A review of the efficacy and safety of denosumab in postmenopausal women with osteoporosis

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Abstract: Denosumab, a fully human monoclonal antibody to RANK ligand [RANK-L]) was approved for the treatment of postmenopausal osteoporosis in June 2010, and is highly effective in reducing the risk of vertebral, nonvertebral, and hip fracture risk. The registration of denosumab was the culmination of the discovery and clarification of the internal bone microenvironment regulation of bone remodeling: the osteoblast-produced competitors: RANK-L and osteoprotogerin; and the osteoclast receptor: RANK. When RANK-L is upregulated in the estrogen-deficient state and exceeds the amount of osteoprotogerin, there is an increase in osteoclastogenesis and bone resorption, and this is the major mechanism for bone mass loss and osteoporotic fractures in the postmenopausal state. The subsequent development of the human monoclonal antibody to RANK-L (denosumab) was the first product developed to reduce bone resorption by inhibiting RANK-L binding to RANK. Denosumab does not accumulate in bone, and has a unique pharmacokinetics so that its biological effect at the registered dose of 60 mg by subcutaneous injection every 6 months is no longer effective, at least as measured by an increase in the bone resorption marker collagen-cross-link C-telopeptide and a decline in bone mineral density as measured by dual energy X-ray absorptiometry. This unique pharmacokinetic profile thus suggests that in order to maintain the effectiveness of denosumab, continuous administration might be necessary. Extension of the registration trial ('FREEDOM') 5-year data indicates continued safety and efficacy, and will be extended to 10 years so that even longer-term data will be forthcoming. The profound but reversible suppression of bone turnover that is seen with denosumab partly explains the continuous increase in bone mineral density seen through 8 years of the phase II clinical trials. Denosumab offers a highly effective and safe parenteral therapy for osteoporosis and is being studied long term with the extension of the FREEDOM trial, and in other osteoporotic states – in men and glucocorticoid-induced osteoporosis.

Keywords:

Introduction

A new therapeutic advance in the treatment of postmenopausal osteoporosis is denosumab. Densoumab is a fully human monoclonal antibody to an activator of osteoclastic differentiation and activity, soluble RANK ligand (RANK-L). RANK-L is receptor activator (RANK being the receptor on osteoclasts, also called nuclear factor κB). RANK-L, a glycoprotein produced in the osteoblast, is a member of the superfamily of ligands, and is also known as a tumor necrosis factor (TNF) activation-induced cytokine and osteoclast activator [Bekker *et al.* 2004; Boyle *et al.* 2003; Eghbali-Fatourechi *et al.* 2003; Suda *et al.* 1999]. Also produced in the osteoblast is the decoy receptor for RANK-L, osteoprotogerin (OPG) [Bekker *et al.* 2001]. RANK-L and OPG are molecular competitors and are either upregulated or downregulated by opposing biological control systems. For example, in the estrogen-deficient state, there is an upregulation of RANK-L (e.g. more RANK-L is produced). When RANK-L concentrations in the bone marrow microenvironment exceed the concentration of OPG, then more RANK-L can bind to its receptor on the osteoclast, RANK. When this RANK-L–RANK binding occurs, there is a marked increase in osteoclast differentiation and

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activity, and thus, increase in bone resorption. If there is a greater concentration of OPG produced by the osteoblast, then less RANK-L is assessable to the osteoclast receptor, RANK, and there is more inhibition of osteoclast differentiation and activity and less bone resorption. Hence the balance between osteoblast-produced RANK-L and OPG is the determining factor for the internal bone microenvironment regulation of bone resorption (Figure 1) [Boyle *et al.* 2003]. Denosumab (anti-RANK-L antibody) thus acts like OPG, preventing RANK-L from binding to RANK and reducing bone resorption.

In the initial basic science development of these remarkable bone biological regulatory pathways, the development company, Amgen (Thousand Oaks, California, USA) first introduced parenteral administration of OPG as a means of reducing bone resorption, by then allowing less RANK-L to become available to the osteoclast receptor, RANK. OPG was first discovered from an immune cellular system, hence the concerns regarding how manipulation of this pathway might alter immune function or pose a risk for infection. However, the development of neutralizing antibodies to OPG prevented its development as a therapeutic agent, something which has not been seen with the administration of the anti-RANK-L antibody denosumab. As a culmination of this pioneering basic science work and the solid antifracture data observed in the pivotal registration trial, denosumab received international registration for the treatment of postmenopausal osteoporosis in June 2010 at a dose of 60 mg subcutaneously every 6 months [Cummings *et al.* 2009].

Basic science and pharmacokinetics of denosumab

The discovery of the RANK-L–OPG–RANK pathways was a giant leap in the understanding of the regulation of bone remodeling within the bone microenvironment *per se* [Eghbali-Fatourechi *et al.* 2003]. Regulation of bone remodeling, especially osteoblast activity, had been known for years to be under the control of systemic factors (e.g. parathyroid hormone, 1,25 dihydroxyvitamin D, bone morphogenic proteins, etc.); and there was also knowledge that within the bone marrow microenvironment the bone cell lines, especially the osteoblast and the osteoclast, were 'coupled in a directional manner, e.g. when one cell line activity was increased, the other cell line would be increase in time as well, and vice-versa' [Atfi and Baron, 2010; Bilezikian *et al.* 2009; Eriksen, 2010; Rosen, 2008]. While some data show that within the bone microenvironment *per se* the osteoblast activity was increased by bone matrix derived insulin growth factors and their binding proteins released into the microenvironment during bone resorption, regulation of coupling from the osteoclast to the osteoblast side of the equation was ill defined until the RANK-L–OPG–RANK systems were defined [Mackie, 2003]. The clarification of the RANK-L–OPG–RANK pathway closed the loop. Now, the regulation of the entire bone remodeling cycle from within the bone *per se* had become defined. In addition, there is increasing understanding of how the dominant cell in bone, the osteocyte, is also linked to the regulation of bone turnover through the osteocyte production of fibroblast growth factor 23 (FGF 23) and sclerostin, providing the foundation of the mechanostat mechanism and bone formation [Bonewald, 2011; Juppner, 2011; Price *et al.* 2011; Wang *et al.* 2008]. While the effect on bone mineralization of FGF 23 is not yet fully characterized, sclerostin clearly can inhibit a major activator of osteoblast activity, the Wnt pathway [Atkins *et al.* 2011; Silverman, 2010]. FGF 23, a phosphaturic glycoprotein, also reduces renal production of 1,25 dihydroxyvitamin D, and reduces parathyroid hormone secretion in short-term studies [Juppner, 2011].

While the RANK-L–RANK–OPG glycoproteins have multiple nonskeletal tissue expression, there is little knowledge of the physiological or pathological role for these proteins in other tissues. The bone microenvironment is the most well understood pathway for the RANK L–RANK–OPG system.

Denosumab is produced in the Chinese hamster embryo oophocyte, and is metabolized by the reticulo-endothelial system [Wypych *et al.* 2008]. It shows a nonlinear pharmacokinetic profile. This IgG 2 monoclonal antibody has pharmacokinetic characteristics (peak, half life, serum concentrations), as shown in Figure 2 [Bekker *et al.* 2004]. Neutralizing antibodies do not develop to anti-RANK-L antibodies as they do to OPG because denosumab does not resemble OPG. In addition, denosumab does not bind to TNF-apoptosis-inducing ligand (TRAIL, a survival factor for tumor cells), and has no significant effect on lymphocyte counts overall (CD3), T cells (CD 4, CD 8, CD 54), or B cells (CD 20) [Suda *et al.* 1999].

