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Denosumab: Targeting the RANKL pathway to treat Rheumatoid Arthritis

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Abstract

Introduction—Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by focal pathologic bone resorption due to excessive activity of osteoclasts (OC). Receptor activator of nuclear factor kappa B ligand (RANKL) is essential for the proliferation, differentiation, and survival of OC. Denosumab (DMab) is a humanized monoclonal antibody that binds to RANKL with high affinity and blocks its subsequent association with its receptor RANK on the surface of OC precursors.

Area Covered—The authors review the molecular and cellular mechanisms underlying therapeutic applications of DMab, provide recent highlights on pharmacology, efficacy and safety of DMab, and discuss the potential of DMab as a novel therapeutic option for the treatment of rheumatoid arthritis.

Expert opinion—Clinical results suggest that DMab is efficient both in systemic and articular bone loss in RA with limited side effects. Diminished bone erosion activity was also noted in RA patients on corticosteroids and bisphosphonates. Combination of DMab with an anti-TNF agent was not associated with increased infection rates. Collectively, these data indicate that DMab, in combination with methotrexate and possibly other conventional synthetic Disease Modifying Anti-Rheumatic Drugs (csDMARDs), is an effective, safe and cost-effective option for the treatment of RA.

1. Introduction

DMab is a human monoclonal IgG_2 antibody that inhibits bone resorption by binding and inhibiting receptor activator of NF-kB ligand (RANKL), an essential cytokine for osteoclast (OC) formation, activity, and survival (1, 2). Association of DMab with RANKL will inhibit the binding of RANKL to the RANK receptor expressed on the cell surface of OC, an essential activation step for OC differentiation. The Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) trial and its Extension provide long-term information on denosumab for treating postmenopausal osteoporosis (3-5). DMab

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Declaration of Interest:

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treatment for up to 8 years significantly decreased bone turnover (6), increased bone mineral density (BMD)(7), improved bone microstructure of both cortical and trabecular bone (7, 8), and reduces the risk of bone fracture and osteoporosis (3, 5, 9-12). Bone biopsies confirmed potent and sustained effects of DMab on bone quality with continuous DMab treatment for 5-8 years (3, 4). The beneficial effects of DMab, however, can be fully reversed at the tissue level within 2 years of discontinuation, indicating that the skeletal effects of DMab are directed towards regulation of bone turnover to inhibit resorption and maintain bone mineral density (BMD).

The properties of denosumab are summarized in Box 1. DMab was approved by the FDA for 1) postmenopausal women with osteoporosis at high risk for fracture; 2) fractures arising from metastastic bone cancer; and 3) prevention of skeletal-related events in patients with bone metastases from solid tumors 4) adults and skeletally mature adolescents with giant cell tumor of bone that is unresectable or where surgical resection is likely to result in severe morbidity (13-18). Elevated OC activity coupled with increased OC in bone are shared features of disorders that respond to DMab. Effective control of cancer metastasis by DMab is related to its regulation of immune cell profiles and inflammatory cytokines through the regulation of RANKL concentration (19, 20). Based on its ability to enhance bone quality and limit the progression of inflammatory bone diseases and cancer metastasis, the efficacy and safety of DMab has been examined in ongoing and completed trials. Thus, despite divergent molecular mechanisms, DMab exerts beneficial effects in cancer, inflammatory arthritis and osteoporosis that far outweigh the rare and often minor side effects including hypocalcemia and local infection (18, 21, 22). Herein we provide an update on the role of DMab in RA from the molecular, cellular and clinical perspective.

2. Denosumab - Current Indications

2.1 Profile

Denosumab (DMab) is an FDA-approved humanized monoclonal antibody to treat patients with osteoporosis (23-25) and cancer patients with bone metastasis (16, 23, 26, 27). DMab was commercialized by Amgen under two brand names, XGEVA and Prolia (Box 1). Completed and ongoing clinical trials of DMab suggest the clinical benefits of DMab outweigh its side effects (Table 1 and website: clinicaltrials.gov) (28-33). Phase III clinical trials demonstrated that DMab can (i) decrease bone turnover; (ii) reduce the risk of bone fracture in patients with osteoporosis; and (iii) increase bone mineral density with minimal side effects (23).

