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ORIGINAL

Denosumab prevents bone loss in newly diagnosed malignant lymphoma patients undergoing corticosteroid-containing chemotherapy: a prospective, non-randomized study

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ABSTRACT

Background: Malignant lymphoma patients have a high risk of bone mineral density (BMD) loss caused by corticosteroid-containing chemotherapy. Bisphosphonates have been used to prevent bone loss: however, little is known about effects of denosumab, a fully humanized monoclonal antibody inhibiting osteoclast-mediated bone resorption.

Methods: This clinical trial was conducted in newly diagnosed lymphoma patients undergoing corticosteroid-containing chemotherapy. BMD was evaluated at baseline, and patients with a lumbar spine T-score of ≤ -1 were subcutaneously administered once with 60 mg of denosumab ("Denosumab" group). Patients with a T-score > -1 were allocated to the "No treatment" group. BMD was reevaluated at 24 weeks after enrollment. Bone turnover markers (BTMs) were collected at 0, 2, and 24 weeks.

Results: Forty-three patients were enrolled (19 in the "Denosumab" group and 24 in the "No treatment" group). Patients in the "No treatment" group had decreased T-scores for the lumbar spine or femoral neck ($P < 0.0001$ or $P = 0.0029$, respectively) at 24 weeks after enrollment, whereas both T-scores were stable in the "Denosumab" group. Of the 18 patients in the "Denosumab" group, 12 had a T-score change from baseline (ΔT-score) of ≥ 0, whereas the remaining six patients had a ΔT-score < 0. These six patients had severely low T-scores at enrollment. Osteoclastic BTMs were strongly suppressed during the 24 weeks in the "Denosumab" group. The probability of major osteoporotic fracture or hip fracture in the "No treatment" group increased during the 24 weeks ($P = 0.0195$ or $P = 0.0289$, respectively), whereas pretreatment with denosumab prevented increased risks of both types of fractures.

Conclusions: Our data suggests that BMD screening at diagnosis of lymphoma should be considered so that the bone health of lymphoma survivors can be improved with denosumab.

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Key words: Lymphoma, Chemotherapy, Bone mineral density, Bone turnover markers, Denosumab

1 Background

Health promotion, including maintenance of bone health, is essential for the well-being cancer survivors, which are growing in number[1)]. Aging, natural menopause, and cancer treatments such as surgical oophorectomy, gonadotropin-releasing hormone (GnRH) agonists, chemotherapy-induced ovarian failure, androgen deprivation therapy (ADT), and aromatase inhibitors (AIs) all cause bone loss, which potentially increases the risk of osteoporosis and subsequent bone fractures[1)]. Although there are many recommendations for the improvement of bone health in cancer survivors, most of these guidelines address women with breast cancer receiving AIs or GnRH agonists and men with prostate cancer receiving ADT[2-4)].

Prostate cancer (43%) and breast cancer (41%) are the most common cancers among male and female cancer survivors, respectively[5)]. Therefore, bone health of these cancer survivors is a matter of high concern.

Meanwhile, non-Hodgkin's lymphoma (NHL) is the fifth (4%) and sixth (4%) most common among male and female cancer survivors, respectively, with 569,820 NHL survivors living in the United States in 2014[5].

We may need to pay more attention to the bone health of NHL patients, as compared to other cancer patients, because the standard chemotherapy regimens for NHL include the use of corticosteroids, which are known to increase the risk of osteoporosis and fractures[6]. High-dose corticosteroids and alkylating agents such as cyclophosphamide, which often cause gonadal dysfunction, are thought to be the major causes of osteoporosis in patients with malignant lymphoma[7]. In fact, a previous study demonstrated that the use of chemotherapy is associated with significantly increased risk of fracture and osteoporosis in elderly patients with NHL[6]. Similarly, another study showed that adult lymphoma patients receiving chemotherapy experienced osteoporotic fractures and significant bone mineral density (BMD) loss in the lumbar spine and proximal femur[8].

