2001;142(12):5050–5055.
- Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell*. 1998;93(2):165–176.
- Schoppet M, Preissner KT, Hofbauer LC. RANK ligand and osteoprotegerin: paracrine regulators of bone metabolism and vascular function. *Arterioscler Thromb Vasc Biol*. 2002;22(4):549–553.
- Kong YY, Yoshida H, Sarosi I, et al. OPG is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature*. 1999;397(6717):315–323.
- Nakagawa N, Kinosaki M, Yamaguchi K, et al. RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. *Biochem Biophys Res Commun*. 1998;253(2):395–400.
- Liu C, Walter TS, Huang P, et al. Structural and functional insights of RANKL-RANK interaction and signaling. *J Immunol*. 2010;184(12):6910–6919.
- Bord S, Ireland DC, Beavan SR, Compston JE. The effects of estrogen on osteoprotegerin, RANKL, and estrogen receptor expression in human osteoblasts. *Bone*. 2003;32(2):136–141.
- Khosla S, Atkinson EJ, Dunstan CR, O'Fallon WM. Effect of estrogen versus testosterone on circulating osteoprotegerin and other cytokine levels in normal elderly men. *J Clin Endocrinol Metab*. 2002;87(4):1550–1554.
- Eghbali-Fatourehchi G, Khosla S, Sanyal A, Boyle WJ, Lacey DL, Riggs BL. Role of RANK ligand in mediating increased bone resorption in early postmenopausal women. *J Clin Invest*. 2003;111(8):1221–1230.
- Chen Q, Kaji H, Kanatani M, Sugimoto T, Chihara K. Testosterone increases osteoprotegerin mRNA expression in mouse osteoblast cells. *Horm Metab Res*. 2004;36(10):674–678.
- Hofbauer LC, Hicok KC, Chen D, Khosla S. Regulation of osteoprotegerin production by androgens and anti-androgens in human osteoblastic lineage cells. *Eur J Endocrinol*. 2002;147(2):269–273.
- Proell V, Xu H, Schuler C, Weber K, Hofbauer LC, Erben RG. Orchiectomy upregulates free soluble RANKL in bone marrow of aged rats. *Bone*. 2009;45(4):677–681.
- Li X, Ominsky MS, Stolina M, et al. Increased RANK ligand in bone marrow of orchietomized rats and prevention of their bone loss by the RANK ligand inhibitor osteoprotegerin. *Bone*. 2009;45(4):669–676.

