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Optimizing bone health in lymphoma survivors: denosumab superiority to alendronate for R-CHOP-like therapy (the DENOSULY phase III randomized controlled trial)

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Short Title

Denosumab vs alendronate in lymphoma survivors

Keywords
Lymphoma patients; glucocorticoid-induced osteoporosis; bone mineral density; denosumab; alendronate.

Disclosures

No conflicts of interest to disclose.

Contributions

T.S. conceived and designed the study; R.H., T.F., Y.N., T.M., K.O., Y.K., and A.K. collected the data; S.K. and E.N. performed the data analysis and interpretation; T.S. and N.H.D. drafted the manuscript; Y.M., T.M., and T.Y. verified the data; and all authors contributed to revisions of the manuscript, and read and approved the final version of the manuscript.

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Data-sharing statement

The datasets generated and/or analyzed during the current study are not

publicly available due to privacy and ethical restrictions but are available from the corresponding author on reasonable request.

Abstract

Glucocorticoid-induced osteoporosis (GIOP) poses a critical long-term complication for lymphoma survivors, with cumulative incidence of fractures following R-CHOP-like therapy. Existing GIOP guidelines, typically based on chronic low-dose steroid use, are insufficient for managing this acute, high-risk toxicity of lymphoma therapy-related GIOP (LTR-GIOP). This prospective, multi-center, phase 3 randomized controlled trial (n=100; median age 74-years) compared the efficacy and safety of denosumab versus alendronate in newly diagnosed lymphoma patients receiving R-CHOP-like therapy. Patients were randomized into two groups: one received a total of two subcutaneous injections of denosumab every 6 months, and the other received oral alendronate once a week for 12 months. This study was named DENOSULY and the cases were collected by the Hokuriku Hematology Oncology Study Group. The primary endpoint was the percentage change in lumbar spine (LS) bone mineral density (BMD) at 12 months. Consequently, denosumab demonstrated superiority over alendronate in LS(L1-L4) BMD change (denosumab: +2.8%±4.4% vs. alendronate: −1.3%±5.6%; p=0.0010). Crucially, denosumab also showed superiority at the femoral neck (denosumab: +2.8%±5.8% vs. alendronate: −3.6%±10.3%; p=0.0020), a site where superiority is infrequently demonstrated in non-LTR-GIOP comparisons. Denosumab achieved stronger suppression of the bone resorption marker TRACP-5b (p=0.0003). Since denosumab showed significant superiority over alendronate at both the lumbar spine and femoral neck, denosumab may be the preferred agent in LTR-GIOP. Recognizing R-CHOP recipients as a very high-risk population, our findings underscore the need for immediate, enhanced prophylaxis with denosumab to prevent LTR-GIOP and improve long-term survivorship and quality of life. This trial was registered at www.umin.ac.jp as UMIN000038881.

Introduction

The introduction of rituximab has transformed the treatment of B-cell lymphomas. For instance, the R-CHOP regimen has significantly improved survival outcomes in patients with diffuse large B-cell lymphoma (DLBCL) [1,2]. However, this success has illuminated long-term complications, specifically the decrease in bone mineral density (BMD) associated with chemotherapy.

In the treatment of lymphoma, corticosteroids are indispensable, serving a dual role: they exert a powerful cytotoxic effect that kills lymphoma cells and, concurrently, mitigate chemotherapy side effects such as nausea and vomiting. On the other hand, the detrimental effect of steroids on bone—specifically, glucocorticoid-induced osteoporosis (GIOP)—is the most common cause of iatrogenic osteoporosis and is estimated to occur in approximately 30–50% of chronic steroid users[3]. This steroid-induced imbalance in bone remodeling results in a characteristic rapid loss of bone mass within a short period[4]. Despite the well-known effects of steroids on bone, the impact of steroid-containing lymphoma treatment on BMD reduction has not been sufficiently addressed until recently, with several important clinical studies examining this issue being published in recent years.

Prospective studies have demonstrated a significant reduction in BMD within the first year of chemotherapy in lymphoma patients[5]. This reduction is particularly pronounced in patients receiving a higher number of cycles and those of older age[6].

Importantly, this BMD reduction was associated with bone-related events. Specifically, long-term registry studies involving DLBCL and Follicular Lymphoma survivors treated with R-CHOP-like regimens demonstrated a significantly elevated 10-year cumulative risk of osteoporotic events compared to the general population (16.3% vs 13.5%)[7]. Furthermore, a retrospective analysis targeting elderly DLBCL patients treated with R-CHOP therapy revealed an alarming cumulative fracture incidence of 11.4% at just 18 months post-treatment[8]. Moreover, a significant proportion of patients (23%) had pre-

existing fractures prior to treatment, which were often unrecognized and dramatically increased the risk of new fractures during therapy[9]. As recently demonstrated by Douglas et al. using opportunistic bone density assessment on routine staging 18F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT), the early identification of skeletal fragility in lymphoma patients is becoming an established clinical possibility[10]. However, while identifying at-risk patients is increasingly achievable, definitive evidence for the "optimal interventional agent" to manage these high-risk individuals remains insufficient.

To mitigate BMD loss, several intervention trials have evaluated bisphosphonates. While agents such as alendronate[11], pamidronate[12], and zoledronic acid[13] have shown efficacy in stabilizing BMD, definitive evidence for the optimal interventional agent in this high-risk population remains insufficient.

On the other hand, denosumab is an osteoporosis treatment with a mechanism of action distinct from that of bisphosphonates. It is a monoclonal antibody that suppresses osteoclast function by neutralizing Receptor Activator of NF-kappaB Ligand (RANKL), which is essential for the formation, activation, and survival of bone-resorbing osteoclasts. We previously conducted a non-randomized prospective study evaluating the efficacy of denosumab in lymphoma patients[14]. In this study, we stratified 43 newly diagnosed lymphoma patients receiving steroid-containing chemotherapy by T-score: patients with a baseline T-score of ≤-1 received denosumab, and patients with a T-score of >-1 were assigned to a non-intervention group. The results indicated that while BMD reduction was observed in the lumbar spine and femoral neck of the non-intervention group, BMD remained stable in the denosumab group. This study suggested that denosumab is a promising option for lymphoma therapy-related GIOP (LTR-GIOP). However, the non-randomized design of this study required caution in interpreting the obtained results. Furthermore, the intervention group included more elderly patients who were at a higher

risk of bone loss, thereby limiting strict scientific comparison. This previous work served as a crucial pilot study, the results of which provided the clinical rationale for the design of our current phase 3 trial.

The four aforementioned intervention trials offered different levels of evidence. The pamidronate study provided the strongest clinical rationale, as it demonstrated a reduction not only in the surrogate marker of BMD improvement but also in the clinically relevant endpoint of fracture[12]. The SIESTA trial also confirmed that oral bisphosphonates stabilize BMD, although with the caveat that this surrogate endpoint alone may not correlate with fracture reduction in small cohorts[11]. The zoledronic acid study, an RCT with the phase 3 study design, provided high-quality evidence indicating that prophylactic intervention is effective in stabilizing BMD[13]. In contrast, our previous work with denosumab was a pilot/proof of concept study, resulting in a lower level of evidence[14]. To obtain higher quality evidence, we therefore conducted a multi-center phase 3 trial comparing denosumab and alendronate, which was evaluated in the aforementioned SIESTA trial[11], as the subject of this current paper.

Methods

Detailed methods are available in the Online Supplementary Appendix.