2.2 Side effects

Low Ca^{2+} and phosphate in the blood, muscle cramps, cellulitis and numbness are known DMab-associated adverse effects (21-24). Hypocalcemia is the most common side effect in DMab-treated patients (34-37). Ca^{2+} levels should be evaluated and low Ca^{2+} and Vitamin D concentration should be corrected before the initiation of DMab treatment (38, 39). It is also known that patients treated with zoledronic acid prior to DMab have a higher risk of DMab-induced hypocalcemia ($p<0.05$)(36). In addition to hypocalcemia, osteonecrosis of the jaw (ONJ) is a rare complication in DMab-treated RA patients (40-44). Histopathologic Analysis

of bone tissue from patients who developed ONJ on DMab for bone metastases revealed a decrease number of osteoclasts with few nuclei. This morpohology is strikingly different from ONJ linked to bisphosphonates where osteoclast numbers are increased with giant, hypernucleated and detached and undergoing apoptosis (16, 45-47). Thus, a careful screening of RA patients for hypocalcemia and ONJ is recommended before DMab administration. Atypical femur Fracture is another uncommon side effect reported in RA patients on DMab. (48-50). The fractures share radiologic features with stress fractures and patients can show periosteal reactions from presumed microtrauma weeks prior to the femur fracture(s) (51). It is recommended that patients be informed about the risk of atypical femur fractures and to report new onset thigh, hip or groin pain. Rebound fractures, usually associated with rebound osteoclast activity in the absence of anti-resorptive reagents (52), have been reported in patients on DMab following withdrawal of the agent (53).

2.3 Denosumab and Bisphosphonate: Contrasting disease mechanisms

2.3.1 Molecular Mechanism—The divergent molecular mechanisms underlying the protective effect of DMab and bisphosphonates on bone have been reviewed by Baron et al., (54). DMab prevents RANKL from binding to RANK receptor, thereby inhibiting osteoclast differentiation from osteoclast precursors. In contrast, bisphosphonates bind to calcium in bone and inhibit mature osteoclast function through induction of apoptotic pathways or blocking cytoskeletal assembly by inhibition of lipid modification of Ras, Rho, and Rac proteins.

2.3.2 Cellular distribution and action—DMab is a circulating antibody that can reach inflammatory sites. Bone penetration of DMab was demonstrated by its presence in blood vessels and the tibial cortex s (54). In contrast, bisphosphonates bind to mineral surfaces throughout the bone and agents with the strongest avidity may not reach the deeper trabecular surfaces (55). Osteoporosis clinical studies were performed to examine whether accumulation of bisphosphonates leads to a continuous decline in bone remodeling (54, 56, 57). Findings from these studies suggest that bisphosphonates accumulate in bone but the effect on resorption is not well understood (54). Distinct cellular distribution and action could explain the difference in the degree, speed and action of anti-resorptive effects between DMab and bisphosphonate (18, 31, 58). In contrast to bisphosphonate, DMab significantly reduced bone-specific alkaline phosphatase at 6 and 12 months compared with pretreatment, but had no effect on tartrate-resistant acid phosphatase 5b levels, emphasizing the effect of DMab more on bone formation than resorption rate(58). In addition, effects of DMab on the reduction of bone resorption are more reversible and profound than bisphosphonates (54). One recent study showed that neither DMab nor bisphosphonates suppress rheumatoid inflammation (58). However, DMab, but not bisphosphonates, significantly suppressed bone metabolism in a cohort of Japanese RA patients not previously treated for osteoporosis. These findings suggest distinct cellular mechanisms underlying DMab- and bisphosphonate-based RA therapy (4, 58). DMab exerts its protective effects likely through the WNT/β-catenin signaling pathway via regulating DKK-1 (25, 59). A decreased expression of DKK-1 was detected only with DMab and not bisphosphonates therapy (60). This observation may account for the distinct densitometric therapeutic duration window (larger without apparent plateau) observed with DMab therapy.

2.4 Market Potential

The sale of Xgeva and Prolia continue to show upward trends according to the market report on July 29, 2016. During the year 2015-16, Xgeva sales rose 11% to $378 million and Prolia sales increased 29% to $352 million (61). Collectively, Prolia and Xgeva earn a combined $730 million in revenue annually, which accounts for more than 16% of the market share for osteoporosis and cancer- associated bone metastasis in the United States.