Figure 1. The mechanism of action of anti-RANK ligand (RANK-L) antibody on bone turnover. Denosumab binds to osteoblast-produced RANK-L, thereby preventing RANK-L from binding to the osteoclast receptor, RANK. By preventing RANK-L from binding to RANK, there is less osteoclast differentiation and activity so that bone resorption decreases. Illustration courtesy of Alessandro Baliani © [2011].

RANK-L, RANK ligand; CFU-M, colony-forming unit-macrophage

The phase II denosumab dose-ranging clinical trial – the longest-term denosumab data available: 2-, 4-, 6-, and 8-year data.

The initial dose-ranging studies in postmenopausal women randomized patients to seven different doses of denosumab given at different intervals *versus* alendronate (70 mg/week) *versus* placebo [McClung *et al.* 2006]. The initial 1-year and 2 year data showed that these doses increased bone mineral density (BMD) at all skeletal sites and

Figure 2. The pharmacokinetics of denosumab. The 1.0 mg/kg dose had no measurable level by 6 months. Therefore, the 6-month dosing interval at this strength was selected for clinical trial development. Adapted with permission from [Bekker *et al.* 2004] copyright 2011, Wiley.

EC_{50}, half maximal effective concentration.

reduced biochemical markers of bone turnover more than alendronate or placebo [Lewiecki *et al.* 2007]. This initial 2-year study was then extended to 4, 6, and now 8 years [Miller *et al.* 2008, 2011; McClung *et al.* 2011]. The 4- and 8-year data show a few unique highlights: the 4-year group had a subgroup that discontinued denosumab for 2 years after the initial 2 years of treatment; and a group that, after 1 year of discontinuation, were rechallenged with denosumab. In the continuous discontinuation group the bone resorption marker (CTX) increased and even went above the pretreatment baseline by the sixth month after discontinuation, reached a peak at month 36, but returned to baseline during the fourth year without any further treatment. Likewise, the BMD responses were the mirror image of the bone turnover markers (BTMs) (Figure 3(a)) [Miller *et al.* 2008]. The reasons for the return of CTX and BMD back to baseline without any additional denosumab being provided are unknown but might be related to the bone mechanostat mechanism [Bonewald, 2011; Juppner, 2011; Price *et al.* 2011; Wang *et al.* 2008]. In addition, in the retreatment group, the response to denosumab was retained, such that upon rechallenge with denosumab, the BMD increased and the CTX decreased, with these responsive changes having the same slope as seen in the first year of treatment (Figure 3(b)) [Miller *et al.* 2011]. Thus, as expected from the initial pharmacokinetic and pharmacodynamic data, the human biological data show that the effect of denosumab is reversible yet retains responsiveness after treatment, discontinuation and retreatment.

The 6- and 8-year phase II data provide some additional interesting insights into the pharmacodynamics of denosumab [Miller *et al.* 2011; McClung *et al.* 2011]. During these periods the lumbar spine and total hip BMD continue to increase and not plateau as is seen with other antiresorptive agents. The mechanism for this progressive increase in BMD is not yet defined, but may be related to the larger degree of suppression of bone turnover than that seen with alendronate, and prolongation of the time for secondary mineralization or greater closure of the remodeling space [Miller *et al.* 2011]. In addition,

Figure 3. (a) The phase II, 4-year data showing the effect of discontinuation and retreatment of denosumab on bone turnover markers and bone mineral density. The data show that after 2 years of use, the bone turnover markers increase to baseline and above by month 6 after discontinuation but return to treatment levels when retreated with denosumab. Adapted with permission from [Miller *et al.* 2008] copyright 2011, Elsevier. (b) Likewise, after treatment then discontinuation, the bone mineral density decreases within 6 months to baseline, but then increases again when treatment is restarted. Adapted with permission from [Miller *et al.* 2011] Copyright 2011, The Endocrine Society.

LS, least squares; SE, standard error.

the serum bone resorption marker (CTX) does not continue to decline with repeated doses of denosumab, and in fact has a trend to increase over time though remain within the premenopausal normal reference range (Figure 4) [Miller *et al.* 2011]. These data are reassuring in that continuous administration of denosumab is not associated with reduction of BTMs to levels below the normal reference range. The mechanism by which CTX tends to increase over time despite continual administration of denosumab is unknown but may have more to do with the mechanostat than tachyphylaxis because the reductions in CTX 1 month after dosing were consistent at year 1 and year 5 [Miller *et al.* 2011].

The phase III clinical trials: 'DEFEND', 'DECIDE', 'STAND', and the registration trial: 'FREEDOM'

Defend

The DEFEND trial was a prevention trial. It included 332 early postmenopausal younger white

Figure 4. Six-year exposure to denosumab (DMAb), 60 mg every 6 months, does not result in reduction on bone resorption marker (serum CTX) below the premenopausal normal reference range. In fact, the serum CTX seems to drift up (but remain in the premenopausal reference range) during the fourth to sixth year of therapy. Adapted with permission from [Miller *et al.* 2011] Copyright 2011, The Endocrine Society.

IQR, interquartile range.

women with a mean age of 56 years [Bone *et al.* 2008]. These women were treatment naive, at low risk for fracture (mean lumbar spine T-score −1.6), postmenopausal and were randomized to either denosumab (60 mg subcutaneously every 6 months) or subcutaneous placebo in a 1:1 ratio. The first 2 years of the study showed that denosumab significantly increased BMD at all skeletal sites, both from baseline and compared with placebo, and significantly reduced the level of BTMs. After the first 2 years of the DEFEND trial, 252 of the initial study participants continued into an extension study for an additional 2 years [Bone *et al.* 2011]. During these 'treatment-off' 2 years, all women received placebo, so the initial placebo group had a total of 4 years of placebo, and the initial denosumab-treated group came off therapy to receive 2 years of placebo. During the extension study the BMD in the initial denosumab-treated group declined to baseline at all skeletal sites during months 24–36, but then leveled off at the lumbar spine while it continued to decline at the total hip and one-third radius. Likewise, the BTMs increased to baseline and above within 6 months of discontinuation (as was seen in the phase II trials), but then returned to baseline during the fourth year without any additional therapy (also as was seen in the phase II trials). Thus, examining the registered dose (60 mg subcutaneously every 6 months) for this entire 4-year study showed that the effects of denosumab are reversible upon discontinuation and suggest that treatment may need to be continued to maintain an effect on BMD and BTMs. What is unknown in this phase II study, like the phase II trial, is what the effect on bone strength (fractures) is upon discontinuation.

Decide

The DECIDE trial was a prospective randomized comparison of alendronate (70 mg/week) to denosumab (60 mg subcutaneously every 6 months) in 1189 early postmenopausal women with low bone mass [Brown *et al.* 2009]. At the total hip, denosumab resulted in a significantly greater increase in BMD compared with alendronate (3.5% *versus* 2.6%, $p < 0.0001$). The larger increases in BMD with denosumab administration were also seen at all skeletal sites measured. In addition, denosumab induced a significantly greater reduction in BTMs compared with alendronate. There are no prespecified fracture data in DECIDE.