Study Design and Objectives

This prospective, multi-center, randomized, open-label phase 3 trial compared the efficacy and safety of denosumab versus alendronate in newly diagnosed lymphoma patients undergoing corticosteroid-containing chemotherapy. The primary endpoint was the 12-month percentage change in lumbar spine BMD (comparison to baseline). Secondary endpoints included BMD changes at the

femoral neck and total hip at 6 and 12 months, and changes in bone turnover markers (BTMs) at 6 months. The protocol was approved by the Institutional Review Board of Toyama University Hospital (protocol no. R2019137) and registered at UMIN-CTR (ID: UMIN000038881). All participants provided written informed consent.

Patients and Treatment

We enrolled newly diagnosed lymphoma patients aged ≥65 years scheduled for steroid-containing chemotherapy. Key exclusion criteria included hypersensitivity to denosumab or bisphosphonates, baseline hypocalcemia, or current osteoporosis treatment. Patients were stratified by age (≥80 years) and sex, then randomized (permuted block method) to receive either: 1) Denosumab Group: 60 mg subcutaneously at baseline and 6 months or 2) Alendronate Group: 35 mg orally once weekly for 12 months. All patients received daily oral calcitriol (0.25 μg) for 12 months. BMD was assessed by dual-energy X-ray absorptiometry (DXA). BTMs included tartrate-resistant acid phosphatase 5b (TRACP-5b) and total type I procollagen N-terminal propeptide (total P1NP).

Adverse Events

Adverse events (AEs) were categorized following standard criteria (see Supplemental Methods). Predetermined AEs of interest (Grade ≥3) included hypocalcemia and osteonecrosis of the jaw (ONJ). Standard chemotherapy-related toxicities (e.g., myelosuppression) were not reported unless serious.

Rationale for target sample size

The sample size was determined by referencing a comparative study of denosumab and alendronate for patients with nephritis[15].

Statistical Methods

Analyses used GraphPad Prism v9.0. Values are mean ± SD. Significance was determined via Student's t-test (normal distribution) or Mann-Whitney U test. Categorical variables were evaluated by Fisher's exact test and correlations by Spearman coefficient. All tests were two-sided (p<0.05).

Results

Enrollment, allocation, and follow-up of patients

Patient disposition is shown in Figure 1.

Characteristics of patients

In the 48 patients in the alendronate group and 52 patients in the denosumab group, the median age (years) was 75 and 73, the percentage of male patients (%) was 54.2 and 55.8, and the proportion of DLBCL among lymphoma subtypes (%) was 93.8 and 84.6, respectively. No statistically significant difference was found between the two groups for any of these characteristics. Furthermore, BMD was measured for the lumbar spine (L2-L4 and L1-L4), total hip, and femoral neck, and BTMs measured were TRACP-5b and total P1NP. No significant differences were observed between the alendronate and denosumab groups for any of these parameters. Similarly, the cumulative dose of prednisolone did not differ significantly between the two groups (Table 1). The detailed breakdown of lymphoma histology and treatment regimens is provided in Supplementary Table 1.

Bone turnover markers

Figure 2 illustrates the percentage change in BTMs at 6 months post-study initiation. For TRACP-5b (Figure 2A), the alendronate group showed 0 ± 44% change, while the denosumab group showed -36 ± 42%, indicating that denosumab significantly suppressed TRACP-5b more than alendronate (p=0.0003). In contrast, no significant difference was observed between the two groups for total P1NP (Figure 2B).

Bone mineral density

Supplementary Figure 1 shows the percentage change in BMD at 6 months post-study initiation. There were no significant differences between the alendronate and denosumab groups for L2-L4, L1-L4, and total hip (Supplementary Figure 1A, B, and C). However, at the femoral neck (Supplementary Figure 1D), the alendronate group showed -1.9 ± 6.7% change, and the denosumab group showed 4.5 ± 16.3%, demonstrating that denosumab significantly increased BMD compared to alendronate (p=0.0338). Significantly, the superiority of denosumab over alendronate became even more pronounced at 12 months post-study initiation (Figure 3). Specifically, denosumab was superior to alendronate in L2-L4, L1-L4, and femoral neck, excluding the total hip. In detail, for L2-L4, the alendronate group showed -1.3 ± 5.6% and the denosumab group showed 2.8 ± 4.4% (p=0.0010); for L1-L4, the alendronate group showed -1.0 ± 4.8% and the denosumab group showed 2.7 ± 4.5% (p=0.0054); and for the femoral neck, the alendronate group showed -3.6 ± 10.3% and the denosumab group showed 2.8 ± 5.8% (p=0.0020).

Subgroup analysis of patients with DLBCL (n=89) yielded results consistent with the findings in the overall population (Supplementary Figure 2).

Correlation analysis

These results suggest a model in which denosumab prevents BMD reduction or induces BMD increase by substantially lowering TRACP-5b among BTMs, thereby sufficiently suppressing osteoclast-mediated bone resorption.

Therefore, we analyzed whether the reduction in TRACP-5b at 6 months correlated with the increase in BMD at 12 months in the denosumab group using simple linear regression (Table 2). Contrary to expectations, the percentage change in BMD at 12 months did not correlate with the percentage change in TRACP-5b at 6 months in any of the sites measured: L2-L4, L1-L4, total hip, and femoral neck. Furthermore, it did not correlate with the percentage change in total P1NP at 6 months, the total cumulative dose of corticosteroids, or the baseline BMD prior to treatment initiation. Conversely, patient age showed a weak negative correlation with the percentage change in L2-L4 and L1-L4 BMD. The specific correlation graphs are shown in Supplementary Figure 3. The correlation coefficients were -0.384 (p=0.0131) for L2-L4 and -0.342 (p=0.0409) for L1-L4.

Adverse events

Regarding serious adverse events (SAEs) (Table 3), it is important to note that, as predefined in our protocol and Supplementary Methods, common chemotherapy-related toxicities such as myelosuppression were only reported as SAEs if they met the strict criteria for being 'serious' (e.g., resulting in hospitalization or life-threatening events). Based on these criteria, the alendronate group had 1 patient with Grade <2 events and 9 patients with Grade ≥3 events. The Denosumab group had 0 patients with Grade <2 events and 10 patients with Grade ≥3 events. In all cases, no direct causal relationship was suspected between the adverse events and alendronate or denosumab. Notably, the three neoplasms observed in the denosumab group were gastric cancer (1 patient), colorectal cancer (1 patient), and esophageal cancer (1 patient). All three were discovered during the pre-treatment systemic screening performed for staging purposes, prior to denosumab administration.

Endoscopic or surgical treatments were subsequently performed following chemotherapy for lymphoma. Furthermore, Supplementary Table 2 presents the characteristic adverse events associated with alendronate and denosumab. Two particular items of note are hypocalcemia and ONJ. Regarding these adverse events of interest, no cases of Grade ≥3 events were reported.

Discussion

This study is the first randomized controlled trial to demonstrate the superiority of denosumab over alendronate in preventing GIOP in newly diagnosed lymphoma patients receiving steroid-containing chemotherapy. Denosumab significantly surpassed alendronate in the primary endpoint, the percentage change in lumbar spine BMD at 12 months. Similar superiority was also demonstrated at the femoral neck.

The main therapeutic options for GIOP include bisphosphonates and denosumab. Bisphosphonates (e.g., alendronate, risedronate, etidronate, zoledronate) suppress bone resorption by depositing onto the bone surface and inhibiting specific enzymes, such as farnesyl pyrophosphate synthase, within osteoclasts[16]. On the other hand, denosumab is a fully humanized monoclonal antibody that specifically binds to and inhibits the function of RANKL, a key regulator of bone metabolism. This interaction leads to the suppression of osteoclast formation, function, and survival, resulting in a powerful inhibition of bone resorption and a subsequent increase in BMD[17].