Despite the fact that DMab is approved for treatment of osteoporosis and cancer patients with bone metastasis, it has not yet approved for treatment of inflammatory bone conditions such as RA and psoriatic arthritis (PsA). To reduce the medical cost of RA treatment with biologic DMARDs, several completed studies (28, 32, 33, 62, 63) evaluated the effects of DMab in combination with methotrexate in RA (discussed below). Although the molecular mechanism underlying DMab are not fully understood, DMab is a cost-effective alternative to current RA therapies with the potential to limit systemic and articular bone loss.

3. RANKL in RA Pathogenesis

3.1 Mechanism

RA is a chronic systemic inflammatory autoimmune disease characterized by bone loss that predates bone erosion on radiographs. RA has a range of extra-articular manifestations that include rheumatoid nodules/vasculitis, granulomatous skin disorders, and neutrophilic dermatoses (64). RA patients typically experience progressive joint damage associated with physical pain and functional impairment. It is now widely accepted that inflammatory bone diseases are initiated by dysregulated bone remodeling due to imbalance between bone resorption and formation. In RA, pathologic bone loss is not compensated by osteoblast-mediated repair because these pathways are inhibited as a result of synovial inflammation (65). Intriguingly, impaired osteoblast-mediated bone repair in RA is likely also inhibited by elevated DKK-1 expression, which correlates with bone erosions and systemic bone loss (66). Thus, controlling bone-resorption by OC via regulating RANKL and DKK-1 is of central importance for developing effective RA therapies.

The complex pathobiology of RA, is characterized by infiltration of the synovial membrane with cells of the innate and acquired immune system, hyperplasia of the joint lining and a progressive localized destruction of bone and cartilage mediated by fibroblastoid cells and OCs (65, 67). Given that elevated serum and tissue RANKL concentration were detected in RA patients (19, 68-71), rheumatoid bone and joint destruction in RA are likely mediated by RANKL-induced OC differentiation (Figure 1). Many cell types, including T lymphocytes, B cells and osteoblasts (OB), release RANKL during the course of RA (72, 73). Several cellular factors including PTH (74) and CXCL16 are involved in the regulation of RANKL (20). Intriguingly, the level of RANKL in RA sera is not only positively correlated with other RA-specific disease biomarkers including anti-citrullinated protein antibodies (ACPA), but also with the degree of bone erosion (19). Considering the fact that bone repair is rarely detected in RA once bone erosion is established (75), preventing initial erosion by targeting RANKL remains an effective option for therapeutic intervention.

3.2 Denosumab on osteoclast differentiation

Many proinflammatory cytokines (TNF, IL-6, M-CSF, RANKL, IL-1, IL-17, IL-15, IL-33 and DKK-1) contribute to joint inflammation and structural damage in RA, however, not all proinflammatory cytokines trigger bone loss (25, 67). Each cytokine exerts direct or indirect actions which affect immune regulation and/or osteoclastogenesis, in RA (67). Among these regulators, RANKL and M-CSF are two mediators essential for osteoclastogenesis, a process by which naïve osteoclast precursors (OCPs) differentiate into mature multinucleated osteoclasts with bone resorption activity. Dual signals, one from RANKL and a second from Immunoreceptor Tyrosine Activation Motif (ITAM)-bearing molecules including Triggering Receptor Expressed on Myeloid cells-2 (TREM)-2, and/or Osteoclast Associated Receptor (OSCAR), are required to trigger the initiation of osteoclastogenesis (76-78). As shown in Figure 2, binding of RANKL to the RANK receptor on the cell surface of osteoclasts initiates a complex signaling pathway which involves TRAF6, TRAF3 and c-Fos proteins. Binding of RANKL, together with activation signals from OSCAR and TREM-2, modulate intracellular calcium oscillation which in turn induces NFATc1 nuclear translocation and turns on a cassette of genes related to osteoclastogenesis. RANKL-dependent osteoclastogenesis is a complex pathway which involves many intermediate signaling regulators and mediators, which are not depicted in Figure 1. Without RANKL, osteoclast precursors fail to initiate the NFATc1-Ca^{2+} signaling cascade and differentiate into mature osteoclasts. The cellular mechanisms underlying the protective effects of DMab in RA via RANKL-mediated pathway are illustrated in Figure 2. Increased RANKL concentration promotes osteoclast formation and subsequently enhances bone-resorbing activities (Figure 2). Binding of DMab to RANKL effectively blocks RANK::RANKL association, and results in an impaired osteoclast differentiation, decreased bone erosion and joint damage (Figure 2).