18. Lee SK, Lorenzo JA. Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation. *Endocrinology*. 1999;140(8):3552–3561.
19. Ma YL, Cain RL, Halladay DL, et al. Catabolic effects of continuous human PTH (1–38) in vivo is associated with sustained stimulation of RANKL and inhibition of osteoprotegerin and gene-associated bone formation. *Endocrinology*. 2001;142(9):4047–4054.
20. Seck T, Diel I, Bismar H, Ziegler R, Pfeilschifter J. Serum parathyroid hormone, but not menopausal status, is associated with the expression of osteoprotegerin and RANKL mRNA in human bone samples. *Eur J Endocrinol*. 2001;145(2):199–205.
21. Kudlacek S, Schneider B, Woloszczuk W, Pietschmann P, Willvonseder R; Austrian Study Group on Normative Values of Bone Metabolism. Serum levels of osteoprotegerin increase with age in a healthy adult population. *Bone*. 2003;32(6):681–686.
22. Ponchel F, Cuthbert RJ, Goëb V. IL-7 and lymphopenia. *Clin Chim Acta*. 2010 Sep 15. [Epub ahead of print].
23. Cao JJ, Kurimoto P, Boudignon B, Rosen C, Lima F, Halloran BP. Aging impairs IGF-I receptor activation and induces skeletal resistance to IGF-I. *J Bone Miner Res*. 2007;22(8):1271–1279.
24. Zhao HY, Liu JM, Ning G, et al. Relationships between insulin-like growth factor-I (IGF-I) and OPG, RANKL, bone mineral density in healthy Chinese women. *Osteoporos Int*. 2008;19(2):221–226.
25. Akiyama T, Dass CR, Shinoda Y, Kawano H, Tanaka S, Choong PF. PEDF regulates osteoclasts via osteoprotegerin and RANKL. *Biochem Biophys Res Commun*. 2010;391(1):789–794.
26. Hsu YH, Niu T, Terwedow HA, et al. Variation in genes involved in the RANKL/RANK/OPG bone remodeling pathway are associated with bone mineral density at different skeletal sites in men. *Hum Genet*. 2006;118(5):568–577.
27. Roshandel D, Holliday KL, Pye SR, et al. Genetic variation in the RANKL/RANK/OPG signaling pathway is associated with bone turnover and bone mineral density in men. *J Bone Miner Res*. 2010; 25(8):1830–1838.
28. Cao J, Venton L, Sakata T, Halloran BP. Expression of RANKL and OPG correlates with age-related bone loss in male C57BL/6 mice. *J Bone Miner Res*. 2003;18(2):270–277.
29. Cao JJ, Wronski TJ, Iwaniec U, et al. Aging increases stromal/osteoblastic cell-induced osteoclastogenesis and alters the osteoclast precursor pool in the mouse. *J Bone Miner Res*. 2005;20(9):1659–1668.
30. Mazziotti G, Amato G, Sorvillo F, et al. Increased serum osteoprotegerin values in long-lived subjects: different effects of inflammation and bone metabolism. *Eur J Endocrinol*. 2006;154(3):373–377.
31. Pulsatelli L, Dolzani P, Silvestri T, et al. Soluble receptor activator of nuclear factor-kappaB ligand (sRANKL)/osteoprotegerin balance in ageing and age-associated diseases. *Biogerontology*. 2004;5(2): 119–127.
32. Nabipour I, Larjani B, Vahdat K, et al. Relationships among serum receptor of nuclear factor-kappaB ligand, osteoprotegerin, high-sensitivity C-reactive protein, and bone mineral density in postmenopausal women: osteoimmunity versus osteoinflammatory. *Menopause*. 2009;16(5):950–955.
33. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Spelsberg TC, Riggs BL. Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. *Endocrinology*. 1999;140(9):4367–4370.
34. Michael H, Harkonen PL, Vaananen HK, Hentunen TA. Estrogen and testosterone use different cellular pathways to inhibit osteoclastogenesis and bone resorption. *J Bone Miner Res*. 2005;20(12):2224–2232.
35. Oh ES, Rhee EJ, Oh KW, et al. Circulating osteoprotegerin levels are associated with age, waist-to-hip ratio, serum total cholesterol, and low-density lipoprotein cholesterol levels in healthy Korean women. *Metabolism*. 2005;54(1):49–54.
36. Mezquita-Raya P, de la Higuera M, Garcia DF, et al. The contribution of serum osteoprotegerin to bone mass and vertebral fractures in postmenopausal women. *Osteoporos Int*. 2005;16(11):1368–1374.
37. Han KO, Choi JT, Choi HA, et al. The changes in circulating osteoprotegerin after hormone therapy in postmenopausal women and their relationship with oestrogen responsiveness on bone. *Clin Endocrinol (Oxf)*. 2005;62(3):349–353.
38. Wang YD, Wang L, Li DJ, Wang WJ. Dehydroepiandrosterone inhibited the bone resorption through the upregulation of OPG/RANKL. *Cell Mol Immunol*. 2006;3(1):41–45.
39. Rogers A, Saleh G, Hannon RA, Greenfield D, Eastell R. Circulating estradiol and osteoprotegerin as determinants of bone turnover and bone density in postmenopausal women. *J Clin Endocrinol Metab*. 2002;87(10):4470–4475.
40. Khosla S, Arrighi HM, Melton LJ 3rd, et al. Correlates of osteoprotegerin levels in women and men. *Osteoporos Int*. 2002;13(5):394–399.
41. Fahrleitner-Pammer A, Dobnig H, Piswanger-Soelkner C, et al. Osteoprotegerin serum levels in women: correlation with age, bone mass, bone turnover and fracture status. *Wien Klin Wochenschr*. 2003;115(9):291–297.
42. Indridason OS, Franzson L, Sigurdsson G. Serum osteoprotegerin and its relationship with bone mineral density and markers of bone turnover. *Osteoporos Int*. 2005;16(4):417–423.
43. Liu JM, Zhao HY, Ning G, et al. Relationships between the changes of serum levels of OPG and RANKL with age, menopause, bone biochemical markers and bone mineral density in Chinese women aged 20–75. *Calcif Tissue Int*. 2005;76(1):1–6.
44. Oh KW, Rhee EJ, Lee WY, et al. Circulating osteoprotegerin and receptor activator of NF-kappaB ligand system are associated with bone metabolism in middle-aged males. *Clin Endocrinol (Oxf)*. 2005;62(1):92–98.
45. Jorgensen L, Vik A, Emaus N, et al. Bone loss in relation to serum levels of osteoprotegerin and nuclear factor-kappaB ligand: the Tromso study. *Osteoporos Int*. 2010;21(6):931–938.
46. Chiba Y, Onouchi T, Ikeda T, Adachi J, Tamura Y, Horiuchi T. Implications of measuring soluble receptor activators of nuclear factor-kappaB ligand and osteoprotegerin in bone metabolism of elderly women. *Gerontology*. 2009;55(3):275–280.
47. Stern A, Laughlin GA, Bergstrom J, Barrett-Connor E. The sex-specific association of serum osteoprotegerin and receptor activator of nuclear factor kappaB ligand with bone mineral density in older adults: the Rancho Bernardo study. *Eur J Endocrinol*. 2007;156(5):555–562.
48. Jorgensen HL, Kusk P, Madsen B, Fenger M, Lauritzen JB. Serum osteoprotegerin (OPG) and the A163G polymorphism in the OPG promoter region are related to peripheral measures of bone mass and fracture odds ratios. *J Bone Miner Metab*. 2004;22(2):132–138.
49. Nakamichi Y, Udagawa N, Kobayashi Y, et al. Osteoprotegerin reduces the serum level of receptor activator of NF-kappaB ligand derived from osteoblasts. *J Immunol*. 2007;178(1):192–200.
50. Uemura H, Yasui T, Miyatani Y, et al. Circulating profiles of osteoprotegerin and soluble receptor activator of nuclear factor kappaB ligand in postmenopausal women. *J Endocrinol Invest*. 2008;31(2):163–168.
51. Findlay D, Chehade M, Tsangari H, et al. Circulating RANKL is inversely related to RANKL mRNA levels in bone in osteoarthritic males. *Arthritis Res Ther*. 2008;10(1):R2.
52. Szulc P, Hofbauer LC, Heufelder AE, Roth S, Delmas PD. Osteoprotegerin serum levels in men: correlation with age, estrogen, and testosterone status. *J Clin Endocrinol Metab*. 2001;86(7):3162–3165.
53. Gannage-Yared MH, Fares F, Semaan M, Khalife S, Jambart S. Circulating osteoprotegerin is correlated with lipid profile, insulin sensitivity, adiponectin and sex steroids in an ageing male population. *Clin Endocrinol (Oxf)*. 2006;64(6):652–658.
54. Kostenuik PJ, Nguyen HQ, McCabe J, et al. Denosumab, a fully human monoclonal antibody to RANKL, inhibits bone resorption and increases BMD in knock-in mice that express chimeric (murine/human) RANKL. *J Bone Miner Res*. 2009;24(2):182–195.
55. Bekker PJ, Holloway DL, Rasmussen AS, et al. A single-dose placebo-controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women. *J Bone Miner Res*. 2004;19(7):1059–1066.