Concerning non-LTR-GIOP, multiple meta-analyses report that denosumab is superior to bisphosphonates in improving lumbar spine BMD[18,19]. However, results regarding femoral BMD remain inconsistent; while one meta-analysis showed denosumab superiority[18], another found no significant difference between the two agents[19]. This divergence may be explained by the lower proportion of metabolically active trabecular bone in the femoral neck compared to the lumbar spine[20,21].

In contrast, our current data on LTR-GIOP demonstrated that denosumab was significantly superior to alendronate at both the lumbar spine and, notably, the

femoral neck at 6 and 12 months. This striking difference suggests that BMD degradation in LTR-GIOP may be pathologically more severe than in non-LTR-GIOP, acutely affecting even cortical bone integrity. Indeed, the reported 1-year femoral neck BMD reduction in rheumatoid patients (non-LTR-GIOP) was -1.40%[22], whereas it reached -2.2% in LTR-GIOP patients[5].

We hypothesize that the intensive nature of LTR-GIOP—high-dose pulse therapy (100 mg prednisolone/day for 5 days, repeated every 21 days)—may exert a more profound skeletal toxicity than previously assumed. This cyclical high-dose regimen may cause unexpectedly rapid and intense suppression of bone formation. For instance, high-dose methylprednisolone can reduce bone formation markers by over 50–80% within just two days[23]. Our findings suggest that in context of such profound bone-turnover suppression characteristic of LTR-GIOP, the potent anti-resorptive effect of denosumab provides a crucial advantage over alendronate in preserving both trabecular and cortical bone.

Denosumab is widely approved as an anti-osteoporosis agent, yet strong concerns persist among clinicians involved in treating multiple myeloma (MM) and solid tumors with bone metastases regarding adverse events such as hypocalcemia and ONJ. This apprehension arises because denosumab is used in these oncology settings at the "oncology dose" of 120 mg subcutaneously every 4 weeks, a regimen frequently associated with such toxicities. Indeed, according to a data integrated analysis of three major randomized, phase 3 trials involving patients with breast cancer, prostate cancer, other solid tumors, or MM, the incidence of severe (Grade 3 or 4) hypocalcemia in the denosumab arm was reported to be 3.1%, and the overall incidence of ONJ was reported as 1.8%[24].

However, the regimen adopted in the present study for LTR-GIOP prophylaxis is the standard dose for osteoporosis, which is 60 mg subcutaneously every 6 months. A key report demonstrating the safety of this "osteoporosis dose" involves a multicenter, double-blind study of men receiving androgen

deprivation therapy for prostate cancer[25]. The results showed that, in association with denosumab treatment, the incidence of Grade 2 hypocalcemia was less than 1%, and no cases of ONJ were reported.

In our phase 3 trial targeting LTR-GIOP, no serious adverse events of Grade ≥ 3 including either hypocalcemia or ONJ were observed in the denosumab group (Supplementary Table 2). This result clearly demonstrates that "osteoporosis dose" of denosumab used for LTR-GIOP prophylaxis can safely deliver the high BMD increase observed in our study. Therefore, excessive concern regarding severe adverse events should not be a barrier to the adoption of denosumab in the bone health management of lymphoma survivors.

The findings obtained in this study may provide important implications for existing guidelines and clinical practice recommendations regarding the treatment of GIOP. Many clinical guidelines, including those from the American College of Rheumatology (ACR)[26] and the Japanese Society for Bone and Mineral Research (JSBMR)[27], recommend bisphosphonates and denosumab as equivalent options for the prevention and treatment of GIOP. These recommendations are primarily based on evidence derived from patient populations where relatively low doses of steroids are administered over a long period, such as in rheumatoid arthritis or asthma.

However, in the treatment protocol for lymphoma, high doses of steroids, such as 100 mg of prednisolone per day, are administered to patients short-term and cyclically. This unique administration regimen may induce a bone metabolism pathology that differs from the conventional GIOP model. Therefore, a reexamination is necessary as to whether the aforementioned guidelines and clinical practice recommendations should be applied directly to LTR-GIOP. Indeed, previous meta-analyses reported no significant difference between the two agents regarding femoral neck BMD[19]. However, our study demonstrated the superiority of denosumab over alendronate in LTR-GIOP, not only at the lumbar spine but also at the femoral neck. The results of this study suggest that in the specific clinical scenario of lymphoma treatment, the two

therapeutic agents should not be recommended as equivalent, and that denosumab may be a superior choice. Therefore, existing guidelines might need to be amended or revised to include additional considerations for LTR-GIOP. We propose that future guidelines should explicitly recommend enhanced Intervention: Classifying R-CHOP recipients as a "very high risk" population, necessitating the consideration of powerful antiresorptive agents like denosumab, deviating from the typical stepwise approach often recommended for chronic low-dose GC users.

This study yielded important clinical insights into the comparison of denosumab and bisphosphonates in LTR-GIOP. However, several limitations must be considered regarding the generalizability of our findings. First, the number of patients examined in this study may result in insufficient statistical power. Furthermore, direct clinical outcomes, most importantly fractures, were not examined, which remains a primary limitation of this study. Second, a slightly higher tendency for dropout was observed in the bisphosphonate group than in the denosumab group. This difference in dropout rates cannot be ruled out as a potential factor influencing the final efficacy assessment between the two groups. Third, the bisphosphonate chosen for comparison in this study was only alendronate; while other more potent bisphosphonates, such as zoledronate, were not selected for comparison.

To overcome these limitations and validate the hypotheses derived from this study, a larger, long-term, multi-center randomized controlled trial targeting LTR-GIOP patients is necessary. Such a large-scale study should set fracture risk reduction as the primary endpoint and would require detailed evaluation of the long-term safety profile and cost-effectiveness of both agents. Furthermore, zoledronate would be an appropriate comparator for denosumab, as it is administered intravenously. This route of administration would allow for a more precise evaluation of efficacy by eliminating the concerns associated with poor adherence to oral medications—a common challenge in long-term GIOP prophylaxis.

In summary, this study represents the first clinical trial comparing denosumab and alendronate specifically in LTR-GIOP. Our results yielded the important finding that denosumab demonstrated a superior BMD-increasing effect compared to bisphosphonates, even at the femoral neck, a site typically considered less metabolically active. This phenomenon may be related to the high-dose, cyclical steroid administration characteristic of lymphoma treatment. Given these findings, denosumab may be particularly beneficial for patients with a low baseline BMD at the femoral neck, who are at a higher risk of hip fractures during or after intensive chemotherapy.

In conclusion, our data complement the recommendations of general GIOP practice guidelines. For this specific patient population—LTR-GIOP patients—denosumab should perhaps be more actively recommended over alendronate, suggesting that the individualization of intervention is crucial for the effective management of fracture risk in this group.

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Table 1. Characteristics of patients.

	Alendronate (n=48)	Denosumab (n=52)	*p* value
Age, median (range)	75 (64-87)	73 (62-86)	0.978
Male sex, n (%)	26 (54.2)	29 (55.8)	0.872
Type of lymphoma, n (%)			0.144
DLBCL	45 (93.8)	44 (84.6)	
Others	3 (6.2)	8 (15.4)	
FL	1	1	
PTCL	1	3	
AITL	1	1	
BL	0	1	
IVLBCL	0	2	
BMD (g/cm^2), mean (±SD)			
L2-4	1.14 (±0.30)	1.12 (±0.24)	0.654
L1-4	1.13 (±0.29)	1.11 (±0.21)	0.720
Total hip	0.84 (±0.14)	0.86 (±0.18)	0.480
Femoral neck	0.77 (±0.15)	0.78 (±0.20)	0.768
BTM, mean (±SD)			
TRACP-5b (mU/dL)	468 (±216)	412 (±165)	0.142
total P1NP (ng/mL)	53.1 (±30.3)	53.2 (±24.7)	0.977
Cumulative dose of PSL (mg)	2707 (±706)	2565 (±721)	0.386

BMD, bone mineral density; BTM, Bone turnover marker; PSL, prednisolone; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; PTCL, peripheral T-cell lymphoma; AITL, angioimmunoblastic T-cell lymphoma; BL, Burkitt lymphoma; IVLBCL, intravascular large B-cell lymphoma.

