

# UNIVERSITY OF BIRMINGHAM

University of Birmingham  
Research at Birmingham

## Osteoprotegerin (OPG) pathways in bone diseases and its application in therapeutic perspectives

Jayash, Soher; Al-Namnam, Nisreen Mohammed ; Shaghayegh, Gohar

DOI:

[10.33263/BRIAC102.193200](https://doi.org/10.33263/BRIAC102.193200)

License:

Creative Commons: Attribution (CC BY)

*Document Version*

Publisher's PDF, also known as Version of record

*Citation for published version (Harvard):*

Jayash, S, Al-Namnam, NM & Shaghayegh, G 2020, 'Osteoprotegerin (OPG) pathways in bone diseases and its application in therapeutic perspectives', *Biointerface Research in Applied Chemistry*, vol. 10, no. 2, pp. 5193-5200. <https://doi.org/10.33263/BRIAC102.193200>

[Link to publication on Research at Birmingham portal](#)

### General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

## Osteoprotegerin (OPG) pathways in bone diseases and its application in therapeutic perspectives

Soher Nagi Jayash<sup>1,2\*</sup> , Nisreen Mohammed Al-Namnam<sup>3,\*</sup> , Gohar Shaghayegh<sup>4</sup> 

<sup>1</sup>School of Dentistry, University of Birmingham, Birmingham, United Kingdom

<sup>2</sup>Department of Oral Medicine and Periodontology, Ibb University, Ibb, Yemen

<sup>3</sup>Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Sana'a University, Yemen

<sup>4</sup>Department of ENT Surgery, Faculty of Health and Medical Sciences, Adelaide University, Adelaide, SA, Australia

\*corresponding author e-mail address: [ss.jayash@bham.ac.uk](mailto:ss.jayash@bham.ac.uk), [sohernaji20@yahoo.com](mailto:sohernaji20@yahoo.com), [nis\\_moh2007@yahoo.com](mailto:nis_moh2007@yahoo.com) | Scopus ID [57191577152](https://orcid.org/0000-0001-9151-7152)

### ABSTRACT

Remodeling process results from the action of the tissue-forming osteoblasts and the tissue-resorbing osteoclasts which work together in certain cell units called basic multicellular units (BMUs). The local regulation of the bone cell function includes the receptor activator of nuclear factor  $\kappa$ B ligand (RANKL)/ receptor activator of nuclear factor  $\kappa$ B (RANK) / Osteoprotegerin (OPG) system. These molecules are key factors linking bone formation to resorption during the bone remodeling process. The OPG/RANKL/RANK system has a role in the pathogenesis of bone loss by modulating RANK-induced osteoclastogenesis. This role has provided the rationale for drug development that treat the bone diseases. Systemic OPG therapy was used for bone disease treatment parenteral. According to our knowledge that got from researches, there is no study has been done to explore the use of the local delivery system of OPG in the bone resorption treatment. This approach may have similar osteogenic potential in bone defects that might be capable of treatment the bone loss.

**Keywords:** *Osteoprotegerin, bone remodeling, bone diseases.*

### 1. INTRODUCTION

The discovery of nuclear factor  $\kappa$ B ligand (RANKL)/ RANK/OPG signaling pathway that has a role in the pathogenesis of bone resorption by modulating RANK-induced osteoclastogenesis have provided the rationale for the development of drugs to treat bone diseases [1].

OPG is a secretory glycoprotein of the tumour necrosis factor (TNF) receptor and plays an important role in the control of bone resorption [2]. OPG has one ligand namely RANKL. RANKL is a Type 2 transmembrane glycoprotein that belongs to the TNF family of cytokines. RANKL is showed on the surface of osteoblasts, stromal cells, and T cells. The bound between

RANKL and RANK stimulates differentiation of monocyte/macrophage progenitor cells into active and matured osteoclasts via numerous signaling pathways [3].

The role of OPG in the pathological aspects of bone diseases, such as osteoporosis associated with estrogen deficiency and periodontal disease, has been well established. Since the discovery of OPG as an inhibitor of osteoclast activity and maturation, it has opened up a new research exploration using OPG as a potential therapeutic agent for the treatment of bone diseases. Here, we reviewed the pathways of OPG in bone diseases and its application in therapeutic perspectives.

### 2. OSTEOPROTEGERIN (OPG)

OPG has been identified by Simonet and co-workers [4] as a secretory glycoprotein form of the superfamily of tumor necrosis factor (TNF) receptors. It inhibits osteoclast maturation and protects the physiological process of osteoclast in bone remodeling and ovariectomy-associated bone resorption.

The name osteoprotegerin derived from Latin os for bone and protegere to protect [5]. It is also named as TNF receptor-related molecule-1 (TR-1), tumor necrosis factor receptor superfamily member 11B (TNFRSF11B), osteoclastogenesis inhibitory factor (OCIF) and follicular dendritic cell receptor-1 (FDCLR-1).

OPG mRNA is expressed in bone, skin, liver, lung, stomach, placenta, brain and the range of other tissues. The site(s) at which it exerts its biological function is unnecessarily predictable by the site of its expression. The binding of RANKL to RANK exerts its biological function as OPG is a secreted protein [4].

important for such growth factors as a basic fibroblast growth factor to work.

OPG has no transmembrane and cytoplasmic domain as most of the TNF superfamily receptors and is secreted as soluble protein. OPG is synthesized by osteoblast as a propeptide from which the signal peptide (with 21 amino acids) separates, generating a 380-amino acid matures peptide. It has two terminals, N and C terminus and has seven major domains (domains 1–7).

At the N-terminus of OPG is death domains (D1–D4) which are cysteine-rich structures with a characteristic of the extracellular domains of the TNFR family proteins, these domains are essential for biological activity. At the C-terminus, it has two death domains (D5–D6) that share structure features with the death domains of the TNF receptor p55, Fas and TNF-related apoptosis-inducing ligand (TRAIL) receptor, all mediate apoptosis signals. Furthermore, C-terminus has domain 7 which has the heparin binding site. Binding to heparin or heparin-like molecules is

Despite it does not correlate with the biological activity, but changes in heparin-binding with proteins affect stability, the

rate of clearance, and target cell specificity. Domain 7 is also responsible for the dimerization of OPG by disulfide bond and the dimer TNF protein is a more potent inhibitor [4-7].