Stand

The STAND study (study of transitioning from alendronate to denosumab) was an international, multicenter, randomized, double-dummy, parallel-group phase III trial [Kendler *et al.* 2010]. A total of 504 postmenopausal women (average age 67 years) previously taking alendronate (median 36 months, range 6–192 months) were randomized to receive either brand name alendronate (Fosamax) (70 mg/week) or placebo injections; or denosumab (60 mg subcutaneously every 6 months) or placebo alendronate for 12 months. A repeated-measures model was used as the primary analysis method for the percentage change in BMD at months 6 and 12. The model included treatment, time of BMD assessment, treatment by time of BMD assessment interaction, baseline BMD value, time on prior alendronate therapy stratum, dual-emission X-ray absorptiometry instrument type, and baseline BMD value and instrument type interaction. The primary hypothesis was that denosumab was not inferior to alendronate with respect to the mean percentage change in total hip BMD at month 12. BMD at the total hip increased by 1.90% [95% confidence interval (CI) 1.61–2.18%] at month 12 in women transitioned to denosumab compared with a 1.05% (95% CI 0.76–1.34%) increase from baseline in women continuing on alendronate therapy (Figure 2) [Bekker *et al.* 2004]. The difference between treatment groups was 0.85% (95% CI 0.44–1.25%) greater with denosumab; the lower limit of the confidence interval excluded the prespecified noninferiority margin (0.35%), thus showing the noninferiority of denosumab compared with alendronate. Superiority testing demonstrated the BMD increase with denosumab at the total hip was statistically superior to the change with alendronate ($p < 0.0001$).

At the lumbar spine, denosumab increased BMD by 3.03% (95% CI 2.63–3.44%) at month 12, and alendronate increased BMD by 1.85% (95% CI 1.44–2.26%), for a difference of 1.18% (95% CI 0.63–1.73%), with the lower limit of the confidence interval excluding the noninferiority margin of 0.22. These data demonstrate the noninferiority of denosumab *versus* alendronate at this skeletal site. The increase in BMD with denosumab *versus* alendronate was statistically significant ($p < 0.0001$). Significantly greater increases in BMD with denosumab compared with alendronate were also observed at month 12 at the femoral neck and one-third radius ($p < 0.0121$). Furthermore, significant BMD increases for denosumab compared with alendronate were observed as early as month 6 at the lumbar spine and all measured femoral sites ($p < 0.05$). Thus, the data show that in patients previously on alendronate, changing to denosumab is accompanied by at least stabilization if not further increases in BMD.

Freedom

The large phase III registration trial randomized 7808 postmenopausal women (mean age 72.3 years, average lumbar spine T-score –2.8, WHO defined osteoporosis), 23% of whom had a prevalent vertebral fracture [Cummings *et al.* 2009]. One-half received denosumab (60 mg subcutaneously every 6 months) and the other half placebo (subcutaneously). Over 3 years, denosumab reduced the incidence of morphometric vertebral fractures, the primary endpoint (Figure 5) [relative risk reduction (RRR) 68%, $p < 0.0001$ *versus* placebo], nonvertebral fractures (RRR 20%, $p < 0.01$ *versus* placebo), and hip fractures (RRR 40%, $p < 0.04$) [Cummings *et al.* 2009].

The FREEDOM trial is designed to extend to 10 years. The initial 5-year data of the extension have recently been presented [Papapoulos *et al.* 2011]. The design of the extension trial is as follows: after the first 3 years, 2343 patients from the initial 3902 patients who were randomized to the treatment arm of denosumab continued with treatment (60 mg subcutaneously every 6 months). From the initial randomized placebo group of 3906 patients, 2207 switched over to treatment with denosumab (60 mg subcutaneously every 6 months). In patients who received 5 years of denosumab treatment, the fracture rates continued to remain reduced from the end of the registration trial, and the BMD continued to increase at both the spine and hip. In the patients who initially received placebo and when on to receive denosumab, the BMD increased and the fracture rates declined, similar to the initial results after 2 years of the FREEDOM trial.

Safety data on denosumab

In the collective data from the phase II and III clinical trials, denosumab has been shown to be safe. The clinical concern was the potential risk for infections because of the ubiquitous presence of RANK-L throughout many tissues. In the pivotal registration trial, the only serious adverse event (AE) that was significantly greater than placebo was skin infections (termed either 'cellulitis' or

Figure 5. The results of the pivotal registration fracture trial with denosumab *versus* placebo ('FREEDOM'). Over 3 years denosumab (60 mg as a subcutaneous injection every 6 months) significantly reduced the incidence of vertebral, nonvertebral and hip fractures compared with placebo [Cummings *et al.* 2009].

*Composite measurement excluding pathological fractures and those associated with severe trauma, fractures of vertebrae, skull, face mandible, metacarpals, fingers and toes.

ARR, absolute risk reduction; RRR, relative risk reduction.

'erysipelas') [Cummings *et al.* 2009]. In the AE reports there was a small and nonsignificant difference between the denosumab and placebo groups for skin infections (54 *versus* 53 cases), while serious AEs did reach statistical significance (0.3% *versus* <0.1%, $p < 0.002$) [Cummings *et al.* 2009]. These areas of inflammation could occur at any time after the injection and at a site distant from the injection, however all responded to conservative management. There were no unusual opportunistic infections reported. The US Food and Drug Administration (FDA) label also includes 'adverse reactions': events that occurred more frequently in those that received the investigational product compared with placebo. These events were included on the FDA label even if they were not statistically significantly different from one another. The aim of this was to provide caution for a new drug of its class being exposed to a large population so that physicians could report any trend in experiences in the real world. In this regard, the only adverse event that was significantly greater in the treated *versus* the placebo group was: 'Epidermal and dermal adverse events such as dermatitis, eczema, and rashes occurred at a significantly higher rate in the treated *versus* placebo groups (10.8% *versus* 8.2%, $P < 0.0001$)' [Cummings *et al.* 2009]. No studies have examined the potential for infections in patients who are immunosuppressed or on other biological drugs. However, denosumab has recently received registration to reduce the risk of fractures in patients with metastatic carcinoma of the bone, and in oncology trials, there was no increased risk of infection seen in this group of patients who have more compromised immune systems and receive a much higher dose of denosumab for this indication (120 mg/month) [Stopeck *et al.* 2010]. In addition, the extension data from FREEDOM show that the group who received 5 years of continuous treatment with denosumab had a cellulitis rate that did not increase and was comparable to the rate reported for the placebo group in the first 3 years (< 0.1% *versus* <0.1%) (unpublished data from Amgen). Vigilance is a key word when this very effective human monoclonal antibody is used in large numbers of patients so that any trends in adverse events can be reported to the FDA.

The FDA label for the postmenopausal osteoporosis indication does not have any lower cut-off for renal function. This is because denosumab is not cleared by the kidney but by the reticuloendothelial system, and it may not have any adverse renal effects as may be seen, though rarely, with intravenous bisphosphonates [Miller, 2009]. In a

Figure 6. The *post hoc* analysis from the FREEDOM trial showing that denosumab (60 mg as a subcutaneous injection every 6 months) was effective in reducing incident vertebral fractures and was safe over 3 years in patients with stage 1–4 chronic kidney disease (estimated glomerular filtration rate > 90–15 ml/min) [Jamal *et al.* 2011].