Table 2. Correlation analysis of BMD percent change at 12M.

	BMD at 12M (%)			
	L2-L4 (%)	L1-L4 (%)	Total hip (%)	Femoral neck (%)
Age (year)	-0.384†	-0.342†	-0.119	0.110
Total PSL (mg)	0.233	0.243	0.277	0.049
BMD at 0M (g/cm^2)				
L2-L4	-0.047	0.041	-0.306	-0.072
L1-L4	0.025	-0.014	-0.330	-0.324
Total hip	-0.122	-0.163	0.177	-0.074
Femoral neck	-0.154	-0.077	0.285	0.006
BTM at 12M				
TRACP-5b (%)	-0.190	-0.062	-0.059	-0.072
total P1NP (%)	-0.076	-0.086	-0.210	-0.094

†$p<0.05$

PSL, prednisolone; BMD, bone mineral density; BTM, bone turnover marker.

Table 3. Serious adverse events.

Event	Alendronate (n=48)		Denosumab (n=52)	
	Grade < 2 n (%)	Grade ≥ 3 n (%)	Grade < 2 n (%)	Grade ≥ 3 n (%)
Blood bilirubin increased	0 (0)	0 (0)	0 (0)	1 (1.9)
Neutrophil count decreased	0 (0)	1 (2.1)	0 (0)	1 (1.9)
Lung infection	0 (0)	2 (4.1)	0 (0)	1 (1.9)
Viremia	0 (0)	0 (0)	0 (0)	1 (1.9)
Biliary tract infection	0 (0)	0 (0)	0 (0)	1 (1.9)
Heart failure	0 (0)	1 (2.1)	0 (0)	0 (0)
Atrial fibrillation	1 (2.1)	0 (0)	0 (0)	0 (0)
Enterocolitis	0 (0)	1 (2.1)	0 (0)	0 (0)
Cholecystitis	0 (0)	1 (2.1)	0 (0)	0 (0)
Brain contusion	0 (0)	0 (0)	0 (0)	1 (1.9)
Adjustment disorder	0 (0)	1 (2.1)	0 (0)	0 (0)
Disease progression	0 (0)	2 (4.2)	0 (0)	1 (1.9)
Neoplasms	0 (0)	0 (0)	0 (0)	3 (5.8)
Total	1 (2.1)	9 (18.8)	0 (0)	10 (19.2)

LEGENDS

Figure 1. Patient disposition and study flow diagram.

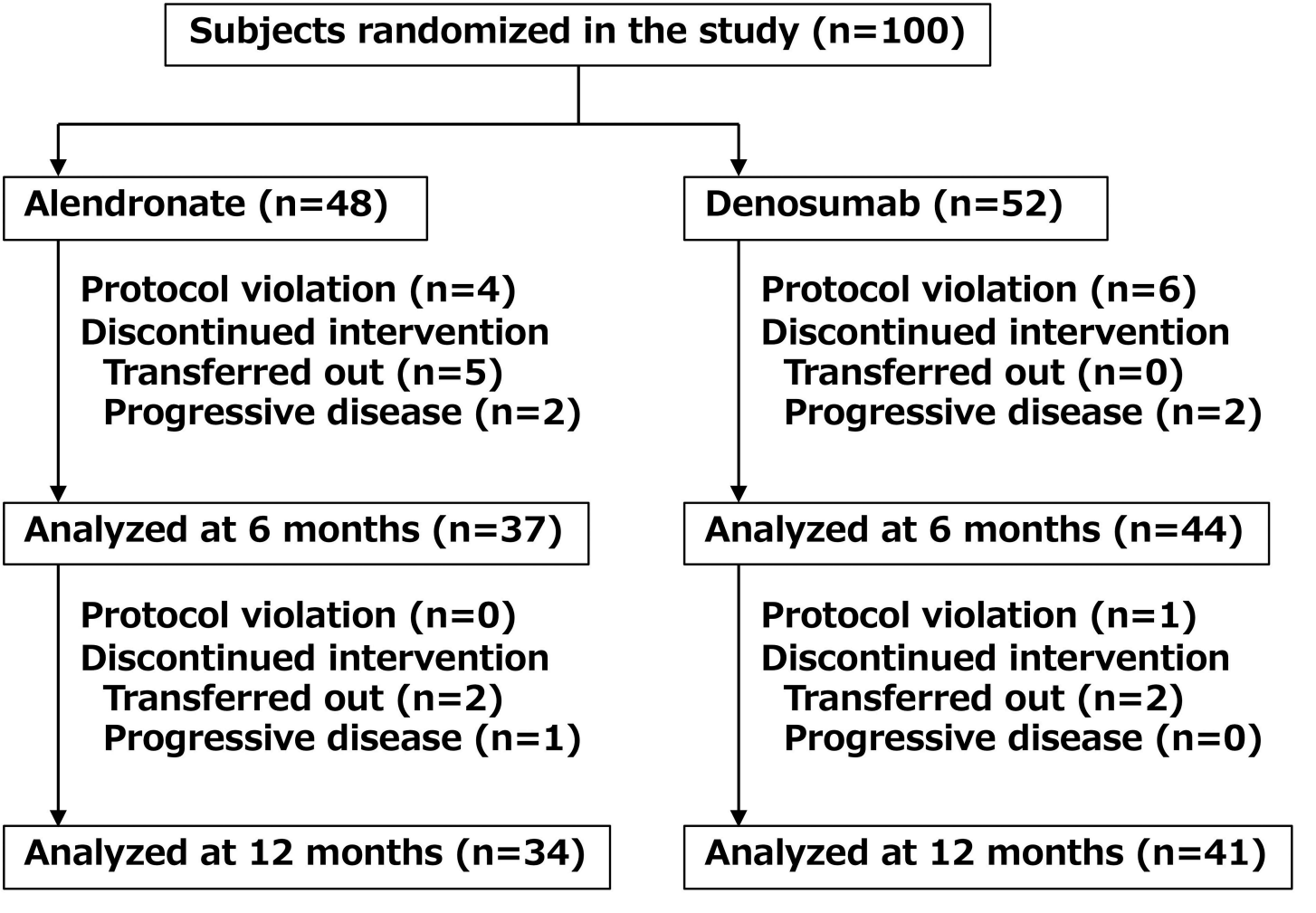
CONSORT flow diagram illustrating the enrollment, randomization, and follow-up of patients in the alendronate and denosumab groups.