OPG exists as homodimeric and monomeric forms. Both of these forms have similar potency in osteoclastogenesis inhibition in vitro as reported by Tomoyasu and co-workers [8]. But in another study, dimeric form of OPG is a more potent RANKL inhibitor

### 3. BIOLOGICAL FUNCTIONS OF OPG

OPG has almost one ligand, RANKL. RANKL is a type 2 transmembrane glycoprotein that belongs to the TNF family of cytokines. It is expressed on the surface of osteoblasts, stromal cells and T cells [12]. RANKL can be expressed in two forms: as a membrane anchored form and as a soluble form. These two forms can work as potential ligands that can interact with RANK and/or OPG receptors with the same biological activity [13].

In bone, RANKL specifically bind the RANK receptors, which are a type I transmembrane protein of the TNF receptor superfamily [14]. RANK receptor expressed on osteoclasts, the surface of hematopoietic osteoclast progenitors, mammary gland epithelial cells, chondrocytes, mature T cells, trophoblast cells, dendritic cells, and hematopoietic precursors [15-17].

The RANK/RANKL binding stimulates the differentiation of the monocyte/macrophage progenitor cells to osteoclasts through the activation of numerous signaling pathways involved in the osteoclast differentiation, activation and survival [2, 3].

### 4. ROLE OF OPG IN THE BONE DISEASE PATHOGENESIS

Various studies suggest that different bone diseases are related to alterations in OPG/RANKL/RANK system. Here we summarize several bone diseases associated with OPG/RANKL/RANK abnormalities that are estrogen secretion deficiency associated with Osteoporosis, drug-induced osteoporosis, hyperparathyroidism, Paget's disease, chronic inflammatory diseases, autoimmune diseases, osteopetrosis, bone tumors and metastases and cushing syndrome.

#### 4.1. Estrogen secretion deficiency associated with osteoporosis.

Estrogens and androgens have direct effects on osteoclasts and inhibit bone resorption in vitro as well as inhibit the production of the pro-resorptive cytokine, interleukin (IL)-6 [21, 22]. The estrogen has a major role in the bone antiresorbing activity by stimulating OPG expression in osteoblasts. Estrogens induce the expression of the OPG gene in vitro and stimulate the OPG production from osteoblasts and inhibit the RANKL production [23]. The decrease of the estrogen secretion associated with decreasing the ovarian function leads to the decrease of the OPG production in the source cells and subsequently increase bone resorption [24].

#### 4.2. Drug-induced osteoporosis.

The most frequent secondary form of osteoporosis is Glucocorticoid-induced osteoporosis. Glucocorticoids down-regulate OPG expression and increase the RANKL expression [25]. After glucocorticoid exposure, the rise in the osteoblastic RANKL/OPG ratio is associated with enhanced osteoclastogenesis [26].

Immunosuppressants such as cyclosporine A, rapamycin, and tacrolimus [27-29] have also been involved in the pathogenesis of post-transplant osteoporosis. These agents have

than the monomeric form, this study was reported in Schneeweis and co-workers [9].

OPG production is imitated by  $1\alpha, 25$ -dihydroxyvitamin D<sub>3</sub>, estrogens, pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF- $\alpha$  as well as transforming growth factor- $\beta$  (TGF- $\beta$ ). OPG production is suppressed by parathyroid hormone and glucocorticoids [10, 11].

As the binding of RANKL to RANK on pre-osteoclasts and osteoclasts is essential for their maturity and activity, OPG deters binding of RANKL to RANK. Subsequently, this binding inhibits the activation of osteoclast and osteoclastogenesis and bone resorption.

Moreover, OPG also plays a key role in cell survival by its interactivity with TNF-related apoptosis inducing ligand (TRAIL). The binding of the OPG to the TRAIL and RANKL has similar affinities. As a result of the TRAIL/OPG interaction, TRAIL-induced apoptosis in several types of cells and many cancer cells is inhibited [18]. This could explain the reason for the phenomena of stimulation OPG production by tumor cells [19]. Pritzker and co-workers [20] considered the OPG as a survival factor for human microvascular endothelial cell survival as it could bind and block TRAIL-induced apoptosis.

been presented to significantly decrease OPG mRNA levels and protein secretion by osteoblast precursor cells in a dose-dependent fashion. It also stimulates RANKL mRNA levels in marrow stromal cells, thus substantially increasing the RANKL/OPG ratio and induce osteoclastogenesis [29]. OPG serum levels were positively correlated with serum levels of osteocalcin, parathyroid hormone, and creatinine. After renal transplantation, patients receiving cyclosporine and glucocorticoids, serum levels of OPG decreased significantly after 2 weeks from receiving drugs compared to baseline levels while creatinine clearance increased [30, 31].

#### 4.3. Hyperparathyroidism (PTH).

Parathyroid hormone stimulates RANKL expression and inhibits OPG production by osteoblasts, and thus promotes osteoclastogenesis [32].

The effect of PTH on the production of OPG is controversial. PTH decreases OPG secretion by the osteoblast in most in vitro studies [33, 34]. However, in vivo observations are conflicting, serum OPG is not decreased in patients with hyperparathyroidism, not changed pre- or postoperatively [35]. On the other side, treatment of rats with PTH decreased OPG mRNA levels in rat femur metaphyseal and diaphyseal bone [31]. One more study showed that hyperparathyroidism is accompanied by a high serum concentration of OPG and RANKL as well as a low OPG/RANKL ratio. Both treatments with alendronate or parathyroidectomy reduce bone resorption, and increase the OPG / RANKL ratio [36, 37].

A new standard of osteoclast regulation is being developing based on the different effects of PTH on RANKL and OPG at different stages of osteoblast development. Osteoclastogenic

activity of PTH takes place primarily by suppression of OPG gene expression in first osteoblasts and raising of RANKL gene expression in mature osteoblasts [38].

#### 4.4. Paget's disease.

The genetic polymorphisms of the OPG gene (TNFRSF11B) and RANK gene (TNFRSF11A) have been associated with Paget's disease of bone. They contribute to increasing the risk for developing the Paget's disease which is characterized by increased bone resorption by osteoclasts and uncontrolled bone remodeling [39].

Juvenile Paget's disease is a rare, autosomal recessive bone disease identified by greatly accelerated bone turnover that presents in infancy or childhood. Juvenile Paget's disease can result from osteoprotegerin deficiency caused by homozygous deletion of the gene that encodes osteoprotegerin [40, 41].