N = number of randomized patients, *N*1= number of randomized patients with an elevation during the time period of interest. There were no patients with a creatinine clearance less than 15 ml/min. $*p < 0.05$.

post hoc analysis of FREEDOM in which the registration population had estimated glomerular filtration rates (eGFRs) divided into quartiles (> 90 to 15–29 ml/min), denosumab showed evidence of reduction in incident vertebral fractures across these quartiles without any adverse renal effects (e.g. change in eGFR over 3 years) (Figure 6) [Jamal *et al.* 2011]. There are no data on changes in BMD or fractures in patients with a GFR less than 15 ml/min. In these patients the diagnosis of osteoporosis becomes far more difficult to establish. There is also concern that in patients with preexisting adynamic renal bone disease, reducing bone turnover further may be associated with an increase in cardiovascular calcification [Hruska *et al.* 2009]. This concern is based on the knowledge that absorbed calcium and/or phosphorus cannot be adequately eliminated by renal clearance, and if bone turnover is low, the capacity of bone to take up these ions is restricted, leaving vascular tissue exposed to calcium and/or phosphorus leading to the risk of vascular calcification. One study has examined the effect of denosumab on vascular calcification in the FREEDOM trial and found that across the eGFR quartiles, there was no greater increase in vascular calcification with denosumab *versus* placebo, at least when assessed by lateral lumbar X-ray assessment of aortic calcification [Egbuna OI *et al.* 2010].

The FDA label includes a caution about the possibility of hypocalcemia after denosumab administration. While all antiresorptive agents may induce a small and transient hypocalcemic effect after administration, clinically significant hypocalcemia (associated with tetany or paresthesias) is not observed in patients with adequate calcium and vitamin D intake, and with intact parathyroid hormone, transient hypocalcemia is normalized. There was no difference in reported hypocalcemia in the FREEDOM trial between the treated and the placebo groups either in the registration (first 3 years) or the extension trial. It is important in patient management to ensure that an adequate amount of calcium and vitamin D are provided.

The effect of denosumab on bone turnover is pronounced. While BTMs do not remain suppressed below the normal premenopausal reference range in long-term denosumab trials and rise rapidly after discontinuation of denosumab, the quantitative bone histomorphometry data show absent tetracycline labels, an indicator of new bone mineralization, in a small number of patients in whom bone biopsies were performed. Absent single tetracycline labels were not seen in any of the 62 placebo biopsies while 19 of 33 (36%) of the biopsies in patients on denosumab had no single labels [Reid *et al.* 2010]. The clinical significance of these findings is unknown. It is reassuring that in the patients who had biopsies the serum CTX overlapped and there was no difference between the biopsies that had no labels *versus* the biopsies that had single or double labels. The tetracycline labels (and therefore mineralization) would be expected to return after denosumab discontinuation since the BTMs would increase. However, this has yet to be studied. In the DECIDE study, bone biopsies were done in patients treated with denosumab and then taken off treatment and were shown to have normal labels. However, it is unknown if any of these patients had absent labels to start with. The maintenance of the low fracture incidence in the FREEDOM extension trial is reassuring because it shows that 5-year use is not associated with any rise in fracture events.

From a safety viewpoint, there have been no cases of osteonecrosis of the jaw (ONJ) or atypical subtrochanteric femur fractures reported in the osteoporosis clinical trials. There have been two adjudicated cases of ONJ in the fourth and fifth year of FREEDOM extension trial. There has also been a low but similar incidence of ONJ in the oncology trials using monthly denosumab *versus* monthly intravenous zoledronic acid (1.3% *versus* 1.8% over 3 years) [Stopeck *et al.* 2010]. Postmarketing data will reveal in time if these rare events occur in the osteoporosis population and with the lower doses of denosumab used in this population.

Finally, a few practical considerations for long-term use of denosumab – questions that, to date, cannot be answered from existing data. One is whether denosumab needs to be continued indefinitely. Since BTMs increase and BMD decreases after discontinuation, it is theoretically possible that in order to maintain antifracture efficacy, continuous administration must be provided. It is also possible that after long-term use (e.g. more than 5 years) denosumab could be discontinued yet bone strength not altered despite a rise in BTMs and a drop in BMD since bone quality (microarchitecture, cortical porosity) might not change. These are vital questions that can only be answered from long-term data . For example, in FREEDOM, approximately 929 patients treated during the first 3 years did not continue into the extension study. It remains to be reported what happened to these patients who are essentially on a denosumab 'drug holiday'. Observational trials of the effects of denosumab in the 'real world' might also provide helpful information, and have been initiated. Since the pharmacokinetics and pharmacodynamics of denosumab differ markedly from bisphosphonates, in that there is neither long-term bone retention nor 'recycling' of denosumab like there is with bisphosphonates, a so-called 'drug holiday' from denosumab might have very different implications [Baron *et al.* 2011]. While there is no 'standard of care' for any of these implementations of continuation *versus* discontinuation with any approved osteoporosis pharmacological agent, prevailing opinion would suggest considering a 'drug holiday' from bisphosphonates after 5 years of use in lower-risk but not in higher-risk patients (patients with preexisting fractures or T-scores at the hip of −2.5 or lower) [Black *et al.* 2006; Schwartz *et al.* 2010]. Hence, both the long-term exposure data and the offset data will be important for us to gain knowledge in the management of patients with the chronic disease of osteoporosis. Finally, data suggest that patients may prefer a subcutaneous injection versus oral administration for long-term osteoporosis therapy [Kendler *et al.* 2010] and that adherence with therapy may be improved with this infrequent route of administration [Kendler *et al.* 2011]. However, it will be important to ascertain whether better adherence translates into better outcomes (e.g. fracture risk reduction). If better outcomes are confirmed, this will facilitate the use of and reimbursement for denosumab.

Conclusions

In a very short period of time, from basic science development to FDA approval, the denosumab story is an exciting and stimulating one. Understanding the bone biology of this fully human monoclonal antibody with a very high specificity to RANK-L has led to the registration of a novel treatment for osteoporosis with robust data for vertebral, nonvertebral and hip fracture risk reduction. Denosumab's safety data continue

to provide reassurance, though continual postmarketing surveillance is very important. The unique pharmacokinetic and pharmacodynamic properties of denosumab provide a challenge and opportunities for long-term management. Hopefully, additional data will allow us to answer pressing yet fundamental questions about treatment duration, discontinuation, and in the future, interactions with other osteoporosis pharmacological agents, both antiresorptive and anabolic agents. Denosumab is both a first-line agent and a second-line agent in patients who cannot tolerate or whose condition is not 'responding' to a previously administered therapy. It provides another treatment option for physicians faced with patients with a multitude of conditions that often accompany aging. Denosumab is a worthy choice since it can be administered parentally, thereby ensuring its delivery to the bone, and it has no drug–drug interactions in a population who are often on many different therapies. We look forward to an abundance of data that will provide continued reassurance as to denosumab's efficacy and safety.

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Conflict of interest statement

The author declares no conflicts of interest in preparing this article.