Bisphosphonates, which are effective inhibitors of osteoclastic bone resorption, have been used to prevent osteoporosis caused by cancer treatment in lymphoma patients[9,10]. The second-generation bisphosphonate, pamidronate, reduces trabecular bone loss and thereby the risk of new vertebral fractures in patients with malignant lymphomas receiving chemotherapy[9]. Treatment with zoledronic acid, a third-generation bisphosphonate, effectively stabilizes BMD and prevents bone loss in patients newly diagnosed with lymphoma receiving chemotherapy[10].

A novel approach to fracture prevention is the use of denosumab[11]. It is a fully humanized monoclonal antibody against the receptor activator of nuclear factor-κB ligand (RANKL), a cytokine essential for the formation, function, and survival of osteoclasts[12]. By binding RANKL, denosumab prevents interaction with its receptor, RANK, on the surface of osteoclasts and osteoclast precursors and reversibly inhibits osteoclast-mediated bone resorption[13]. Denosumab, administered subcutaneously at a dose of 60 mg every six months, has been shown to increase BMD of the lumbar spine and total hip and reduce the incidence of new vertebral, nonvertebral, and hip fractures in postmenopausal women with osteoporosis[11].

Denosumab has been associated with increased BMD at all sites and a reduction in the incidence of new vertebral fractures among men receiving ADT for nonmetastatic prostate cancer[14]. Adjuvant denosumab, administered at 60 mg doses twice a year, reduced the risk of clinical fractures in postmenopausal women with breast cancer receiving AIs[15]. Extending the application of denosumab to lymphoma patients was suggested[16]; however, little is known about the effects of denosumab on bone loss caused by cancer treatment in lymphoma patients.

Therefore, we investigated the effect of denosumab on the prevention of bone loss in newly diagnosed malignant lymphoma patients undergoing corticosteroid-containing chemotherapy in this study. Random allocation was not performed because it was considered unethical. In a previous report[17], denosumab improved BMD in women with osteoporosis with long-term glucocorticoid treatment of autoimmune or inflammatory conditions. Further, it has been demonstrated that denosumab is effective for fracture prevention in patients with prostate[14] or breast[15] cancer.

2 Methods

2・1 Study design and objective

The aim of this single-center, open-label, non-randomized, controlled clinical trial is to evaluate the efficacy and safety of denosumab for prevention of bone loss in newly diagnosed malignant lymphoma patients undergoing corticosteroid-containing chemotherapy. The primary endpoint was a change in BMD and bone turnover markers (BTMs). Secondary endpoints included the incidence rate of vertebral fractures and adverse events (AEs).

2・2 Patients

Eligible patients were age between 20 and 90 years with newly diagnosed malignant lymphoma and were to undergo chemotherapy containing corticosteroids. Other eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0-2, and an expected survival of more than three months. Adequate pulmonary, cardiac, renal, and hepatic function was required. Exclusion criteria included previous use of bisphosphonate; active concomitant malignancy; severe psychiatric disorders; women who are pregnant or in lactation; hypersensitivity to denosumab; currently active dental problems; and unexplained hypocalcemia.

2・3 Treatment

Newly diagnosed malignant lymphoma patients

were divided into two groups based on the level of their T-scores, which was the BMD measured by dual-energy X-ray absorptiometry (DXA). Patients with a T-score of ≤ -1.0 for the lumbar spine were subcutaneously administered a single 60 mg dose of denosumab one day before the start of corticosteroid-containing chemotherapy. All patients who received denosumab were also administered an oral nutritional supplement containing at least 600 mg calcium and 400 IU vitamin D (denotas®), daily, throughout the study period. Patients with a T-score of > -1.0 for the lumbar spine underwent corticosteroid-containing chemotherapy without denosumab.