#### 4.5. Chronic inflammatory diseases.

The T cells seem to be the link between the bone resorption and inflammation. RANKL expression occurs at the surface of activated, but not quiescent, murine T cells. RANKL derived from T cell has as a signal included in optimal T cell activation. Also, T cell derived RANKL can regulate the osteoclast development and activation resulting in bone resorption so it plays a major role in the bone remodeling [42]. RANKL expression is upregulated by many soluble factors affecting bone loss, including the proinflammatory cytokines and interleukin-1 and TNF- $\alpha$ . T cells present a cell-surface membrane-bound RANKL which is split by metalloproteinases into a soluble form. Possibly, there are some functional differences between membrane-bound and soluble RANKL, with cell-bound OPG being more functional mediators of osteoclastogenesis when measured by *in vitro* assays [42, 43]. Proinflammatory cytokines such as IL1 and IL6 and TNF- $\alpha$  increase the production of the RANKL and decrease the OPG production. These mediators and change of OPG production are associated with the chronic local inflammation and viral infections. [44, 45]. OPG act as a decoy receptor for RANKL, competing with RANK for binding with RANKL, effectively inhibiting osteoclastogenesis. Therefore, the evaluation of RANKL levels with OPG levels must be accompanied as the balance of both will determine whether osteoblastic or osteoclastic activity dominates [44, 46].

#### 4.6. Periodontitis.

In the normal periodontium, OPG is expressed by cultured human gingival fibroblasts, periodontal ligament cells and dental pulp cells, but not epithelial cells [45]. OPG is continually released by resident periodontal fibroblasts and potentially endothelial cells [47].

Macrophages and T lymphocytes that infiltrate the gingival connective tissue in periodontitis release inflammatory mediators for example, IL-1, IL-6, TNF- $\alpha$  and prostaglandin E2. These mediators stimulate osteoblasts to produce RANKL and induce bone resorption indirectly. T lymphocytes can also promote alveolar bone resorption by direct production of RANKL [48]. The various forms of periodontitis are differentially regulated by the expression of RANKL and OPG and it acts as indicative of disease occurrence [49].

Crotti and co-workers [50] compared the RANKL and OPG expression in the granulomatous tissue adjacent to the areas of alveolar bone loss from periodontitis patients to the tissue

without periodontitis. Their results revealed the significantly higher levels of RANKL protein in the periodontitis tissue.

Mogi et al. [51] and Bostanci et al [49] studied the alteration of RANKL and OPG levels in gingival crevice fluid (GCF) of patients with periodontitis. They found that an increase of RANKL/OPG in GCF from periodontitis patients.

In the same line Koide and coworkers [52] reported that the alveolar bone loss associated with OPG-deficient mice may be prevented by OPG which is produced locally.

#### 4.7. Periapical disease.

OPG and RANKL have been identified in odontoblasts, ameloblasts, pulp cell lines, and periodontal ligament cells, and their expression is considered to play a role in osteoclastogenesis and bone resorption [53, 54]. In periapical disease, abnormal RANKL and OPG expression are detected, RANKL level is increased, OPG level is decreased significantly which associated with the bone absorption. The bone absorption in the periapical cyst is more active than in periapical granuloma [55].

#### 4.8. Autoimmune diseases.

The RANKL/RANK/OPG system plays as an important link between the immune system and bone metabolism. The functioning of the OPG/RANKL system is identical to that of the interleukin-cytokine system [56].

Rheumatoid arthritis is a chronic disease that is defined by progressive synovial inflammation and joint destruction. The RANK/RANKL/OPG system plays a significant part in the pathogenesis of local and generalized bone loss in rheumatoid arthritis [57]. The synovial fibroblasts, osteoblastic stromal cells, and activated T-cells express the RANKL lead to increase the RANKL/OPG ratio, that results in enhancing osteoclastogenic in erosive arthritis [58, 59]. Otherwise, some studies have shown that serum levels of OPG and RANKL in patients with rheumatoid arthritis are higher than those in healthy people. These can be explained by anti-TNF therapy, whereby regulates the OPG/RANKL balance by stimulating the bone erosion in arthritis [59, 60].

#### 4.9. Osteopetrosis.

Osteopetrosis is a rare inherited disorder whereby bone mass increase as a result of decreasing the osteoclastogenesis and bone resorption [61]. OPG, RANKL and their signaling pathway may play a major role in the pathogenesis of osteopetrosis. The inhibition effect of OPG on osteoclastogenesis was approved *in vitro* and *in vivo* [4].

#### 4.10. Bone tumors and metastases.

In bone metastases, the RANK / RANKL pathway is essential in the pathology of bone destruction. Bone tumor cells can activate the osteoclasts by causing increased RANKL levels and/or decreasing OPG levels locally, resulting in excessive osteoclast activity [62, 63]. In severe osteolysis, RANKL/OPG ratio is increased and involved in the development of benign and malignant bone tumors and the progress of osteolytic lesions by tumor metastases [64, 65]. RANKL expression is increased in bone metastases associated with different types of solid tumors, including breast cancer while the expression of OPG is inhibited. These changes caused by secretion of the factors such as IL-1, IL-11 and PGE2 by tumor cells present in the bone marrow [66, 67].

#### 4.11. Cushing syndrome.

Cushing syndrome defined as the signs and symptoms associated with prolonged exposure to incorrectly high levels of the hormone cortisol. This can result from diseases that caused the excess cortisol, adrenocorticotropic hormone levels, taking glucocorticoid drugs [68].

Serum OPG levels also positively correlated with morning serum cortisol. In patients with Cushing syndrome, serum OPG levels are higher than healthy control subjects, but it remained unchanged after the recovery, even the bone is restored. The elevation of OPG level could be due to persistent damage to the cardiovascular system by glucocorticoid [69-71].

### 5. OVERVIEW OF THERAPEUTIC PERSPECTIVES OF OPG IN BONE DISEASES

As the OPG has a key role in the bone diseases, there are various experimental and clinical trials on the use of OPG in treatment of different bone diseases. It was previously reported that OPG-chitosan matrices and gel increase the in vitro proliferation of normal human osteoblasts and fibroblasts cells that used later to treat the critical size defects on parietal bone of rabbit. The results of this studies showed that bioresorbable OPG-chitosan material induced the formation of a significant quantity of bone in a critical-sized parietal bone defect in a rabbit model [72-74].

Simonet et al. [4] and Yasuda et al. [6] reported that the increasing in bone mass in mice after treatment with OPG at 10 mg/kg/day for 7 days and 24 mg/ kg/day for 14 days. Furthermore, OPG treatment at a dose of 5 mg/ kg/day for 14 days has been completely prevented ovariectomy-induced bone loss in rats [4]. There is another study confirmed the hypocalcemia effects of OPG after injection in normal rats. The result showed that increases of density of bone mineral and volume of bone accompanied by a decrease of osteoclast number. The facts suggested that the reduction of the calcium concentration in serum of normal rats by administration of OPG is due to suppression of bone resorption by inhibition of osteoclastogenesis, especially maturation of osteoclasts [8].