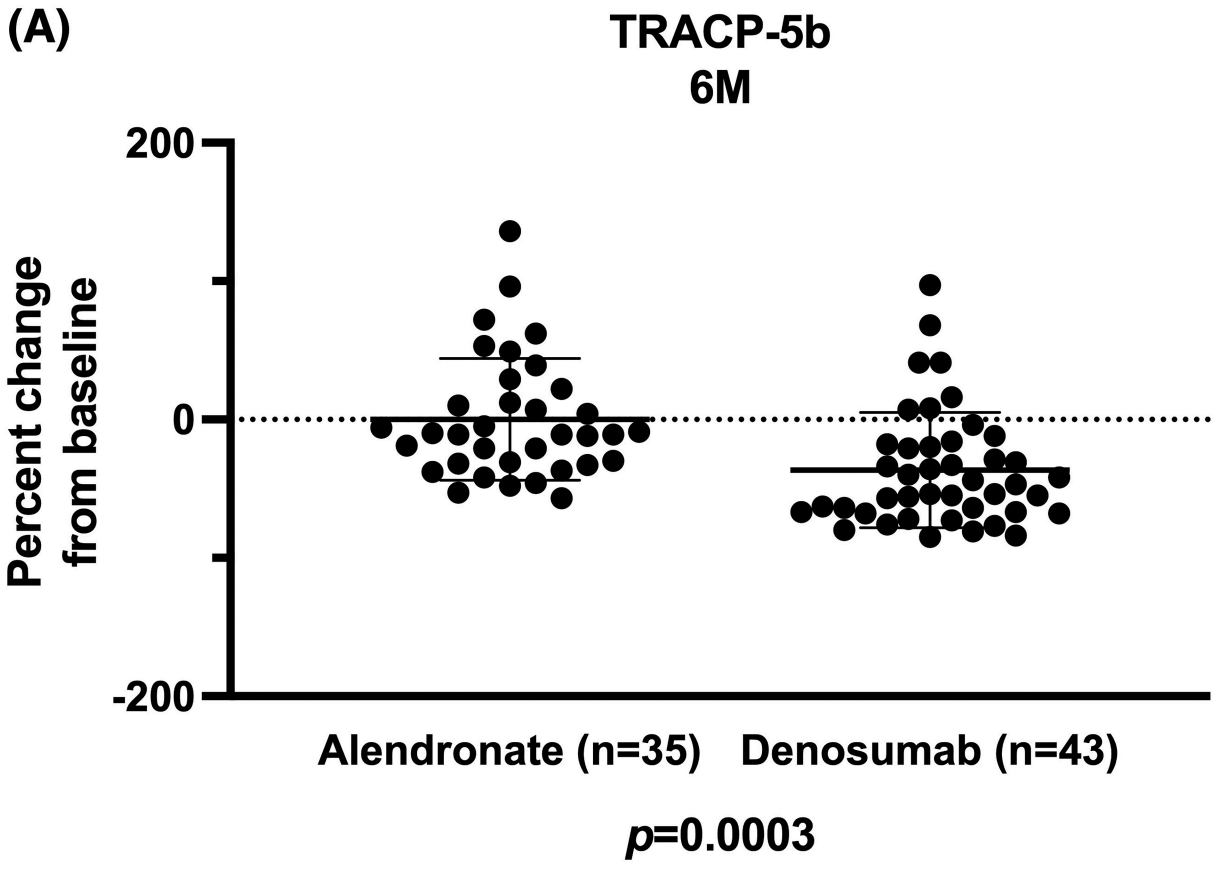
Figure 2. Comparison of percentage changes in bone turnover markers (BTMs) at 6 months.

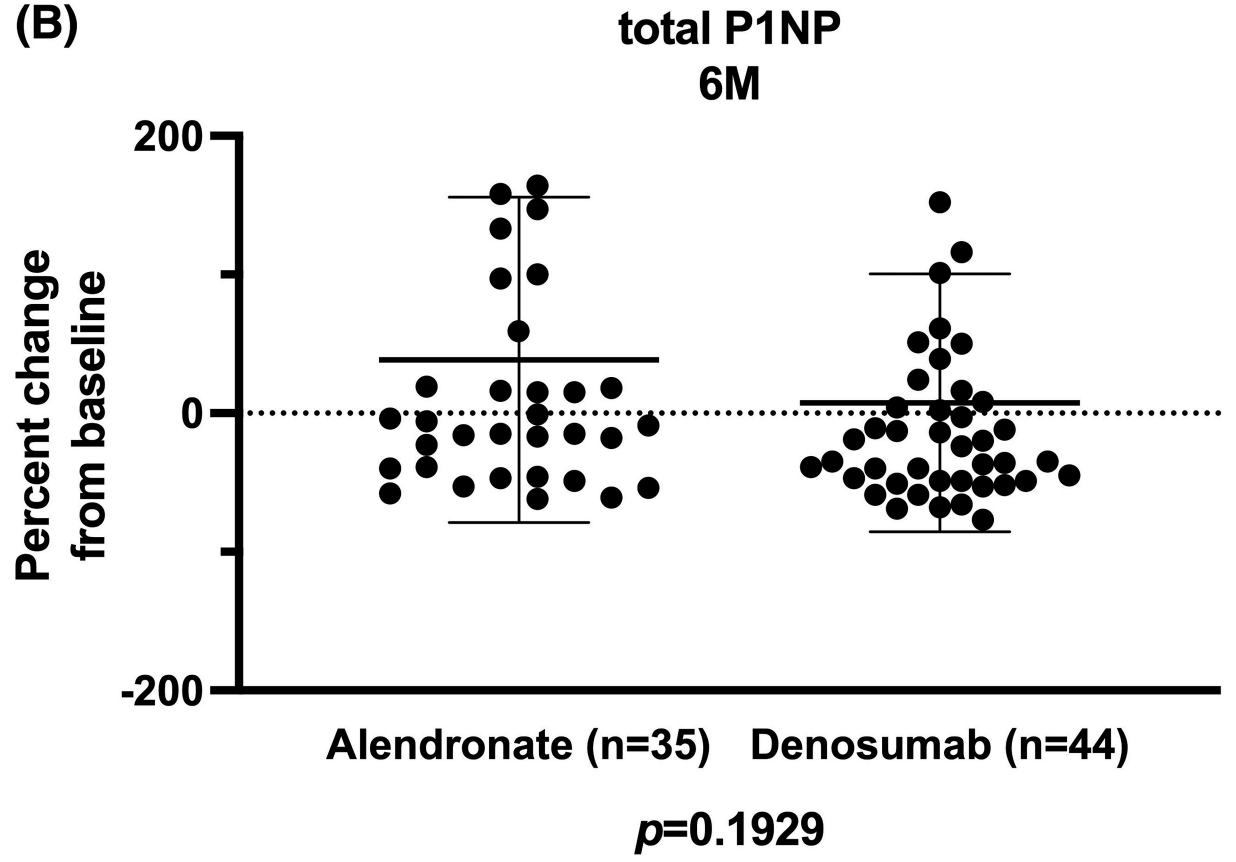
Percentage change from baseline in (A) TRACP-5b and (B) total P1NP between the alendronate and denosumab groups.