2・4 Bone mineral density and bone turnover markers

We measured BMD of the lumbar spine (L2 to L4) and the femoral neck using DXA (Hologic QDR-4500A; Bedford, MA) at baseline and 24 weeks after the administration of denosumab. Concentrations of the following BTMs were measured from fasting serum samples collected in the morning at baseline, 2, and 24 weeks after the administration of denosumab: tartrate-resistant acid phosphatase 5b (TRACP-5b), serum type I collagen cross-linked N-telopeptide (sNTx), intact procollagen type I N-terminal propeptide (I-PINP), osteocalcin (OC), and bone alkaline phosphatase (BAP). BTMs were evaluated by the central laboratory, SRL (Tokyo, Japan). TRACP-5b was analyzed by the enzyme immunoassay (EIA), sNTx by the enzyme-linked immunosorbent assay (ELISA), I-PINP and OC by the radioimmunoassay (RIA), and BAP by the chemiluminescent enzyme immunoassay (CLEIA).

2・5 Fracture Assessment

Lateral thoracic and lumbar spine radiographs were obtained at baseline and 24 weeks after the administration of denosumab. Prevalent vertebral fractures were diagnosed using standard criteria[18]. The fracture risk was assessed using the online WHO Fracture Risk Assessment Tool, FRAX® at http://www.shef.ac.uk/FRAX/tool.jsp, as described previously[18]. In brief, completion of the following 12 fields was required: age (years); sex (male or female); height (cm); weight (kg); history of previous fracture; history of parental hip fracture; current smoking; oral glucocorticoids exposure (more than three months at a dose of 5 mg of prednisolone daily or more); diagnosis of rheumatoid arthritis; secondary osteoporosis; daily alcohol intake of more than three units; and femoral neck DXA score (g/cm^2).

2・6 Adverse events

All patients were questioned concerning AEs at each visit. All AEs were assessed, regardless of determination of causality by the investigators. Safety laboratory tests including serum chemistry and hematology were assessed three times a week for three weeks after the administration of denosumab. An AE was considered "serious" if it resulted in any of the following outcomes: death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or a substantial disruption in the ability to conduct normal life functions, or a congenital anomaly or birth defect[17]. We prespecified AEs of interests, such as hypocalcemia, bacterial cellulitis, infection, eczema, events potentially related to hypersensitivity, cardiovascular disorder, malignant or unspecified tumors, fracture healing complication, atypical fracture of femur, and osteonecrosis of the jaw, as previously described[19]. This was based on the Food and Drug Administration (FDA) safety analysis, announced at a meeting of the Advisory Committee for Reproductive Health Drugs on August 13, 2009[17], which identified the following as "AEs of special interest" for denosumab: infection, new malignancy, tumor progression, dermatologic events, hypocalcemia, osteonecrosis of the jaw, bone histomorphometry findings, hypersensitivity/immunogenicity, cardiovascular adverse events, pancreatitis, and ocular adverse events.

2・7 Statistical methods

All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA). All values are presented as mean ± standard error of the mean (SEM). 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.

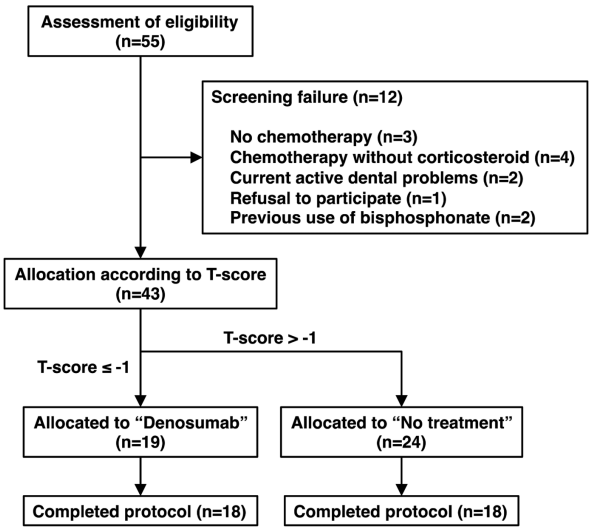

Figure 1. Enrollment, allocation, and follow-up of the study patients.