56. McClung MR, Lewiecki EM, Cohen SB, et al. Denosumab in postmenopausal women with low bone mineral density. *N Engl J Med*. 2006;354(8):821–831.
57. Lewiecki EM, Miller PD, McClung MR, et al. Two-year treatment with denosumab (AMG 162) in a randomized phase 2 study of postmenopausal women with low BMD. *J Bone Miner Res*. 2007;22(12):1832–1841.
58. Bone HG, Bolognese MA, Yuen CK, et al. Effects of denosumab on bone mineral density and bone turnover in postmenopausal women. *J Clin Endocrinol Metab*. 2008;93(6):2149–2157.
59. Cummings SR, San Martin J, McClung MR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med*. 2009;361(8):756–765.
60. Geusens P. Emerging treatments for postmenopausal osteoporosis – focus on denosumab. *Clin Interv Aging*. 2009;4:241–250.
61. Beck TJ, Lewiecki EM, Miller PD, et al. Effects of denosumab on the geometry of the proximal femur in postmenopausal women in comparison with alendronate. *J Clin Densitom*. 2008;11(3):351–359.
62. Brown JP, Prince RL, Deal C, et al. Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: a randomized, blinded, phase 3 trial. *J Bone Miner Res*. 2009;24(1):153–161.
63. Lewiecki EM. Denosumab—an emerging treatment for postmenopausal osteoporosis. *Expert Opin Biol Ther*. 2010;10(3):467–476.
64. Brennan TC, Rybchyn MS, Green W, Atwa S, Conigrave AD, Mason RS. Osteoblasts play key roles in the mechanisms of action of strontium ranelate. *Br J Pharmacol*. 2009;157(7):1291–1300.
65. Caudrillier A, Hurtel-Lemaire AS, Wattel A, et al. Strontium ranelate decreases receptor activator of nuclear factor- κ B ligand-induced osteoclastic differentiation in vitro: involvement of the calcium sensing receptor. *Mol Pharmacol*. 2010;78(4):569–576.
66. Atkins GJ, Welldon KJ, Halbout P, Findlay DM. Strontium ranelate treatment of human primary osteoblasts promotes an osteocyte-like phenotype while eliciting an osteoprotegerin response. *Osteoporos Int*. 2009;20(4):653–664.
67. Lewiecki EM. Current and emerging pharmacologic therapies for the management of postmenopausal osteoporosis. *J Womens Health (Larchmt)*. 2009;18(10):1615–1626.
68. Meunier PJ, Slosman DO, Delmas PD, et al. Strontium ranelate: dose-dependent effects in established postmenopausal vertebral osteoporosis—a 2-year randomized placebo controlled trial. *J Clin Endocrinol Metab*. 2002;87(5):2060–2066.
69. Meunier PJ, Roux C, Seeman E, et al. The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. *N Engl J Med*. 2004;350(5):459–468.

Clinical Interventions in Aging

Publish your work in this journal

Clinical Interventions in Aging is an international, peer-reviewed journal focusing on evidence-based reports on the value or lack thereof of treatments intended to prevent or delay the onset of maladaptive correlates of aging in human beings. This journal is indexed on PubMed Central, MedLine, the American Chemical Society's 'Chemical Abstracts Ser-

Submit your manuscript here: <http://www.dovepress.com/clinical-interventions-in-aging-journal>

vice' (CAS), Scopus and the Elsevier Bibliographic databases. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Dovepress