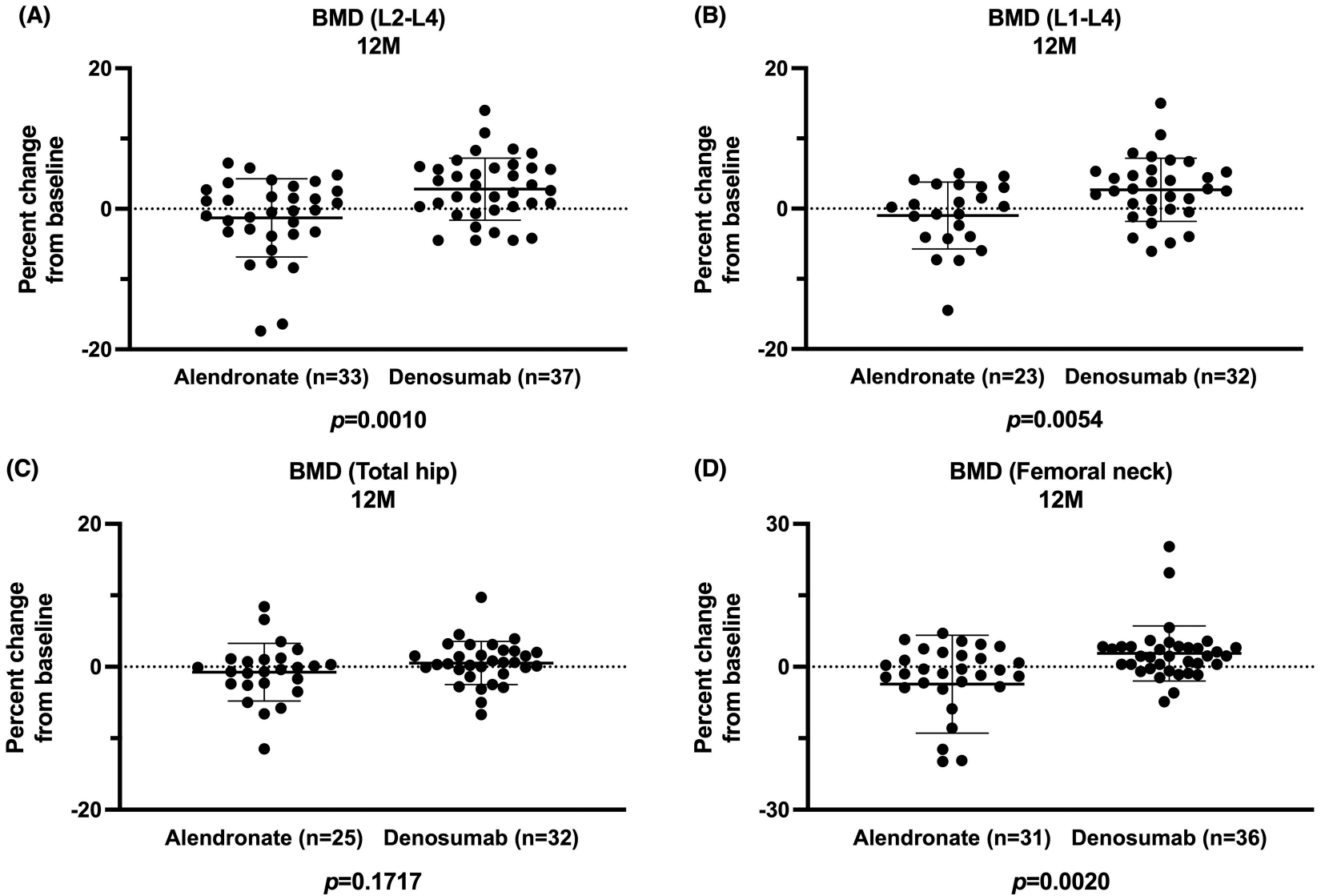
Figure 3. Comparison of percentage changes in bone mineral density (BMD) at 12 months.

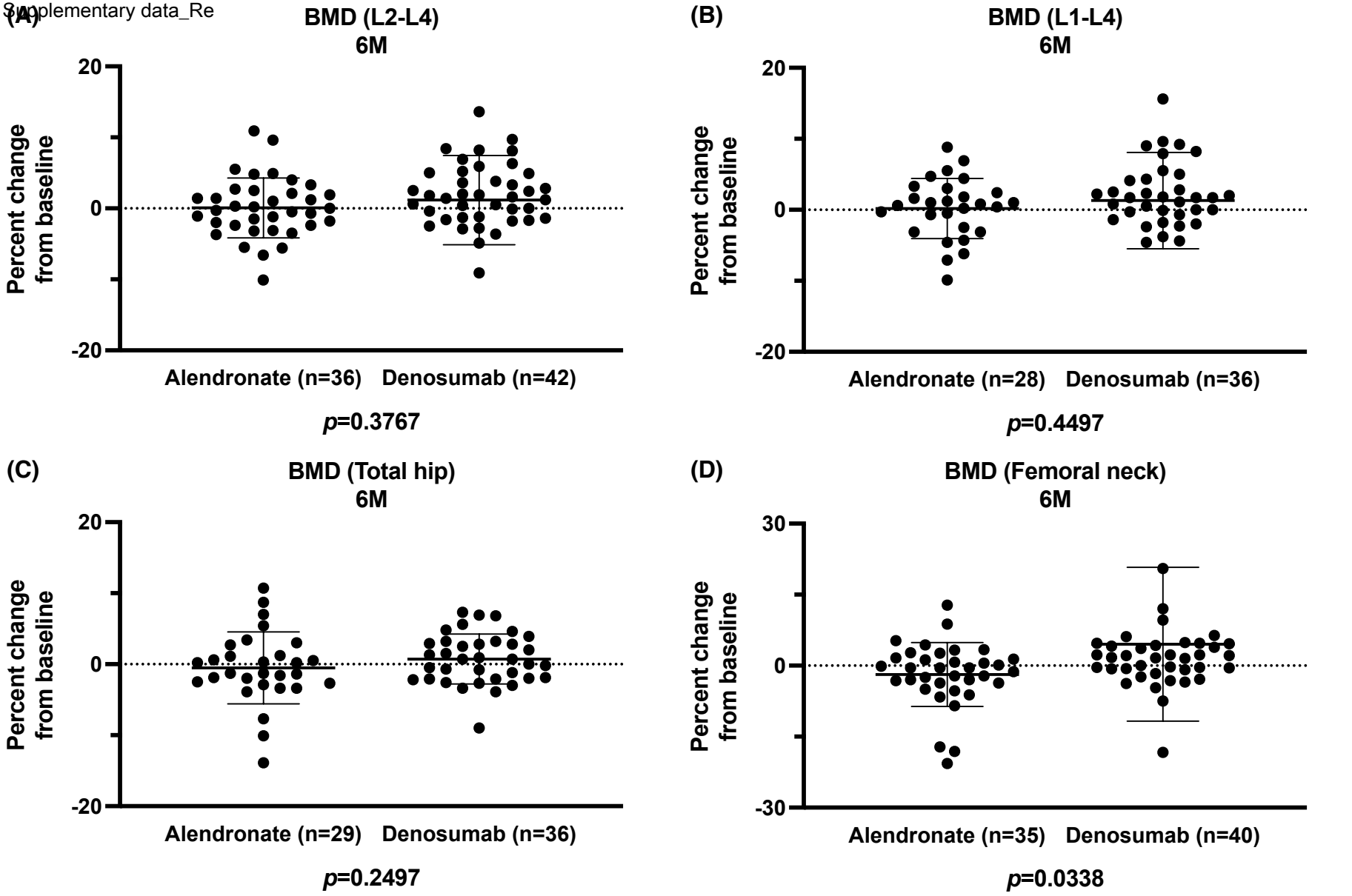
Percentage change from baseline between the alendronate and denosumab groups at (A) L2–L4; (B) L1–L4; (C) total hip; and (D) femoral neck.


Subjects randomized in the study (n=100)
Alendronate (n=48)
Denosumab (n=52)
Protocol violation (n=4)
Discontinued intervention
Transferred out (n=5)
Progressive disease (n=2)
Protocol violation (n=6)
Discontinued intervention
Transferred out (n=0)
Progressive disease (n=2)
Analyzed at 6 months (n=37)
Analyzed at 6 months (n=44)
Protocol violation (n=0)
Discontinued intervention
Transferred out (n=2)
Progressive disease (n=1)
Protocol violation (n=1)
Discontinued intervention
Transferred out (n=2)
Progressive disease (n=0)
Analyzed at 12 months (n=34)
Analyzed at 12 months (n=41)