Recently, a leucine-rich repeat-containing G-protein-coupled receptor 4 (LGR4) was identified as a second receptor for RANKL. Binding of RANKL to LGR4 induces apoptosis in mature OC providing a negative feedback loop to regulate OC survival. Studies in murine models of osteoporosis show efficacy with a fusion protein that contains the LGR4 extracellular domain (79). LGR4 competes with RANK to bind RANKL and suppresses canonical RANK signaling during OC differentiation. Injection of LGR4-extracellular domain inhibited in vitro OC differentiation *in vitro* and osteroporosis in 3 murine models. The finding that LGR4 expression is induced by RANKL-NFATC1 signaling may explain why mature OC undergo apoptosis in the presence of RANKL-LGR4 and provide a negative feedback signal that limits survival of mature OC. In an accompanying editorial, Zaidi and Lqbal acknowledge the therapeutic potential of LGR4 but also point out that LGR family members bind to R-spondins which regulate cell proliferation, differentiation and cell fate as well as tumor suppressors in the intestine (80). Additional studies will help determine the therapeutic utility and safety of strategies that target LGR4.

3.3 DMab and immune regulation

The role of RANKL-RANK-OPG on immune regulation was reviewed by Walsh and Choi and several others (81-85). Briefly, RANKL is an essential survival factor of dendritic cells (DC) (86). RANKL secreted by T cells significantly enhanced immunity by promoting the

survival and function of DCs. RANKL-expressing Th17 cells stimulate mature but non-resorptive osteoclasts to resorb bone (87, 88). In addition, RANKL secreted by memory B cells promotes bone erosion in RA (73) and OC formation in an ovariectomy model of osteoporosis (89). Lastly, RANKL was known to induce immune tolerance by promoting the differentiation of Treg cells (90-92). RANKL knock out (KO) mice are osteopetrotic, lack lymph nodes and show alterations in B and T cell maturation (93). Interestingly, in the serum transfer model, however, inflammation was equivalent in the wild type and KO mice (94).

Considering the essential role of RANKL/OPG (95-97) in the development of the immune system and the expression of these molecules by immune cells that release co-stimulatory factors for the activation of T and dendritic cells (98), it is conceivable that RANKL antagonists may influence immune regulation. This notion is further supported by data which demonstrated that DMab exhibits effects on non-skeletal systems including immune and vascular cells (54). Blockade of RANKL signaling by RANK-Fc or OPG-Fc inhibits dendritic cell-dependent T cell activation in IL-2 knockout or CD40 knockout mice, two autoimmune disease models (99, 100). The evidence outlined above support involvement of RANKL in immune regulation but there is no evidence that it significantly alters immune function at the approved doses for osteoporosis. Whether DMab directly interferes immune responses in RA remains controversial (101).

3.3.1 DMab changes the profile of cell subsets in immune and vascular systems—RANKL, RANK, osteoprotegerin are key mediators of osteoimmunology and vascular diseases (102, 103). RANKL inhibition by DMab changes immune cell profiles (104) and cell populations that are involved in bone remodeling including osteoblasts and osteocytes (54). In addition, RANKL has been known to increase vascular smooth muscle cell calcification through a RANK-BMP4-dependent pathway (105). Blocking of RANKL by DMab may affect the vascular smooth muscles and reduce calcium deposition as shown in the huRANKL mice (106). Additional studies are necessary to elucidate a possible involvement of DMab, either directly or indirectly, in immune regulation and vascular diseases.