Bolon and co-workers transfer OPG gene mediated vehicle by a single injection of a recombinant adenovirus carrying the OPG-Fc gene, prevents bone density loss induced by ovariectomy period [75]. In addition, a single intravenous injection of OPG in young growing rats leads to a significant increase in bone volume

and density, which are associated with suppression of osteoclastic bone resorption [76].

Other preclinical studies demonstrated a potential therapeutic role of OPG in the prevention and reduction of lytic bone lesion associated with skeletal tumor. Lamoureux et al. [77] suggested the targeting of RANKL may has the potential to modify the osteosarcoma and suppress its development.

In experimental periodontitis, human OPG has strong preventive effects on alveolar bone loss in rats [78]. Yao et al. [79] revealed that OPG with BMP-2 improves osteoblastogenesis and new bone formation by recruitment of mesenchymal stem cells [79]

OPG treatment is used in preclinical bone metastasis models. The result revealed that the proliferation of tumor cell is decreased and apoptosis of tumor cell osteoclast activity is decreased. Whereby the bone turnover and the pro-tumor growth factors releasing from the bone matrix are prevented [64].

Additionally, there are a few clinical trials on the use of OPG systemically. One of these studies is done by using a single subcutaneous dose of human recombinant OPG (3 mg/kg) in post-menopausal women [80, 81]. It was stated that biochemical markers of bone resorption have reduced and OPG can reduce bone turnover.

Another study performed by Body et al. [82], who used recombinant OPG in patients with bone disease related to breast carcinoma or multiple myeloma. The results indicated that recombinant OPG is effective in reducing bone resorption marker levels as an accepted treatment for these diseases.

### 6. CONCLUSIONS

Bone resorption-related diseases, such as periodontitis, osteoporosis or rheumatoid arthritis, affect many people worldwide. New doors have been opened for bone research and drug development. The different studies illuminate the fact of that OPG regulates osteoclastogenesis via the RANKL-OPG pathway. Thus, enhancing OPG production may be accompanied with an increase in bone mass.

Since the discovery of OPG as an inhibitor of osteoclast activity and maturation, it has opened up a new research exploration using OPG as a potential therapeutic agent for the treatment of bone diseases. Up to our knowledge, there is a few

research carried out to explore the use of local OPG therapy in the treatment of bone resorption. As a critical therapeutic concern, it is to target specifically the bone tissue to avoid prospective undesired effects on other tissues. Systemic therapy may lead to unwanted side effects, therefore local therapy of OPG may be efficient as an alternative therapy.

Despite the interest of the various studies targeting the OPG pathways in skeletal diseases, further preclinical and clinical studies are needed. One possible approach is the development of local delivery of OPG that may have similar potential in treatment of bone loss.

### 7. REFERENCES

1. Liu, W.; Zhang, X. Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL)/RANK/osteoprotegerin system in bone and other tissues. *Molecular Medicine Reports* **2015**, *11*, 3212-3218, <https://doi.org/10.3892/mmr.2015.3152>.
2. Theoleyre, S.; Tat, K.S.; Vusio, P.; Blanchard, F.; Gallagher, J.; Richard-Blum, S.; Fortun, Y.; Padrines, M.; Redini, F.; Heymann, D. Characterization of osteoprotegerin binding to glycosaminoglycans by surface plasmon resonance: Role in the

interactions with receptor activator of nuclear factor  $\kappa$ B ligand (RANKL) and RANK. *Biochemical and biophysical research communications* **2006**, *347*, 460-467, <https://doi.org/10.1016/j.bbrc.2006.06.120>.

3. Baud'huin, M.; Duplomb, L.; Teletchea, S.; Lamoureux, F.; Ruiz-Velasco, C.; Maillason, M.; Redini, F.; Heymann, M.F.; Heymann, D. Osteoprotegerin: multiple partners for multiple