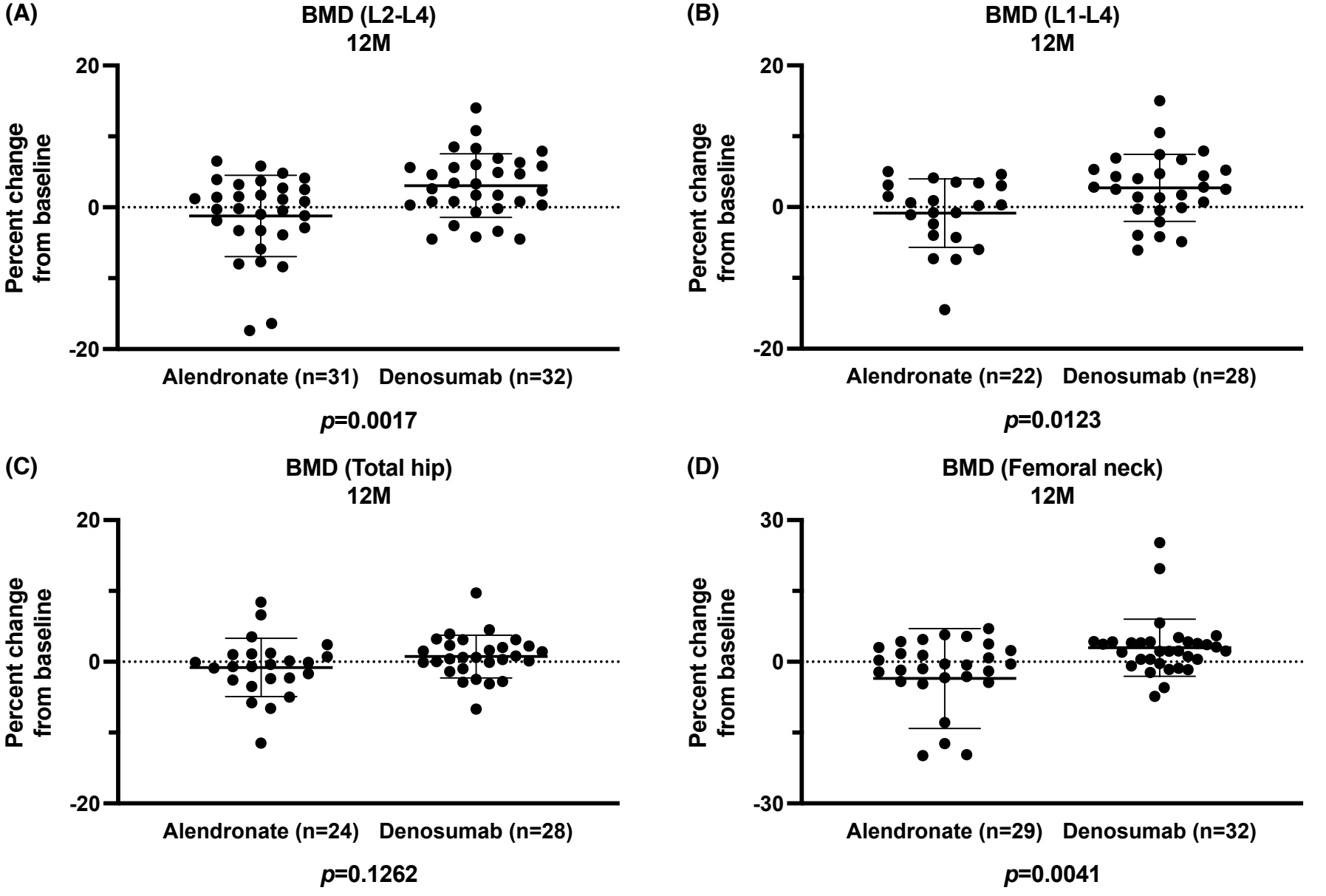

(A)
TRACP-5b
6M
200
0
-200
Percent change from baseline
Alendronate (n=35)
Denosumab (n=43)
p=0.0003


(B)
total P1NP
6M
200
0
-200
Percent change from baseline
Alendronate (n=35)
Denosumab (n=44)
p=0.1929


(A)
BMD (L2-L4)
12M
Percent change from baseline
20
0
-20
Alendronate (n=33)
Denosumab (n=37)
p=0.0010
(B)
BMD (L1-L4)
12M
Percent change from baseline
20
0
-20
Alendronate (n=23)
Denosumab (n=32)
p=0.0054
(C)
BMD (Total hip)
12M
Percent change from baseline
20
0
-20
Alendronate (n=25)
Denosumab (n=32)
p=0.1717
(D)
BMD (Femoral neck)
12M
Percent change from baseline
30
0
-30
Alendronate (n=31)
Denosumab (n=36)
p=0.0020

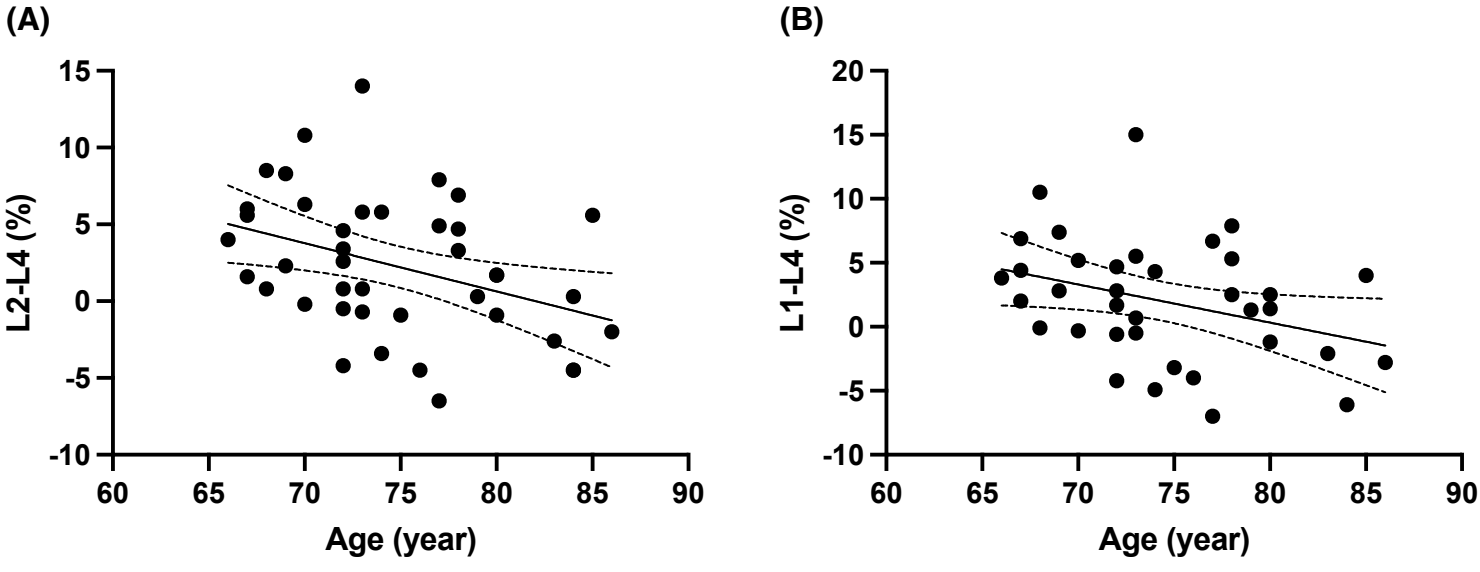


Supplementary Figure 1. Comparison of percentage changes in bone mineral density (BMD) at 6 months.
Percentage change from baseline between the alendronate and denosumab groups at (A) L2–L4; (B) L1–L4; (C) total hip; and (D) femoral neck.



Supplementary Figure 2. Subgroup analysis of percentage change in bone mineral density (BMD) in patients with DLBCL at 12 months.

Comparison between the alendronate and denosumab groups at (A) L2–L4; (B) L1–L4; (C) total hip; and (D) femoral neck.



Supplementary Figure 3. Correlation between patient age and percentage change in bone mineral density (BMD) at 12 months.

Spearman's correlation analysis evaluating the association between age and BMD change at (A) L2–L4 and (B) L1–L4.

Supplementary Table 1. Histology and regimen.