4. Effect of DMab on bone quality

Reduced cortical bone porosity and enhanced bone mineral density (BMD) are two major effects observed following DMab treatment (11, 107, 108). Bone strength is mainly determined by the cortical components. Thus, loss of cortical bone contributes to a higher frequency of bone fractures in the elderly. DMab affects not only the thickness of the cortex but also bone strength, porosity and bone mineral density. Cortical thickness at the distal radius increased 3% following DMab treatment, compared to no significant change in the placebo group (54). Of note, cortical thinning is typically observed before the clinical onset of RA, and correlates with the risk of bone erosion (8, 109). These observations are consistent with the concept that excessive osteoclast resorption precedes radiographic evidence of bone loss and thus blocking osteoclast differentiation in early RA may have a greater impact on inhibition of structural damage. An intriguing mechanism to explain the protective effects of DMab in limiting bone loss in RA may be its action on DKK1. DKK1

levels in RA patients are higher than in healthy controls and correlate with erosion and bone loss (66). Treatment with DMab is associated with a decrease in DKK1 levels (8).

5. DMab in RA

The interplay of activated immune cells, synovial cell hyperplasia and cytokine release characteristic of RA fosters an osteoclastogenic environment fueled by TNF-α and RANKL. Indeed, the presence of local and systemic bone loss in RA patients raised the possibility that inhibition of RANKL may be an effective strategy to limit pathologic bone resorption. Several studies documented a potent effect of DMab on bone erosion and bone mineral density (BMD) in RA (Table 1). In addition, high-resolution quantitative computed tomography studies performed on RA patients revealed partial bone repair (decrease depth, width and volume of erosions) was noted after 6 months of treatment with DMab but not alendronate (110).

In the original phase II double-blind, placebo-controlled trial, 227 patients on baseline methotrexate, were randomized to receive DMab 60 mg or 180 mg every 6 months or placebo for 12 months (28). In patients on 180 mg but not 60 mg, the change in the MRI erosion score at 6 months was significantly less than placebo (mean change for 60 mg 0.13, p=0.118 and 180 mg 0.06, p=0.007). DMab treatment suppressed markers of bone turnover but no decrease in joint space narrowing was detected. Several retrospective analyses were performed on subjects in this trial. Significantly fewer patients demonstrated metacarpal bone loss in both treatment groups compared to placebo at 6 months based on assessment with digital x-ray (29). In a sub-study, bone densitometry (DEXA) of the hands in 56 patients revealed that DMab decreased erosion progression and was associated with higher BMD which declined in the placebo group (30). Lastly, significant increases in bone density were observed in the lumbar spine and hip in the DMab-treated patients compared to placebo at 6 and 12 months, despite the use of glucocorticoids or bisphosphonates (31, 111). In addition, the serum levels of type I C-telopeptide (sCTx-I) and procollagen 1N-terminal peptide (P1NP) declined significantly, regardless of baseline BMD, marker levels or concomitant bisphosphonate or glucocorticoid use in the treatment groups.

In a recent Japanese trial, DMab in patients with Rheumatoid arthritis patients on methotrexate to Validate the Inhibitory effect on bone Erosion (DRIVE), 350 RA patients on baseline methotrexate were randomized to receive DMab 60 mg every 6 months(M), every 3 months or every 2 months or placebo in a 1:1:1:1 ratio for 12 months (112). At 12 months, all doses of DMab were associated with a significant inhibition of radiographic progression assessed by the modified Sharp erosion score. Changes in this score at 12 months from baseline were 0.99 for placebo, 0.27 ($p<.0.0001$ compared to placebo) for Q6M, 0.14 ($p=0.0036$ compared to placebo) for Q3M and 0.09 ($p<0.0001$ compared to placebo) for Q2M. Bone mineral density was maintained in the treatment groups compared to placebo and no effect on joint space narrowing was observed. In all the studies detailed above, joint space narrowing did not decline significantly on DMab, adverse events were not increased compared to placebo and no anti-inflammatory effects were noted in the treatment groups.

Another potential niche for DMab is the treatment of osteoporosis in RA patients. For rheumatologists, this approach is of limited value since many RA patients are taking biologic DMARDs (bDMARDs) which are quite effective at limiting bone loss. Moreover, concerns regarding an increased risk of infections when these agents are taken in combination have greatly limited their use in this population. To address the efficacy and safety of DMab in combination with biologic agents in RA, a retrospective Japanese cohort trial enrolled 80 RA patients on one of the following baseline biologic bDMARDs: infliximab, adalimumab, infliximab, etanercept, abatacept or tocilizumab and randomized them in a 1:1 manner to DMab 60 mg or placebo every 6 months (33). The modified TSS erosion score was significantly less in the group that received DMab compared to placebo at 12 months without a significant increase in adverse events. This study demonstrated that DMab effectively decreased bone erosion even in patients on bDMARDs, corticosteroids or bisphosphonates without major safety signals. Radiographic analyses confirmed an improved efficacy of DMab-bDMARDs combined therapy for RA treatment (33).