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A review of the efficacy and safety of denosumab in postmenopausal women with osteoporosis

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Abstract: Denosumab, a fully human monoclonal antibody to RANK ligand [RANK-L]) was approved for the treatment of postmenopausal osteoporosis in June 2010, and is highly effective in reducing the risk of vertebral, nonvertebral, and hip fracture risk. The registration of denosumab was the culmination of the discovery and clarification of the internal bone microenvironment regulation of bone remodeling: the osteoblast-produced competitors: RANK-L and osteoprotogerin; and the osteoclast receptor: RANK. When RANK-L is upregulated in the estrogen-deficient state and exceeds the amount of osteoprotogerin, there is an increase in osteoclastogenesis and bone resorption, and this is the major mechanism for bone mass loss and osteoporotic fractures in the postmenopausal state. The subsequent development of the human monoclonal antibody to RANK-L (denosumab) was the first product developed to reduce bone resorption by inhibiting RANK-L binding to RANK. Denosumab does not accumulate in bone, and has a unique pharmacokinetics so that its biological effect at the registered dose of 60 mg by subcutaneous injection every 6 months is no longer effective, at least as measured by an increase in the bone resorption marker collagen-cross-link C-telopeptide and a decline in bone mineral density as measured by dual energy X-ray absorptiometry. This unique pharmacokinetic profile thus suggests that in order to maintain the effectiveness of denosumab, continuous administration might be necessary. Extension of the registration trial ('FREEDOM') 5-year data indicates continued safety and efficacy, and will be extended to 10 years so that even longer-term data will be forthcoming. The profound but reversible suppression of bone turnover that is seen with denosumab partly explains the continuous increase in bone mineral density seen through 8 years of the phase II clinical trials. Denosumab offers a highly effective and safe parenteral therapy for osteoporosis and is being studied long term with the extension of the FREEDOM trial, and in other osteoporotic states – in men and glucocorticoid-induced osteoporosis.

Keywords:

Introduction

A new therapeutic advance in the treatment of postmenopausal osteoporosis is denosumab. Densoumab is a fully human monoclonal antibody to an activator of osteoclastic differentiation and activity, soluble RANK ligand (RANK-L). RANK-L is receptor activator (RANK being the receptor on osteoclasts, also called nuclear factor κB). RANK-L, a glycoprotein produced in the osteoblast, is a member of the superfamily of ligands, and is also known as a tumor necrosis factor (TNF) activation-induced cytokine and osteoclast activator [Bekker *et al.* 2004; Boyle *et al.* 2003; Eghbali-Fatourechi *et al.* 2003; Suda *et al.* 1999]. Also produced in the osteoblast is the decoy receptor for RANK-L, osteoprotogerin (OPG) [Bekker *et al.* 2001]. RANK-L and OPG are molecular competitors and are either upregulated or downregulated by opposing biological control systems. For example, in the estrogen-deficient state, there is an upregulation of RANK-L (e.g. more RANK-L is produced). When RANK-L concentrations in the bone marrow microenvironment exceed the concentration of OPG, then more RANK-L can bind to its receptor on the osteoclast, RANK. When this RANK-L–RANK binding occurs, there is a marked increase in osteoclast differentiation and

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activity, and thus, increase in bone resorption. If there is a greater concentration of OPG produced by the osteoblast, then less RANK-L is assessable to the osteoclast receptor, RANK, and there is more inhibition of osteoclast differentiation and activity and less bone resorption. Hence the balance between osteoblast-produced RANK-L and OPG is the determining factor for the internal bone microenvironment regulation of bone resorption (Figure 1) [Boyle *et al.* 2003]. Denosumab (anti-RANK-L antibody) thus acts like OPG, preventing RANK-L from binding to RANK and reducing bone resorption.

In the initial basic science development of these remarkable bone biological regulatory pathways, the development company, Amgen (Thousand Oaks, California, USA) first introduced parenteral administration of OPG as a means of reducing bone resorption, by then allowing less RANK-L to become available to the osteoclast receptor, RANK. OPG was first discovered from an immune cellular system, hence the concerns regarding how manipulation of this pathway might alter immune function or pose a risk for infection. However, the development of neutralizing antibodies to OPG prevented its development as a therapeutic agent, something which has not been seen with the administration of the anti-RANK-L antibody denosumab. As a culmination of this pioneering basic science work and the solid antifracture data observed in the pivotal registration trial, denosumab received international registration for the treatment of postmenopausal osteoporosis in June 2010 at a dose of 60 mg subcutaneously every 6 months [Cummings *et al.* 2009].

Basic science and pharmacokinetics of denosumab

The discovery of the RANK-L–OPG–RANK pathways was a giant leap in the understanding of the regulation of bone remodeling within the bone microenvironment *per se* [Eghbali-Fatourechi *et al.* 2003]. Regulation of bone remodeling, especially osteoblast activity, had been known for years to be under the control of systemic factors (e.g. parathyroid hormone, 1,25 dihydroxyvitamin D, bone morphogenic proteins, etc.); and there was also knowledge that within the bone marrow microenvironment the bone cell lines, especially the osteoblast and the osteoclast, were 'coupled in a directional manner, e.g. when one cell line activity was increased, the other cell line would be increase in time as well, and vice-versa' [Atfi and Baron, 2010; Bilezikian *et al.* 2009; Eriksen, 2010; Rosen, 2008]. While some data show that within the bone microenvironment *per se* the osteoblast activity was increased by bone matrix derived insulin growth factors and their binding proteins released into the microenvironment during bone resorption, regulation of coupling from the osteoclast to the osteoblast side of the equation was ill defined until the RANK-L–OPG–RANK systems were defined [Mackie, 2003]. The clarification of the RANK-L–OPG–RANK pathway closed the loop. Now, the regulation of the entire bone remodeling cycle from within the bone *per se* had become defined. In addition, there is increasing understanding of how the dominant cell in bone, the osteocyte, is also linked to the regulation of bone turnover through the osteocyte production of fibroblast growth factor 23 (FGF 23) and sclerostin, providing the foundation of the mechanostat mechanism and bone formation [Bonewald, 2011; Juppner, 2011; Price *et al.* 2011; Wang *et al.* 2008]. While the effect on bone mineralization of FGF 23 is not yet fully characterized, sclerostin clearly can inhibit a major activator of osteoblast activity, the Wnt pathway [Atkins *et al.* 2011; Silverman, 2010]. FGF 23, a phosphaturic glycoprotein, also reduces renal production of 1,25 dihydroxyvitamin D, and reduces parathyroid hormone secretion in short-term studies [Juppner, 2011].

While the RANK-L–RANK–OPG glycoproteins have multiple nonskeletal tissue expression, there is little knowledge of the physiological or pathological role for these proteins in other tissues. The bone microenvironment is the most well understood pathway for the RANK L–RANK–OPG system.