- functions. *Cytokine & growth factor reviews* **2013**, *24*, 401-409, <https://doi.org/10.1016/j.cytogfr.2013.06.001>.
4. Simonet, W.; Lacey, D.L.; Dunstan, C.R.; Kelley, M.; Chang, M.S.; Luthy, R.; Nguyen, H.Q.; Wooden, S.; Bennett, L.; Boone, T.; Shimamoto, G.; DeRose, M.; Elliott, R.; Colombero, A.; Tan, H.L.; Trail, G.; Sullivan, J.; Davy, E.; Bucay, N.; Renshaw-Gegg, L.; Hughes, T.M.; Hill, D.; Pattison, W.; Campbell, P.; Sander, S.; Van, G.; Tarpley, J.; Derby, P.; Lee, R.; Boyle, W.J. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* **1997**, *89*, 309-319, [https://doi.org/10.1016/S0092-8674\(00\)80209-3](https://doi.org/10.1016/S0092-8674(00)80209-3).
5. Holen, I.; Shipman, C. Role of osteoprotegerin (OPG) in cancer. *Clinical Science* **2006**, *110*, 279-291, <https://doi.org/10.1042/CS20050175>.
6. Yasuda, H.; Shima, N.; Nakagawa, N.; Mochizuki, S.I.; Yano, K.; Fujise, N.; Sato, Y.; Goto, M.; Yamaguchi, K.; Kuriyama, M.; Kanno, T.; Murakami, A.; Tsuda, E.; Morinaga, T.; Higashio, K. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. *Endocrinology* **1998**, *139*, 1329-1337, <https://doi.org/10.1210/endo.139.3.5837>.
7. Yamaguchi, K.; Kinosaki, M.; Goto, M.; Kobayashi, F.; Tsuda, E.; Morinaga, T.; Higashio, K. Characterization of structural domains of human osteoclastogenesis inhibitory factor. *Journal of Biological Chemistry* **1998**, *273*, 5117-5123, <https://doi.org/10.1074/jbc.273.9.5117>.
8. Tomoyasu, A.; Goto, M.; Fujise, N.; Mochizuki, S.I.; Yasuda, H.; Morinaga, T.; Tsuda, E.; Higashio, K. Characterization of monomeric and homodimeric forms of osteoclastogenesis inhibitory factor. *Biochemical and biophysical research communications* **1998**, *245*, 382-387, <https://doi.org/10.1006/bbrc.1998.8443>.
9. Schneeweis, L.A.; Willard, D.; Milla, M.E. Functional dissection of osteoprotegerin and its interaction with receptor activator of NF- $\kappa$ B ligand. *Journal of Biological Chemistry* **2005**, *280*, 41155-41164, <https://doi.org/10.1074/jbc.M506366200>.
10. Bronner, F., M.C. Farach-Carson, E.; Rubin, J. *Bone resorption*. Springer, Volume 2. 2005.
11. Thirunavukkarasu, K.; Miles, R.R.; Halladay, D.L.; Yang, X.; Galvin, R.J.; Chandrasekhar, S.; Martin, T.J.; Onyia, J.E. Stimulation of Osteoprotegerin (OPG) Gene Expression by Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) mapping of the OPG promoter region that mediates TGF- $\beta$  effects. *Journal of Biological Chemistry* **2001**, *276*, 36241-36250, <https://doi.org/10.1074/jbc.M104319200>.
12. Yasuda, H.; Shima, N.; Nakagawa, N.; Yamaguchi, K.; Kinosaki, M.; Mochizuki, S.; Tomoyasu, A.; Yano, K.; Goto, M.; Murakami, A.; Tsuda, E.; Morinaga, T.; Higashio, K.; Udagawa, N.; Takahashi, N.; Suda, T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proceedings of the National Academy of Sciences* **1998**, *95*, 3597-3602, <https://doi.org/10.1073/pnas.95.7.3597>.
13. Lum, L.; Wong, B.R.; Josien, R.; Becherer, J.D.; Erdjument-Bromage, H.; Schlöndorff, J.; Tempst, P.; Choi, Y.; Blobel, C.P. Evidence for a role of a tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. *Journal of Biological Chemistry* **1999**, *274*, 13613-13618, <https://doi.org/10.1074/jbc.274.19.13613>.
14. Anderson, D.M., Maraskovsky, E.; Billingsley, W.L.; Dougall, W.C.; Tometsko, M.E.; Roux, E.R.; Teepe, M.C.; DuBose, R.F.; Cosman, D.; Galibert, L. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* **1997**, *390*, 175-179, <https://doi.org/10.1038/36593>.
15. Neumann, E.; Gay, S.; Müller-Ladner, U. The RANK/RANKL/osteoprotegerin system in rheumatoid arthritis: new insights from animal models. *Arthritis & Rheumatism* **2005**, *52*, 2960-2967, <https://doi.org/10.1002/art.21361>.
16. Fata, J.E.; Kong, Y.Y.; Li, J.; Sasaki, T.; Irie-Sasaki, J.; Moorehead, R.A.; Elliott, R.; Scully, S.; Voura, E.B.; Lacey, D.L.; Boyle, W.J.; Khokha, R.; Penninger, J.M. The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. *Cell* **2000**, *103*, 41-50, [https://doi.org/10.1016/S0092-8674\(00\)00103-3](https://doi.org/10.1016/S0092-8674(00)00103-3).
17. Hsu, H.; Lacey, D.L.; Dunstan, C.R.; Solovyev, I.; Colombero, A.; Timms, E.; Tan, H.L.; Elliott, G.; Kelley, M.J.; Sarosi, I.; Wang, L.; Xia, X.Z.; Elliott, R.; Chiu, L.; Black, T.; Scully, S.; Capparelli, C.; Morony, S.; Shimamoto, G.; Bass, M.B.; Boyle, W.J. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. *Proceedings of the National Academy of Sciences* **1999**, *96*, 3540-3545, <https://doi.org/10.1073/pnas.96.7.3540>.
18. Neville-Webbe, H.; Cross, N.A.; Eaton, C.L.; Nyambo, R.; Evans, C.A.; Coleman, R.E.; Holen, I. Osteoprotegerin (OPG) produced by bone marrow stromal cells protects breast cancer cells from TRAIL-induced apoptosis. *Breast cancer research and treatment* **2004**, *86*, 271-282, <https://doi.org/10.1023/b:brea.0000036900.48763.b3>.
19. Reid, P.E.; Brown, N.J.; Holen, I. Breast cancer cells stimulate osteoprotegerin (OPG) production by endothelial cells through direct cell contact. *Molecular cancer* **2009**, *8*, <https://doi.org/10.1186/1476-4598-8-49>.
20. Pritzker, L.; Scatena, M.; Giachelli, C. The role of osteoprotegerin and tumor necrosis factor-related apoptosis-inducing ligand in human microvascular endothelial cell survival. *Molecular biology of the cell* **2004**, *15*, 2834-2841, <https://doi.org/10.1091/mbc.e04-01-0059>.
21. Bellido, T.; Jilka, R.L.; Boyce, B.F.; Girasole, G.; Broxmeyer, H.; Dalrymple, S.A.; Murray, R.; Manolagas, S.C. Regulation of interleukin-6, osteoclastogenesis, and bone mass by androgens. The role of the androgen receptor. *Journal of Clinical Investigation* **1995**, *95*, 2886, <https://doi.org/10.1172/JCI117995>.
22. Pederson, L.; Kremer, M.; Judd, J.; Pascoe, D.; Spelsberg, T.C.; Riggs, B.L.; Oursler, M.J. Androgens regulate bone resorption activity of isolated osteoclasts in vitro. *Proceedings of the National Academy of Sciences* **1999**, *96*, 505-510, <https://doi.org/10.1073/pnas.96.2.505>.
23. Hofbauer, L.C.; Khosla, S.; Dunstan, C.R.; Lacey, D.L.; Spelsberg, T.C.; Riggs, B.L. Estrogen Stimulates Gene Expression and Protein Production of Osteoprotegerin in Human Osteoblastic Cells\*. *Endocrinology* **1999**, *140*, 4367-4370, <https://doi.org/10.1210/endo.140.9.7131>.
24. Bord, S.; Ireland, D.C.; Beavan, S.R.; Compston, J.E. The effects of estrogen on osteoprotegerin, RANKL, and estrogen receptor expression in human osteoblasts. *Bone* **2003**, *32*, 136-141, [https://doi.org/10.1016/S8756-3282\(02\)00953-5](https://doi.org/10.1016/S8756-3282(02)00953-5).
25. Wang, J.; Gao, H.Y.; Wang, K.Z.; Zhou, R.X.; Li, X.D.; Guo, J.; Lv, H.C. Effect of epimedium extract on osteoprotegerin and RANKL mRNA expressions in glucocorticoid-induced femoral head necrosis in rats. *Nan fang yi ke da xue xue bao = Journal of Southern Medical University* **2011**, *31*, 1714.
26. von Tirpitz, C.; Epp, S.; Klaus, J.; Mason, R.; Hawa, G.; Brinskelle-Schmal, N.; Hofbauer, L.C.; Adler, G.; Kratzer, W.; Reinshagen, M. Effect of systemic glucocorticoid therapy on bone metabolism and the osteoprotegerin system in patients with active Crohn's disease. *European journal of gastroenterology & hepatology* **2003**, *15*, 1165-1170, <https://doi.org/10.1097/00042737-200311000-00003>.