The safety of combining DMAb with a biologic agent in RA patients for treatment of osteoporosis was further examined in a Medicare administrative database that included 5814 patients, 1354 exposed to DMab and 4460 to zolendronic acid (113). A subgroup analysis was also performed on 463 patients in each group matched on infection risk score and several demographic variables. The crude rate of hospitalized infection in the two RA cohorts was not significantly different in patients on DMab compared to zolendronic acid in both the main and subgroup analyses. These results must be interpreted with the understanding that the time on DMab was variable, the infections were limited to hospitalized patients and the hospitalizations were not confirmed. Nevertheless, these data provide the first confirmation that combination of DMab with bDMARDs to treat concomitant osteoporosis does not increase infection risk leading to hospitalization.

6. Expert Opinion

Evidence from 2 phase II trials and one randomized observational trial indicate that DMab inhibits focal and systemic bone loss in RA. These findings are particularly relevant in RA because many of the risk factors associated with systemic bone loss are present including a high prevalence of post-menopausal females, concomitant glucocorticoid use, systemic osteoclast activation and limited functional mobility. For those patients who do require additional treatment for osteoporosis on bDMARDs, DMab may be a good option based on administrative data showing no increase in adverse events in patients on a combination of a bDMARD and DMab. It is important to point out, however, that while DMab does inhibit bone erosion, it does not appear to have a major impact on pathologic cartilage resorption and it does not demonstrate anti-inflammatory properties so its actions are limited and require co-treatment.

Given these findings, is there a role for DMab in RA? This is not a trivial question particularly given that the first pivotal trial was published 8 years ago and this approach has not been formally examined in a phase III trial. The landscape of RA therapy is changing rapidly, however, with the entry of biosimilars, novel biologic agents and the JAK inhibitors. The rapid uptake and high penetration of bDMARDs, particularly in the U.S. market, has

been accompanied by unprecedented price inflation that will be only partially offset by biosimilars due to high development costs. These inflated drug costs are limiting the use of bDMARDs and this problem is expected to increase as we move towards a system focused on population health and cost savings. These trends, coupled with studies that indicate conventional synthetic (cs) DMARDs are appropriate and effective therapy, either alone or in combination, provide new opportunities for regimens that include DMab as a cost savings alternative.

Combination regimens of csDMARDs or bDMARDs with DMab may be appropriate in several different settings. First, methotrexate monotherapy is effective in about 30 percent of patients, some of whom will develop erosive changes on this treatment which could be ameliorated with DMab (114). Second, some patients with early RA present with baseline erosions and the traditional approach has been to initiate a bDMARD in combination with methotrexate (115). Addition of DMab in place of a biologic is significantly less expensive and may provide a similar outcome. Third, many patients cannot afford biologics and a significant percentage may be intolerant, have comorbid diseases that prohibit the use of a bDMARD (multiple sclerosis, congestive heart failure) or fear self-injection. Fourth, several studies have documented subclinical synovitis on imaging studies and histopathology in patients in remission and progressive joint damage has been reported (116, 117). These patients would benefit from an additional agent that targets erosions, particularly given that strategies are under study to taper bDMARDs in patients with remission (118). The addition of DMab will limit structural damage that may arise when the bDMARD is withdrawn. It is anticipated that development of surrogate markers of radiographic damage and treatment stratification biomarkers which can classify responders from non-responders prior to treatment may also allow for improved treatment assignment in relation to csDMARDs and bDMARDs which may facilitate early regimens that include DMab.

Phase III trials are required to discern the magnitude of the inhibitory effect on bone erosions and help to establish an optimal dose. In the initial study by Cohen et al. (28), bone erosions on MRI were not significantly inhibited by 60 mg but 180 mg was effective and in the Takeuchi study, shorter dosing intervals demonstrated greater inhibition of structural damage. The role of DMab in RA patients with osteoporosis is also not well understood. The ability of DMab to reduce cortical porosity may be distinctly advantageous in a disease characterized by focused resorption of cortical bone and this may explain why it was effective even in patients taking bisphosphonates. The ability to prevent fractures in RA patients with multiple risk factors, particularly glucocorticoids also deserves further study.