Denosumab is produced in the Chinese hamster embryo oophocyte, and is metabolized by the reticulo-endothelial system [Wypych *et al.* 2008]. It shows a nonlinear pharmacokinetic profile. This IgG 2 monoclonal antibody has pharmacokinetic characteristics (peak, half life, serum concentrations), as shown in Figure 2 [Bekker *et al.* 2004]. Neutralizing antibodies do not develop to anti-RANK-L antibodies as they do to OPG because denosumab does not resemble OPG. In addition, denosumab does not bind to TNF-apoptosis-inducing ligand (TRAIL, a survival factor for tumor cells), and has no significant effect on lymphocyte counts overall (CD3), T cells (CD 4, CD 8, CD 54), or B cells (CD 20) [Suda *et al.* 1999]

Figure 1. The mechanism of action of anti-RANK ligand (RANK-L) antibody on bone turnover. Denosumab binds to osteoblast-produced RANK-L, thereby preventing RANK-L from binding to the osteoclast receptor, RANK. By preventing RANK-L from binding to RANK, there is less osteoclast differentiation and activity so that bone resorption decreases. Illustration courtesy of Alessandro Baliani © [2011].

RANK-L, RANK ligand; CFU-M, colony-forming unit-macrophage

The phase II denosumab dose-ranging clinical trial – the longest-term denosumab data available: 2-, 4-, 6-, and 8-year data.

The initial dose-ranging studies in postmenopausal women randomized patients to seven different doses of denosumab given at different intervals *versus* alendronate (70 mg/week) *versus* placebo [McClung *et al.* 2006]. The initial 1-year and 2 year data showed that these doses increased bone mineral density (BMD) at all skeletal sites and

Figure 2. The pharmacokinetics of denosumab. The 1.0 mg/kg dose had no measurable level by 6 months. Therefore, the 6-month dosing interval at this strength was selected for clinical trial development. Adapted with permission from [Bekker *et al.* 2004] copyright 2011, Wiley.

EC_{50}, half maximal effective concentration.

reduced biochemical markers of bone turnover more than alendronate or placebo [Lewiecki *et al.* 2007]. This initial 2-year study was then extended to 4, 6, and now 8 years [Miller *et al.* 2008, 2011; McClung *et al.* 2011]. The 4- and 8-year data show a few unique highlights: the 4-year group had a subgroup that discontinued denosumab for 2 years after the initial 2 years of treatment; and a group that, after 1 year of discontinuation, were rechallenged with denosumab. In the continuous discontinuation group the bone resorption marker (CTX) increased and even went above the pretreatment baseline by the sixth month after discontinuation, reached a peak at month 36, but returned to baseline during the fourth year without any further treatment. Likewise, the BMD responses were the mirror image of the bone turnover markers (BTMs) (Figure 3(a)) [Miller *et al.* 2008]. The reasons for the return of CTX and BMD back to baseline without any additional denosumab being provided are unknown but might be related to the bone mechanostat mechanism [Bonewald, 2011; Juppner, 2011; Price *et al.* 2011; Wang *et al.* 2008]. In addition, in the retreatment group, the response to denosumab was retained, such that upon rechallenge with denosumab, the BMD increased and the CTX decreased, with these responsive changes having the same slope as seen in the first year of treatment (Figure 3(b)) [Miller *et al.* 2011]. Thus, as expected from the initial pharmacokinetic and pharmacodynamic data, the human biological data show that the effect of denosumab is reversible yet retains responsiveness after treatment, discontinuation and retreatment.

The 6- and 8-year phase II data provide some additional interesting insights into the pharmacodynamics of denosumab [Miller *et al.* 2011; McClung *et al.* 2011]. During these periods the lumbar spine and total hip BMD continue to increase and not plateau as is seen with other antiresorptive agents. The mechanism for this progressive increase in BMD is not yet defined, but may be related to the larger degree of suppression of bone turnover than that seen with alendronate, and prolongation of the time for secondary mineralization or greater closure of the remodeling space [Miller *et al.* 2011]. In addition,

Figure 3. (a) The phase II, 4-year data showing the effect of discontinuation and retreatment of denosumab on bone turnover markers and bone mineral density. The data show that after 2 years of use, the bone turnover markers increase to baseline and above by month 6 after discontinuation but return to treatment levels when retreated with denosumab. Adapted with permission from [Miller *et al.* 2008] copyright 2011, Elsevier. (b) Likewise, after treatment then discontinuation, the bone mineral density decreases within 6 months to baseline, but then increases again when treatment is restarted. Adapted with permission from [Miller *et al.* 2011] Copyright 2011, The Endocrine Society.

LS, least squares; SE, standard error.

the serum bone resorption marker (CTX) does not continue to decline with repeated doses of denosumab, and in fact has a trend to increase over time though remain within the premenopausal normal reference range (Figure 4) [Miller *et al.* 2011]. These data are reassuring in that continuous administration of denosumab is not associated with reduction of BTMs to levels below the normal reference range. The mechanism by which CTX tends to increase over time despite continual administration of denosumab is unknown but may have more to do with the mechanostat than tachyphylaxis because the reductions in CTX 1 month after dosing were consistent at year 1 and year 5 [Miller *et al.* 2011].

The phase III clinical trials: 'DEFEND', 'DECIDE', 'STAND', and the registration trial: 'FREEDOM'

Defend

The DEFEND trial was a prevention trial. It included 332 early postmenopausal younger white

Figure 4. Six-year exposure to denosumab (DMAb), 60 mg every 6 months, does not result in reduction on bone resorption marker (serum CTX) below the premenopausal normal reference range. In fact, the serum CTX seems to drift up (but remain in the premenopausal reference range) during the fourth to sixth year of therapy. Adapted with permission from [Miller *et al.* 2011] Copyright 2011, The Endocrine Society.

IQR, interquartile range.

women with a mean age of 56 years [Bone *et al.* 2008]. These women were treatment naive, at low risk for fracture (mean lumbar spine T-score −1.6), postmenopausal and were randomized to either denosumab (60 mg subcutaneously every 6 months) or subcutaneous placebo in a 1:1 ratio. The first 2 years of the study showed that denosumab significantly increased BMD at all skeletal sites, both from baseline and compared with placebo, and significantly reduced the level of BTMs. After the first 2 years of the DEFEND trial, 252 of the initial study participants continued into an extension study for an additional 2 years [Bone *et al.* 2011]. During these 'treatment-off' 2 years, all women received placebo, so the initial placebo group had a total of 4 years of placebo, and the initial denosumab-treated group came off therapy to receive 2 years of placebo. During the extension study the BMD in the initial denosumab-treated group declined to baseline at all skeletal sites during months 24–36, but then leveled off at the lumbar spine while it continued to decline at the total hip and one-third radius. Likewise, the BTMs increased to baseline and above within 6 months of discontinuation (as was seen in the phase II trials), but then returned to baseline during the fourth year without any additional therapy (also as was seen in the phase II trials). Thus, examining the registered dose (60 mg subcutaneously every 6 months) for this entire 4-year study showed that the effects of denosumab are reversible upon discontinuation and suggest that treatment may need to be continued to maintain an effect on BMD and BTMs. What is unknown in this phase II study, like the phase II trial, is what the effect on bone strength (fractures) is upon discontinuation.

Decide

The DECIDE trial was a prospective randomized comparison of alendronate (70 mg/week) to denosumab (60 mg subcutaneously every 6 months) in 1189 early postmenopausal women with low bone mass [Brown *et al.* 2009]. At the total hip, denosumab resulted in a significantly greater increase in BMD compared with alendronate (3.5% *versus* 2.6%, $p < 0.0001$). The larger increases in BMD with denosumab administration were also seen at all skeletal sites measured. In addition, denosumab induced a significantly greater reduction in BTMs compared with alendronate. There are no prespecified fracture data in DECIDE.