27. Stein, E.; Ebeling, P.; Shane, E. Post-transplantation osteoporosis. *Endocrinology and metabolism clinics of North America* **2007**, *36*, 937-963, <https://doi.org/10.1016/j.ecl.2007.07.008>.
28. Monegal, A.; Navasa, M.; Guañabens, N.; Peris, P.; Pons, F.; Martínez de Osaba, M.J.; Rimola, A.; Rodés, J.; Muñoz-Gómez, J. Bone mass and mineral metabolism in liver transplant patients treated with FK506 or cyclosporine A. *Calcified tissue international* **2001**, *68*, 83-86, <https://doi.org/10.1007/bf02678145>.
29. Hofbauer, L.C.; Shui, C.; Riggs, B.L.; Dunstan, C.R.; Spelsberg, T.C.; O'Brien, T.; Khosla, S. Effects of immunosuppressants on receptor activator of NF- $\kappa$ B ligand and osteoprotegerin production by human osteoblastic and coronary artery smooth muscle cells. *Biochemical and biophysical research communications* **2001**, *280*, 334-339, <https://doi.org/10.1006/bbrc.2000.4130>.
30. Sato, T.; Tominaga, Y.; Iwasaki, Y.; Kazama, J.J.; Shigematsu, T.; Inagaki, H.; Watanabe, I.; Katayama, A.; Haba, T.; Uchida, K.; Fukagawa, M. Osteoprotegerin levels before and after renal transplantation. *American journal of kidney diseases* **2001**, *38*, S175-S177, <https://doi.org/10.1053/ajkd.2001.27437>.
31. Hofbauer, L.; Kuhne, C.; Viereck, V. The OPG/RANKL/RANK system in metabolic bone diseases. *Journal of Musculoskeletal and Neuronal Interactions* **2004**, *4*, 268, <https://doi.org/10.1053/ajkd.2001.27437>.
32. Tsukii, K.; Shima, N.; Mochizuki, S.; Yamaguchi, K.; Kinoshita, M.; Yano, K.; Shibata, O.; Udagawa, N.; Yasuda, H.; Suda, T.; Higashio, K. Osteoclast Differentiation Factor Mediates an Essential Signal for Bone Resorption Induced by  $1\alpha$ , 25-Dihydroxyvitamin D<sub>3</sub>, Prostaglandin E<sub>2</sub>, or Parathyroid Hormone in the Microenvironment of Bone. *Biochemical and biophysical research communications* **1998**, *246*, 337-341, <https://doi.org/10.1006/bbrc.1998.8610>.
33. Onyia, J.; Miles, R.R.; Yang, X.; Halladay, D.L.; Hale, J.; Glasebrook, A.; McClure, D.; Seno, G.; Churgay, L.; Chandrasekhar, S.; Martin, T.J. In vivo demonstration that human parathyroid hormone 1-38 inhibits the expression of osteoprotegerin in bone with the kinetics of an immediate early gene. *Journal of Bone and Mineral Research* **2000**, *15*, 863-871, <https://doi.org/10.1359/jbmr.2000.15.5.863>.
34. Lee, S.K.; Lorenzo, J.A. 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*, 3552-3561, <https://doi.org/10.1210/endo.140.8.6887>.
35. Stilgren, L.; Hegedüs, L.M.; Beck-Nielsen, H.; Abrahamsen, B. Osteoprotegerin levels in primary hyperparathyroidism: effect of parathyroidectomy and association with bone metabolism. *Calcified tissue international* **2003**, *73*, 210-216, <https://doi.org/10.1007/s00223-002-2100-8>.
36. Szymczak, J.; Bohdanowicz-Pawlak, A. Osteoprotegerin, RANKL, and Bone Turnover in Primary Hyperparathyroidism: The Effect of Parathyroidectomy and Treatment with Alendronate. *Hormone and Metabolic Research* **2013**, *45*, <https://doi.org/10.1055/s-0033-1349842>.
37. Kosari, E.; Hezarkhani, S.; Marjani, A.; Aliakbari, F.; Saifi, A.; Mansourian, A.R. Serum Levels Of Osteoprotegerin in Women with Subclinical Hypothyroidism Before and After Of Levothyroxine Treatment. *Transylvanian Review* **2016**.
38. Huang, J.C.; Sakata, T.; Pflieger, L.L.; Bencsik, M.; Halloran, B.P.; Bikle, D.D.; Nissenson, R.A. PTH differentially regulates expression of RANKL and OPG. *Journal of Bone and Mineral Research* **2004**, *19*, 235-244, <https://doi.org/10.1359/JBMR.0301226>.
39. Chung, P.Y.J.; Van Hul, W. Paget's disease of bone: evidence for complex pathogenetic interactions. *Seminars in arthritis and rheumatism* **2012**, *41*, <https://doi.org/10.1016/j.semarthrit.2011.07.005>.
40. Whyte, M.P.; Obrecht, S.E.; Finnegan, P.M.; Jones, J.L.; Podgornik, M.N.; McAlister, W.H. Mumm, S. Osteoprotegerin deficiency and juvenile Paget's disease. *New England Journal of Medicine* **2002**, *347*, 175-184, <https://doi.org/10.1056/NEJMoa013096>.
41. Cundy, T.; Davidson, J.; Rutland, M.D.; Stewart, C.; DePaoli, A.M. Recombinant osteoprotegerin for juvenile Paget's disease. *New England Journal of Medicine* **2005**, *353*, 918-923, <https://doi.org/10.1056/NEJMoa050893>.
42. Kong, Y.Y.; Feige, U.; Sarosi, I.; Bolon, B.; Tafuri, A.; Morony, S.; Capparelli, C.; Li, J.; Elliott, R.; McCabe, S.; Wong, T.; Campagnuolo, G.; Moran, E.; Bogoch, E.R.; Van, G.; Nguyen, L.T.; Ohashi, P.S.; Lacey, D.L.; Fish, E.; Boyle, W.J.; Penninger, J.M. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature* **1999**, *402*, 43-47, <https://doi.org/10.1038/46303>.
43. Sakata, M.; Shiba, H.; Komatsuzawa, H.; Fujita, T.; Ohta, K.; Sugai, M.; Suginaka, H.; Kurihara, H. Expression of osteoprotegerin (osteoclastogenesis inhibitory factor) in cultures of human dental mesenchymal cells and epithelial cells. *Journal of Bone and Mineral Research* **1999**, *14*, 1486-1492, <https://doi.org/10.1359/jbmr.1999.14.9.1486>.
44. Tunyogi-Csapo, M.; Kis-Toth, K.; Radacs, M.; Farkas, B.; Jacobs, J.J.; Finnegan, A.; Mikecz, K.; Glant, T.T. Cytokine-controlled RANKL and osteoprotegerin expression by human and mouse synovial fibroblasts: Fibroblast-mediated pathologic bone resorption. *Arthritis & Rheumatism* **2008**, *58*, 2397-2408, <https://doi.org/10.1002/art.23653>.
45. Nakashima, T.; Penninger, J.M. RANKL and RANK as novel therapeutic targets for arthritis. *Current opinion in rheumatology* **2003**, *15*, 280-287, <https://doi.org/10.1097/00002281-200305000-00016>.
46. Yeung, R.S. The osteoprotegerin/osteoprotegerin ligand family: role in inflammation and bone loss. *The Journal of rheumatology* **2004**, *31*, 844-846.
47. Kobayashi-Sakamoto, M.; Hirose, K.; Isogai, E.; Chiba, I. NF- $\kappa$ B-dependent induction of osteoprotegerin by *Porphyromonas gingivalis* in endothelial cells. *Biochemical and biophysical research communications* **2004**, *315*, 107-112, <https://doi.org/10.1016/j.bbrc.2004.01.024>.
48. Taubman, M.; Kawai, T. Involvement of T-lymphocytes in periodontal disease and in direct and indirect induction of bone resorption. *Critical Reviews in Oral Biology & Medicine* **2001**, *12*, 125-135, <https://doi.org/10.1177/10454411010120020301>.
49. Bostanci, N.; Ilgenli, T.; Emingil, G.; Afacan, B.; Han, B.; Töz, H.; Atilla, G.; Hughes, F.J.; Belibasakis, G.N. Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. *Journal of Clinical Periodontology* **2007**, *34*, 370-376, <https://doi.org/10.1111/j.1600-051X.2007.01061.x>.
50. Crotti, T.; Smith, M.D.; Hirsch, R.; Soukoulis, S.; Weedon, H.; Capone, M.; Ahern, M.J.; Haynes, D. Receptor activator NF  $\kappa$ B ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. *Journal of Periodontal Research* **2003**, *38*, 380-387, <https://doi.org/10.1034/j.1600-0765.2003.00615.x>.
51. Mogi, M.; Otogoto, J.; Ota, N.; Togari, A. Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. *Journal of dental research* **2004**, *83*, 166-169, <https://doi.org/10.1177/154405910408300216>.
52. Koide, M.; Kobayashi, Y.; Ninomiya, T.; Nakamura, M.; Yasuda, H.; Arai, Y.; Okahashi, N.; Yoshinari, N.; Takahashi, N.; Udagawa, N. Osteoprotegerin-Deficient Male Mice as a Model for Severe Alveolar Bone Loss: Comparison with