DMab, an antibody to RANKL, limits osteoclast proliferation, activation and survival. It is approved in the US for the treatment of postmenopausal osteoporosis and bone metastases associated with solid tumors. Despite demonstrated efficacy for limitation of focal and systemic bone loss in RA, this agent has not been widely adopted for treatment of rheumatoid joint disease or concomitant osteoporosis. Changing market dynamics coupled with that appreciation csDMARDs are effective for some patients, either alone or in combination with other csDMARDs along with the impetus to taper bDMARDs in patients who reach sustained remission, hold promise that DMab may emerge as an important therapy to limit local erosions and maintain BMD. Additional studies will be required to

establish optimal treatment regimens and confirm long-term safety for combination bDMARD and DMab regimens. Future DMab-based RA therapy may not only targeting inflammation but also improve cortical bone porosity and BMD at early stages of RA and lead to better treatment outcomes.

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Drug Summary Box

Drug name	Denosumab
Phase	Pre-Registration
Indication	Rheumatoid Arthritis
Pharmacology description	RANKL antagonist
Route of administration	Subcutaneous
Pivotal trial(s)	[23-28]

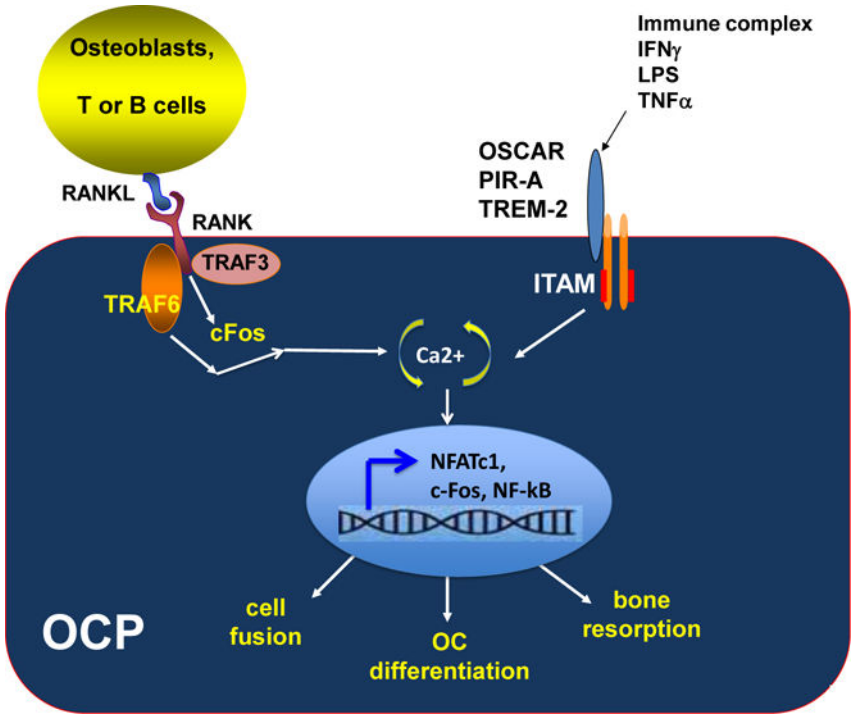

Figure 1. Dual signals, one from RANKL and one from ITAM-bearing receptor, are required for the activation and maturation of osteoclast precursors

Binding of RANKL to the RANK receptor on the cell surface of osteoclasts initiates a complex signaling pathway which involves TRAF6, TRAF3 and c-Fos proteins. This activation signal, together with activation signals from ITAM-bearing receptors such as OSCAR, PIR-A and TREM-2, will modulate intracellular calcium oscillation which in turn induces NFATc1 nuclear translocation and turns on a cassette of genes related to osteoclastogenesis. It is well established that RANKL regulates osteoclastogenesis via the NFATc1/Ca2+ axis (119-122). Of note, many intermediate regulators in the RANKL-mediated osteoclastogenesis pathway are not depicted in this model for simplification purpose. Adapted from [123] with permission of John Wiley and Sons