		Histology					
		DLBCL (n=89)	PTCL (n=4)	FL (n=2)	IVLBCL (n=2)	AITL (n=2)	BL (n=1)
Regimen	R-CHOP (+ RT)	67 (7)	0	1 (0)	0	0	0
	Pola-R-CHP (+ RT)	7 (1)	0	0	2 (0)	0	0
	R-CVP (+ RT)	6 (0)	0	0	0	0	0
	BV-CHP (+ RT)	0	2 (0)	0	0	1 (0)	0
	CHOP (+ RT)	0	2 (0)	0	0	0	0
	DA-EPOCH-R (+ RT)	0	0	0	0	0	1 (1)
	CVP (+ RT)	0	0	0	0	1 (0)	0
	None	9	0	1	0	0	0

DLBCL, diffuse large B-cell lymphoma; PTCL, peripheral T-cell lymphoma; FL, follicular lymphoma; IVLBCL, intravascular large B-cell lymphoma; AITL, angioimmunoblastic T-cell lymphoma; BL, Burkitt lymphoma. R, rituximab; Pola, polatuzumab vedotin; BV, brentuximab vedotin; DA, dose-adjusted; CHOP, cyclophosphamide + hydroxydaunorubicin + vincristine + prednisolone; CHP, cyclophosphamide + hydroxydaunorubicin + prednisolone; CVP, cyclophosphamide + vincristine + prednisolone; EPOCH, etoposide + prednisolone + vincristine + cyclophosphamide + hydroxydaunorubicin; RT, radiation therapy.

Supplementary Table 2. Adverse events of interest.

	Alendronate (n=48)	Denosumab (n=52)
Event	n (%)	n (%)
Hypocalcemia (Grade ≥ 3)	0 (0)	0 (0)
Skin infection (cellulitis) (Grade ≥ 3)	0 (0)	0 (0)
Eczema (Grade ≥ 3)	0 (0)	0 (0)
Anaphylaxis (Grade ≥ 3)	0 (0)	0 (0)
Secondary malignancy (Grade ≥ 3)	0 (0)	0 (0)
Hip fracture (atypical) (Grade ≥ 3)	0 (0)	0 (0)
Osteonecrosis of jaw (Grade ≥ 3)	0 (0)	0 (0)
Fracture healing complication	0 (0)	0 (0)

Supplementary Methods

Study design and objective

This study is a prospective, interventional, multi-center, open-label, randomized trial. Its objective is to compare the efficacy and safety of denosumab versus alendronate in newly diagnosed malignant lymphoma patients undergoing corticosteroid-containing chemotherapy. The primary endpoint was defined as the percentage change in lumbar spine BMD (comparison between baseline and 12 months post-treatment initiation). Secondary endpoints included the percentage change in lumbar spine BMD (comparison between baseline and 6 months), the percentage change in femoral BMD (comparison between baseline and 6 and 12 months), and the percentage change in Bone Turnover Marker (BTM) (comparison between baseline and 6 months). BMD was measured using Dual energy X-ray absorptiometry (DXA). Measurements were performed using Hologic (Bedford, MA, USA) or GE Lunar (Madison, WI, USA) densitometers at each participating center, with daily calibration performed using a standard phantom to ensure precision. The BTMs measured were Tartrate-resistant acid phosphatase 5b (TRACP-5b) and total type I procollagen N-terminal propeptide (total P1NP). The study protocol was approved by the Institutional Review Board of Toyama University Hospital (protocol no. R2019137) and monitored annually by the Data Safety Monitoring Committee of the Toyama University Hospital. All trial participants provided written informed consent. This study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; ID: UMIN000038881).

Patients

The inclusion criteria for participation in this study were as follows: 1) patients newly diagnosed with malignant lymphoma, 2) patients aged 65 years or older, 3) patients scheduled to receive steroid-containing chemotherapy, such as R-CHOP or similar regimens, and 4) patients who provided written informed

consent for study participation. Exclusion criteria included a history of hypersensitivity to denosumab, the presence of hypocalcemia, pregnant or possibly pregnant women, the presence of esophageal transit disorders, the inability to remain upright for 30 minutes or more, hypersensitivity to bisphosphonates, the presence of hypercalcemia or vitamin D intoxication, current or prior treatment for osteoporosis with denosumab or bisphosphonates, or any patient deemed ineligible by the study investigator.

Treatment

Patients were stratified by two factors: age (80 years or older) and sex and were randomly allocated to either the alendronate group or the denosumab group using a permuted block method. The denosumab group received a total of two subcutaneous injections of denosumab 60 mg, at the initiation of treatment and 6 months later. The alendronate group received oral alendronate 35 mg once weekly for 12 months starting from the initiation of treatment. Both groups received oral calcitriol preparation 0.25 μg once daily for 12 months starting from the initiation of treatment. Regarding the steroid protocol, in each cycle of R-CHOP-like therapy, patients typically received oral prednisolone (100 mg/body/day) for 5 days. However, intravenous administration of water-soluble prednisolone was permitted. Depending on the patient's clinical status, salvage chemotherapy or additional corticosteroid doses could be administered at the discretion of the investigator. Ultimately, all corticosteroids administered during the 1-year study period were converted into prednisolone equivalents and reported as the total cumulative dose.

Adverse events

Adverse events were considered "serious" if they met the following criteria: 1) resulted in death, 2) were life-threatening, 3) required inpatient hospitalization or prolongation of existing hospitalization, 4) resulted in persistent or significant disability/incapacity, or 5) resulted in a congenital anomaly or birth

defect in the offspring. For adverse events highly predicted to occur frequently in the target population due to chemotherapy for malignant lymphoma—such as myelosuppression, febrile neutropenia, gastrointestinal symptoms, hepatic or renal dysfunction, and electrolyte abnormalities—reporting was not required if they were not deemed serious. Conversely, the following items, which are considered characteristic adverse events of denosumab or alendronate, required reporting as "adverse events of interest" if they were Grade ≥3: 1) hypocalcemia, 2) skin infection (cellulitis), 3) eczema, 4) anaphylaxis, 5) secondary malignancy, 6) hip fracture (atypical), 7) osteonecrosis of the jaw (ONJ), and 8) fracture healing complication. For the assessment of calcium status, serum calcium levels were measured at the laboratory of each participating institution. Calcium values were corrected for hypoalbuminemia. The severity of hypocalcemia was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Rationale for target sample size

The sample size was determined by referencing a comparative study of denosumab and alendronate for steroid-induced osteoporosis in patients with nephritis. In that report, the percentage change in lumbar spine BMD when comparing baseline with 12 months post-treatment was +5.3% in the denosumab group. The standard error (SE) was 1.0%, the number of analyzed cases was 14, and the standard deviation (SD) was 3.7%. In the alendronate group, the percentage change was +2.0%, the SE was 1.2%, the number of analyzed cases was 14, and the SD was 4.5%. Therefore, for the current trial, we hypothesized a difference in the percentage change between the two groups of 3.0% and a SD of 5.0%. Performing a two-sided test with a significance level alpha of 0.05 and a statistical power of 80%, the required number of cases calculated using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) was 44 patients per group. Assuming a 12% dropout rate due to withdrawal of participation, disease progression, adverse events, or loss to

follow-up, the target sample size was set at 50 patients per group, totaling 100 patients.

Statistical methods

All statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, La Jolla, CA). All values are presented as mean ± SD. Statistical significance was determined using the student's t-test in the case of normally distributed data, otherwise the Mann-Whitney U test was performed. For comparisons of data from the same patient, the paired Student's t-test was used. The Fisher exact test was used to evaluate the association between two categorical variables. The Spearman correlation coefficient was used to estimate the correlation between two continuous variables. All tests were two-sided. p values < 0.05 were considered statistically significant.