- RANKL-Overexpressing Transgenic Male Mice. *Endocrinology* **2013**, *154*, 773-782, <https://doi.org/10.1210/en.2012-1928>.
53. Tobón-Arroyave, S.I.; Franco-Gonzalez, L.M.; Isaza-Guzman, D.M.; Florez-Moreno, G.A.; Bravo, Vasquez, T.; Castaneda-Pelaez, D.A.; Vieco-Duran, B. Immunohistochemical expression of RANK, GRα and CTR in central giant cell granuloma of the jaws. *Oral Oncology* **2005**, *41*, 480-488, <https://doi.org/10.1016/j.oraloncology.2004.11.006>.
54. Qian, Y.; Huang, H.Z. The role of RANKL and MMP-9 in the bone resorption caused by ameloblastoma. *Journal of oral pathology & medicine* **2010**, *39*, 592-598, <https://doi.org/10.1111/j.1600-0714.2009.00882.x>.
55. Meihua, Z.; Yunzhi, Y.; Yu, M. The expression and significance of receptor activator of nuclear factor κB ligand and osteoprotegerin in periapical cyst and periapical granuloma. *West China Journal of Stomatology* **2012**, *30*.
56. Kohli, S.S.; Kohli, V.S. Role of RANKL-RANK/osteoprotegerin molecular complex in bone remodeling and its immunopathologic implications. *Indian journal of endocrinology and metabolism* **2011**, *15*, 175, <https://doi.org/10.4103/2230-8210.83401>.
57. Vega, D.; Maalouf, N.M.; Sakhaee, K. The role of receptor activator of nuclear factor-κB (RANK)/RANK ligand/osteoprotegerin: clinical implications. *Journal of Clinical Endocrinology & Metabolism* **2007**, *92*, 4514-4521, <https://doi.org/10.1210/jc.2007-0646>.
58. Lubberts, E.; van den Bersselaar, L.; Oppers-Walgreen, B.; Schwarzenberger, P.; Coenen-de Roo, C.J.; Kolls, J.K.; Joosten, L.A.; van den Berg, W.B. IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-κB ligand/osteoprotegerin balance. *The Journal of Immunology* **2003**, *170*, 2655-2662, <https://doi.org/10.4049/jimmunol.170.5.2655>.
59. Fili, S.; Karalaki, M.; Schaller, B. Therapeutic implications of osteoprotegerin. *Cancer Cell Int* **2009**, *9*, 26.
60. Oyajobi, B.O. Multiple myeloma/hypercalcemia. *Arthritis research and therapy* **2007**, *9*, S4.
61. Felix, R.; Hofstetter, W.; Cecchini, M.G. Recent developments in the understanding of the pathophysiology of osteopetrosis. *European journal of endocrinology* **1996**, *134*, 143-156, <https://doi.org/10.1530/eje.0.1340143>.
62. Mundy, G.R. Metastasis: Metastasis to bone: causes, consequences and therapeutic opportunities. *Nature Reviews Cancer* **2002**, *2*, 584-593, <https://doi.org/10.1038/nrc867>.
63. Canon, J.; Bryant, R.; Roudier, M.; Branstetter, D.G.; Dougall, W.C. RANKL inhibition combined with tamoxifen treatment increases anti-tumor efficacy and prevents tumor-induced bone destruction in an estrogen receptor-positive breast cancer bone metastasis model. *Breast cancer research and treatment* **2012**, *135*, 771-780, <https://doi.org/10.1007/s10549-012-2222-2>.
64. Grimaud, E.; Soubigou, L.; Couillaud, S.; Coipeau, P.; Moreau, A.; Passuti, N.; Gouin, F.; Redini, F.; Heymann, D. Receptor activator of nuclear factor κB ligand (RANKL)/osteoprotegerin (OPG) ratio is increased in severe osteolysis. *The American journal of pathology* **2003**, *163*, 2021-2031, [https://doi.org/10.1016/s0002-9440\(10\)63560-2](https://doi.org/10.1016/s0002-9440(10)63560-2).
65. Milone, F.; Pivonello, C.; Cariati, F.; Sarnataro, M.; Ramundo, V.; Marotta, V.; Jann, H.; Pape, U.F.; Wiedenmann, B.; Colao, A.; Pavel, M.; Faggiano, A. Assessment and clinical implications of RANK/RANKL/OPG pathway as markers of bone tumor progression in patients with NET harboring bone metastases. *Biomarkers* **2013**, *18*, 121-125, <https://doi.org/10.3109/1354750X.2012.745166>.
66. Clézardin, P.; Teti, A. Bone metastasis: pathogenesis and therapeutic implications. *Clinical & experimental metastasis* **2007**, *24*, 599-608, <https://doi.org/10.1007/s10585-007-9112-8>.
67. Clézardin, P. The role of RANK/RANKL/osteoprotegerin (OPG) triad in cancer-induced bone diseases: physiopathology and clinical implications. *Bulletin du cancer* **2011**, *98*, 837, <https://doi.org/10.1684/bdc.2011.1398>.
68. Abbas, A.K.; Kumar, V.; Fausto, N. *Robbins & Cotran- Patologia*. Elsevier. 2005.
69. Hofbauer, L.C.; Schoppet, M. Clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular diseases. *JAMA: the journal of the American Medical Association* **2004**, *292*, 490-495, <https://doi.org/10.1001/jama.292.4.490>.
70. Dovio, A.; Allasino, B.; Palmas, E.; Ventura, M.; Pia, A.; Saba, L.; Aroasio, E.; Terzolo, M.; Angeli, A. Increased osteoprotegerin levels in Cushing's syndrome are associated with an adverse cardiovascular risk profile. *Journal of Clinical Endocrinology & Metabolism* **2007**, *92*, 1803-1808, <https://doi.org/10.1210/jc.2006-2283>.
71. Ueland, T.; Bollerslev, J.; Godang, K.; Müller, F.; Frøland, S.S.; Aukrust, P. Increased serum osteoprotegerin in disorders characterized by persistent immune activation or glucocorticoid excess--possible role in bone homeostasis. *European journal of endocrinology* **2001**, *145*, 685-690, <https://doi.org/10.1530/eje.0.1450685>.
72. Jayash, S.N.; Hashim, N.M.; Misran, M.; Baharuddin, N.A. Formulation and in vitro and in vivo evaluation of a new osteoprotegerin-chitosan gel for bone tissue regeneration. *Journal of Biomedical Materials Research Part A* **2017**, *105*, 398-407, <https://doi.org/10.1002/jbm.a.35919>.
73. Jayash, S.N.; Hashim, N.M.; Misran, M.; Baharuddin, N.A. In vitro evaluation of osteoprotegerin in chitosan for potential bone defect applications. *PeerJ* **2016**, *4*, e2229, <https://doi.org/10.7717/peerj.2229>.
74. Jayash, S.N.; Hashim, N.M.; Misran, M.; Baharuddin, N.A. Local application of osteoprotegerin-chitosan gel in critical-sized defects in a rabbit model. *PeerJ* **2017**, *5*, e3513, <https://doi.org/10.7717/peerj.3513>.
75. Bolon, B.; Carter, C.; Daris, M.; Morony, S.; Capparelli, C.; Hsieh, A.; Mao, M.; Kostenuik, P.; Dunstan, C.R.; Lacey, D.L.; Sheng, J.Z. Adenoviral Delivery of Osteoprotegerin Ameliorates Bone Resorption in a Mouse Ovariectomy Model of Osteoporosis. *Molecular therapy* **2001**, *3*, 197-205, <https://doi.org/10.1006/mthe.2001.0245>.
76. Capparelli, C.; Morony, S.; Warmington, K.; Adamu, S.; Lacey, D.; Dunstan, C.R.; Stouch, B.; Martin, S.; Kostenuik, P.J. Sustained antiresorptive effects after a single treatment with human recombinant osteoprotegerin (OPG): a pharmacodynamic and pharmacokinetic analysis in rats. *Journal of Bone and Mineral Research* **2003**, *18*, 852-858, <https://doi.org/10.1359/jbmr.2003.18.5.852>.
77. Lamoureux, F.; Richard, P.; Wittrant, Y.; Battaglia, S.; Pilet, P.; Trichet, V.; Blanchard, F.; Gouin, F.; Pitard, B.; Heymann, D.; Redini, F. Therapeutic relevance of osteoprotegerin gene therapy in osteosarcoma: blockade of the vicious cycle between tumor cell proliferation and bone resorption. *Cancer research* **2007**, *67*, 7308-7318, <https://doi.org/10.1158/0008-5472.CAN-06-4130>.
78. Jin, Q.; Park, C.H.; Taba, M.Jr.; Giannobile, W.V. RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis. *Journal of periodontology* **2007**, *78*, 1300-1308, <https://doi.org/10.1902/jop.2007.070073>.
79. Yao, Y.; Wang, G.; Wang, Z.; Wang, C.; Zhang, H.; Liu, C. Synergistic enhancement of new bone formation by recombinant human bone morphogenetic protein-2 and osteoprotegerin in trans-sutural distraction osteogenesis: a pilot study in dogs. *Journal of Oral and Maxillofacial Surgery* **2011**, *69*, e446-e455, <https://doi.org/10.1016/j.joms.2011.07.002>.