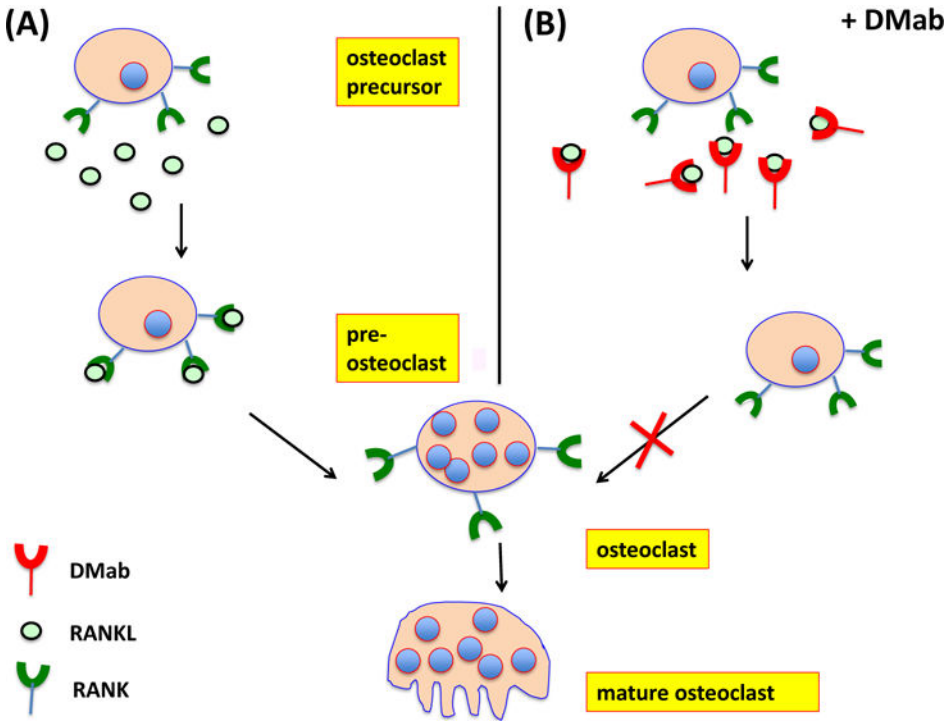

Figure 2. Mechanisms of DMab action in inhibiting osteoclast differentiation

(A) Osteoclast precursors (OCPs) are present in bone marrow, circulation and inflamed joints. OCPs express RANK and c-Fms receptors on their cell surfaces. Binding of RANK to RANK ligand (RANKL) and c-FMS to M-CSF, respectively, are essential for the initiation of RANKL-dependent OC differentiation. **(B)** Through binding to RANKL, DMab blocks engagement with its receptor, RANK on OCPs and inhibits the subsequent activation and maturation of OCPs. Consequently, DMab decreases bone resorption and fosters an overall increase in bone mineral density (BMD).

Table 1

Denosumab clinical trials in RA.

Trials	Number of subjects	Type	Concomitant Medications	Key findings in Denosumab vs. PBO	Reference
Amgen	218	Phase II RDBPCT	MTX	Significant decrease in erosions in hands, wrists (MRI, plain x-ray)	Cohen et al. 2008 (23)
Amgen	218	Post-hoc analysis	MTX	Significantly less metacarpal bone loss	Sharp et al. 2010 (24)
Amgen	56	Post-hoc analysis	MTX	Significant increase in hand BMD and decreased erosion	Deodhar et al. 2010 (25)
Amgen	218	Post-hoc analysis	MTX	Significant increase in spine and hip BMD and reduced sCTx-1 and PiNP levels	Dore et al. 2010 (26)
DRIVE	350	Phase II RPCT	MTX	Significant decrease in hand and wrist erosions	Takeuchi et al. 2016 (27)
Keio University	160	Cohort	TNFi	Significant decrease in erosion score	Hasegawa et al. 2016 (28)

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Table 2

Potential therapeutic value of Denosumab for treatment of RA

1	Early response to csDMARD*
2	Early RA with baseline erosions
3	Unable to take bDMARDs** (cost, intolerance, comorbidity)
4	In clinical remission with synovitis on imaging
5	In clinical remission and withdrawal of bDMARDs**

* csDMARD : conventional synthetic DMARD

** bDMARDs : biological DMARD