Stand

The STAND study (study of transitioning from alendronate to denosumab) was an international, multicenter, randomized, double-dummy, parallel-group phase III trial [Kendler *et al.* 2010]. A total of 504 postmenopausal women (average age 67 years) previously taking alendronate (median 36 months, range 6–192 months) were randomized to receive either brand name alendronate (Fosamax) (70 mg/week) or placebo injections; or denosumab (60 mg subcutaneously every 6 months) or placebo alendronate for 12 months. A repeated-measures model was used as the primary analysis method for the percentage change in BMD at months 6 and 12. The model included treatment, time of BMD assessment, treatment by time of BMD assessment interaction, baseline BMD value, time on prior alendronate therapy stratum, dual-emission X-ray absorptiometry instrument type, and baseline BMD value and instrument type interaction. The primary hypothesis was that denosumab was not inferior to alendronate with respect to the mean percentage change in total hip BMD at month 12. BMD at the total hip increased by 1.90% [95% confidence interval (CI) 1.61–2.18%] at month 12 in women transitioned to denosumab compared with a 1.05% (95% CI 0.76–1.34%) increase from baseline in women continuing on alendronate therapy (Figure 2) [Bekker *et al.* 2004]. The difference between treatment groups was 0.85% (95% CI 0.44–1.25%) greater with denosumab; the lower limit of the confidence interval excluded the prespecified noninferiority margin (0.35%), thus showing the noninferiority of denosumab compared with alendronate. Superiority testing demonstrated the BMD increase with denosumab at the total hip was statistically superior to the change with alendronate ($p < 0.0001$).

At the lumbar spine, denosumab increased BMD by 3.03% (95% CI 2.63–3.44%) at month 12, and alendronate increased BMD by 1.85% (95% CI 1.44–2.26%), for a difference of 1.18% (95% CI 0.63–1.73%), with the lower limit of the confidence interval excluding the noninferiority margin of 0.22. These data demonstrate the noninferiority of denosumab *versus* alendronate at this skeletal site. The increase in BMD with denosumab *versus* alendronate was statistically significant ($p < 0.0001$). Significantly greater increases in BMD with denosumab compared with alendronate were also observed at month 12 at the femoral neck and one-third radius ($p < 0.0121$). Furthermore, significant BMD increases for denosumab compared with alendronate were observed as early as month 6 at the lumbar spine and all measured femoral sites ($p < 0.05$). Thus, the data show that in patients previously on alendronate, changing to denosumab is accompanied by at least stabilization if not further increases in BMD.

Freedom

The large phase III registration trial randomized 7808 postmenopausal women (mean age 72.3 years, average lumbar spine T-score –2.8, WHO defined osteoporosis), 23% of whom had a prevalent vertebral fracture [Cummings *et al.* 2009]. One-half received denosumab (60 mg subcutaneously every 6 months) and the other half placebo (subcutaneously). Over 3 years, denosumab reduced the incidence of morphometric vertebral fractures, the primary endpoint (Figure 5) [relative risk reduction (RRR) 68%, $p < 0.0001$ *versus* placebo], nonvertebral fractures (RRR 20%, $p < 0.01$ *versus* placebo), and hip fractures (RRR 40%, $p < 0.04$) [Cummings *et al.* 2009].

The FREEDOM trial is designed to extend to 10 years. The initial 5-year data of the extension have recently been presented [Papapoulos *et al.* 2011]. The design of the extension trial is as follows: after the first 3 years, 2343 patients from the initial 3902 patients who were randomized to the treatment arm of denosumab continued with treatment (60 mg subcutaneously every 6 months). From the initial randomized placebo group of 3906 patients, 2207 switched over to treatment with denosumab (60 mg subcutaneously every 6 months). In patients who received 5 years of denosumab treatment, the fracture rates continued to remain reduced from the end of the registration trial, and the BMD continued to increase at both the spine and hip. In the patients who initially received placebo and when on to receive denosumab, the BMD increased and the fracture rates declined, similar to the initial results after 2 years of the FREEDOM trial.

Safety data on denosumab

In the collective data from the phase II and III clinical trials, denosumab has been shown to be safe. The clinical concern was the potential risk for infections because of the ubiquitous presence of RANK-L throughout many tissues. In the pivotal registration trial, the only serious adverse event (AE) that was significantly greater than placebo was skin infections (termed either 'cellulitis' or

Figure 5. The results of the pivotal registration fracture trial with denosumab *versus* placebo ('FREEDOM'). Over 3 years denosumab (60 mg as a subcutaneous injection every 6 months) significantly reduced the incidence of vertebral, nonvertebral and hip fractures compared with placebo [Cummings *et al.* 2009].

*Composite measurement excluding pathological fractures and those associated with severe trauma, fractures of vertebrae, skull, face mandible, metacarpals, fingers and toes.

ARR, absolute risk reduction; RRR, relative risk reduction.

'erysipelas') [Cummings *et al.* 2009]. In the AE reports there was a small and nonsignificant difference between the denosumab and placebo groups for skin infections (54 *versus* 53 cases), while serious AEs did reach statistical significance (0.3% *versus* <0.1%, $p < 0.002$) [Cummings *et al.* 2009]. These areas of inflammation could occur at any time after the injection and at a site distant from the injection, however all responded to conservative management. There were no unusual opportunistic infections reported. The US Food and Drug Administration (FDA) label also includes 'adverse reactions': events that occurred more frequently in those that received the investigational product compared with placebo. These events were included on the FDA label even if they were not statistically significantly different from one another. The aim of this was to provide caution for a new drug of its class being exposed to a large population so that physicians could report any trend in experiences in the real world. In this regard, the only adverse event that was significantly greater in the treated *versus* the placebo group was: 'Epidermal and dermal adverse events such as dermatitis, eczema, and rashes occurred at a significantly higher rate in the treated *versus* placebo groups (10.8% *versus* 8.2%, $P < 0.0001$)' [Cummings *et al.* 2009]. No studies have examined the potential for infections in patients who are immunosuppressed or on other biological drugs. However, denosumab has recently received registration to reduce the risk of fractures in patients with metastatic carcinoma of the bone, and in oncology trials, there was no increased risk of infection seen in this group of patients who have more compromised immune systems and receive a much higher dose of denosumab for this indication (120 mg/month) [Stopeck *et al.* 2010]. In addition, the extension data from FREEDOM show that the group who received 5 years of continuous treatment with denosumab had a cellulitis rate that did not increase and was comparable to the rate reported for the placebo group in the first 3 years (< 0.1% *versus* <0.1%) (unpublished data from Amgen). Vigilance is a key word when this very effective human monoclonal antibody is used in large numbers of patients so that any trends in adverse events can be reported to the FDA.

The FDA label for the postmenopausal osteoporosis indication does not have any lower cut-off for renal function. This is because denosumab is not cleared by the kidney but by the reticuloendothelial system, and it may not have any adverse renal effects as may be seen, though rarely, with intravenous bisphosphonates [Miller, 2009]. In a

Figure 6. The *post hoc* analysis from the FREEDOM trial showing that denosumab [60 mg as a subcutaneous injection every 6 months] was effective in reducing incident vertebral fractures and was safe over 3 years in patients with stage 1–4 chronic kidney disease [estimated glomerular filtration rate > 90–15 ml/min] [Jamal *et al.* 2011].