80. Bekker, P.J.; Nakanishi, H.D.; Arrighi, A.; Dunstan, C.R. Osteoprotegerin (OPG) has potent and sustained anti-resorptive activity in postmenopausal women. *J Bone Miner Res.* **1999**, *14*, 1190.

81. Hofbauer, L.C.; Heufelder, A.E. The role of receptor activator of nuclear factor- $\kappa$ B ligand and osteoprotegerin in the pathogenesis and treatment of metabolic bone diseases. *Journal of Clinical Endocrinology & Metabolism* **2000**, *85*, 2355-2363, <https://doi.org/10.1007/s001090100226>.

82. Body, J.J.; Greipp, P.; Coleman, R.E.; Facon, T.; Geurs, F.; Feraud, J.P.; Harousseau, J.L.; Lipton, A.; Mariette, X.; Williams, C.D.; Nakanishi, A.; Holloway, D.; Martin, S.W.; Dunstan, C.R.; Bekker, P.J. A Phase I study of AMG-007, a recombinant osteoprotegerin construct, in patients with multiple myeloma or breast carcinoma related bone metastases. *Cancer* **2003**, *97*(S3), 887-892, <https://doi.org/10.1002/cncr.11138>.

## 8. ACKNOWLEDGEMENTS

We thank the University of Birmingham and Cara for their support.



© 2020 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).