N = number of randomized patients, *N*1 = number of randomized patients with an elevation during the time period of interest. There were no patients with a creatinine clearance less than 15 ml/min. $^*p < 0.05$.

post hoc analysis of FREEDOM in which the registration population had estimated glomerular filtration rates (eGFRs) divided into quartiles (> 90 to 15–29 ml/min), denosumab showed evidence of reduction in incident vertebral fractures across these quartiles without any adverse renal effects (e.g. change in eGFR over 3 years) (Figure 6) [Jamal *et al.* 2011]. There are no data on changes in BMD or fractures in patients with a GFR less than 15 ml/min. In these patients the diagnosis of osteoporosis becomes far more difficult to establish. There is also concern that in patients with preexisting adynamic renal bone disease, reducing bone turnover further may be associated with an increase in cardiovascular calcification [Hruska *et al.* 2009]. This concern is based on the knowledge that absorbed calcium and/or phosphorus cannot be adequately eliminated by renal clearance, and if bone turnover is low, the capacity of bone to take up these ions is restricted, leaving vascular tissue exposed to calcium and/or phosphorus leading to the risk of vascular calcification. One study has examined the effect of denosumab on vascular calcification in the FREEDOM trial and found that across the eGFR quartiles, there was no greater increase in vascular calcification with denosumab *versus* placebo, at least when assessed by lateral lumbar X-ray assessment of aortic calcification [Egbuna OI *et al.* 2010].

The FDA label includes a caution about the possibility of hypocalcemia after denosumab administration. While all antiresorptive agents may induce a small and transient hypocalcemic effect after administration, clinically significant hypocalcemia (associated with tetany or paresthesias) is not observed in patients with adequate calcium and vitamin D intake, and with intact parathyroid hormone, transient hypocalcemia is normalized. There was no difference in reported hypocalcemia in the FREEDOM trial between the treated and the placebo groups either in the registration (first 3 years) or the extension trial. It is important in patient management to ensure that an adequate amount of calcium and vitamin D are provided.

The effect of denosumab on bone turnover is pronounced. While BTMs do not remain suppressed below the normal premenopausal reference range in long-term denosumab trials and rise rapidly after discontinuation of denosumab, the quantitative bone histomorphometry data show absent tetracycline labels, an indicator of new bone mineralization, in a small number of patients in whom bone biopsies were performed. Absent single tetracycline labels were not seen in any of the 62 placebo biopsies while 19 of 33 (36%) of the biopsies in patients on denosumab had no single labels [Reid *et al.* 2010]. The clinical significance of these findings is unknown. It is reassuring that in the patients who had biopsies the serum CTX overlapped and there was no difference between the biopsies that had no labels *versus* the biopsies that had single or double labels. The tetracycline labels (and therefore mineralization) would be expected to return after denosumab discontinuation since the BTMs would increase. However, this has yet to be studied. In the DECIDE study, bone biopsies were done in patients treated with denosumab and then taken off treatment and were shown to have normal labels. However, it is unknown if any of these patients had absent labels to start with. The maintenance of the low fracture incidence in the FREEDOM extension trial is reassuring because it shows that 5-year use is not associated with any rise in fracture events.

From a safety viewpoint, there have been no cases of osteonecrosis of the jaw (ONJ) or atypical subtrochanteric femur fractures reported in the osteoporosis clinical trials. There have been two adjudicated cases of ONJ in the fourth and fifth year of FREEDOM extension trial. There has also been a low but similar incidence of ONJ in the oncology trials using monthly denosumab *versus* monthly intravenous zoledronic acid (1.3% *versus* 1.8% over 3 years) [Stopeck *et al.* 2010]. Postmarketing data will reveal in time if these rare events occur in the osteoporosis population and with the lower doses of denosumab used in this population.

Finally, a few practical considerations for long-term use of denosumab – questions that, to date, cannot be answered from existing data. One is whether denosumab needs to be continued indefinitely. Since BTMs increase and BMD decreases after discontinuation, it is theoretically possible that in order to maintain antifracture efficacy, continuous administration must be provided. It is also possible that after long-term use (e.g. more than 5 years) denosumab could be discontinued yet bone strength not altered despite a rise in BTMs and a drop in BMD since bone quality (microarchitecture, cortical porosity) might not change. These are vital questions that can only be answered from long-term data . For example, in FREEDOM, approximately 929 patients treated during the first 3 years did not continue into the extension study. It remains to be reported what happened to these patients who are essentially on a denosumab 'drug holiday'. Observational trials of the effects of denosumab in the 'real world' might also provide helpful information, and have been initiated. Since the pharmacokinetics and pharmacodynamics of denosumab differ markedly from bisphosphonates, in that there is neither long-term bone retention nor 'recycling' of denosumab like there is with bisphosphonates, a so-called 'drug holiday' from denosumab might have very different implications [Baron *et al.* 2011]. While there is no 'standard of care' for any of these implementations of continuation *versus* discontinuation with any approved osteoporosis pharmacological agent, prevailing opinion would suggest considering a 'drug holiday' from bisphosphonates after 5 years of use in lower-risk but not in higher-risk patients (patients with preexisting fractures or T-scores at the hip of –2.5 or lower) [Black *et al.* 2006; Schwartz *et al.* 2010]. Hence, both the long-term exposure data and the offset data will be important for us to gain knowledge in the management of patients with the chronic disease of osteoporosis. Finally, data suggest that patients may prefer a subcutaneous injection versus oral administration for long-term osteoporosis therapy [Kendler *et al.* 2010] and that adherence with therapy may be improved with this infrequent route of administration [Kendler *et al.* 2011]. However, it will be important to ascertain whether better adherence translates into better outcomes (e.g. fracture risk reduction). If better outcomes are confirmed, this will facilitate the use of and reimbursement for denosumab.

Conclusions

In a very short period of time, from basic science development to FDA approval, the denosumab story is an exciting and stimulating one. Understanding the bone biology of this fully human monoclonal antibody with a very high specificity to RANK-L has led to the registration of a novel treatment for osteoporosis with robust data for vertebral, nonvertebral and hip fracture risk reduction. Denosumab's safety data continue

to provide reassurance, though continual postmarketing surveillance is very important. The unique pharmacokinetic and pharmacodynamic properties of denosumab provide a challenge and opportunities for long-term management. Hopefully, additional data will allow us to answer pressing yet fundamental questions about treatment duration, discontinuation, and in the future, interactions with other osteoporosis pharmacological agents, both antiresorptive and anabolic agents. Denosumab is both a first-line agent and a second-line agent in patients who cannot tolerate or whose condition is not 'responding' to a previously administered therapy. It provides another treatment option for physicians faced with patients with a multitude of conditions that often accompany aging. Denosumab is a worthy choice since it can be administered parentally, thereby ensuring its delivery to the bone, and it has no drug–drug interactions in a population who are often on many different therapies. We look forward to an abundance of data that will provide continued reassurance as to denosumab's efficacy and safety.

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Conflict of interest statement

The author declares no conflicts of interest in preparing this article.

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