3 Results

3・1 Patient enrollment

Fifty-five patients with newly diagnosed malignant lymphomas seen at our department from October 2013 until September 2015 were screened for eligibility to enter this trial (Fig. 1). Twelve patients were excluded for the following reasons: no chemotherapy undertaken (N = 3); chemotherapy without corticosteroids undertaken (N = 4); currently active dental problems (N = 2); refusal to participate (N = 1); and previous use of bisphosphonate (N = 2). Finally, 43 patients consented for this study. Nineteen patients with T-scores of ≤ -1.0 for the lumbar spine were treated with denosumab before chemotherapy containing corticosteroids ("Denosumab" group). Meanwhile, 24 patients with T-scores of > -1 for the lumbar spine underwent corticosteroid-containing chemotherapy without pretreatment with denosumab ("No treatment" group). The data cut-off date for this analysis was March 27, 2016. Eighteen patients in the "Denosumab" group and 18 patients in the "No treatment" group completed the protocol.

Patient demographics and baseline characteristics are described in Table 1. Both, T-scores and BMDs, for lumbar spines and femoral necks of patients in the "No treatment" group were higher than thoses of "Denosumab" patients because patients with lumbar spine T-scores of > -1.0 were allocated to the "No treatment" group. The male/female ratio in the "No treatment" group was higher than that in the "Denosumab" group. The "No treatment" and "Denosumab" groups had similar demographics in terms of age, type of lymphoma, and chemotherapy regimen.

We examined the following five BTMs at baseline (Table 1): TRACP-5b, sNTx, I-PINP, OC, and BAP. TRACP-5b and sNTx are BTMs for osteoclastic bone resorption[20]. The former is a surrogate marker of osteoclast number and the latter is that of osteoclast function. I-PINP, OC, and BAP are BTMs of osteoblastic bone formation[21]. Although there were no differences between the two groups in the serum levels of TRACP-5b, sNTx, I-PINP, and BAP, OC of patients

Table 1. Characteristics of patients

	No treatment (n=24)	Denosumab (n=19)	P value
T-score, mean (±SD)			
Lumbar spine (L1-L4)	0.6 (±1.4)	-1.9 (±0.7)	<0.0001
Femoral neck	-0.3 (±0.8)	-1.5 (±0.9)	<0.0001
BMD (g/cm^2), mean (±SD)			
Lumbar spine (L1-L4)	1.12 (±0.19)	0.80 (±0.08)	<0.0001
Femoral neck	0.90 (±0.12)	0.71 (±0.10)	<0.0001
Age, median (range)	66 (20-86)	66 (54-81)	0.1459
Male sex, n (%)	16 (66.7)	3 (15.8)	0.0016
Type of lymphoma, n (%)			
DLBCL	16 (66.7)	14 (73.7)	0.7433
Others	8 (33.3)	5 (26.3)	
Chemotherapy, n (%)			
R-CHOP-like regimen	22 (91.7)	17 (89.5)	1.0000
Others	2 (8.3)	2 (10.5)	
BTM, mean (±SD)			
TRACP-5b (mU/dL)	516 (±304)	526 (±266)	0.9032
sNTx (nmol BCE/L)	20.1 (±6.7)	24.0 (±9.9)	0.1312
I-PINP (μg/L)	36.1 (±14.3)	43.6 (±16.9)	0.1348
OC (ng/mL)	5.0 (±2.4)	7.2 (±2.0)	0.0034
BAP (μg/L)	13.8 (±3.7)	13.2 (±3.4)	0.5586

in the "No treatment" group was significantly lower than that of patients in the "Denosumab" group (P = 0.0034). Furthermore, the serum level of OC in all patients was negatively correlated with T-scores for the lumbar spine (P = 0.0090).

3・2 Bone mineral density

As shown in Fig. 2, T-scores of lumbar spines or femoral necks of patients in the "No treatment" group decreased during the 24 weeks of corticosteroid-containing chemotherapy (P < 0.0001 or P = 0.0029, respectively). Pretreatment with denosumab, yielded sustained T- scores throughout the 24 weeks. Upon the assessment of BMDs (Fig. 3), almost the same data as that displayed in Fig. 2 was obtained: BMD decreased during corticosteroid-containing chemotherapy, however, the pretreatment of denosumab sustained BMD.

Further analysis revealed that the lumbar spine

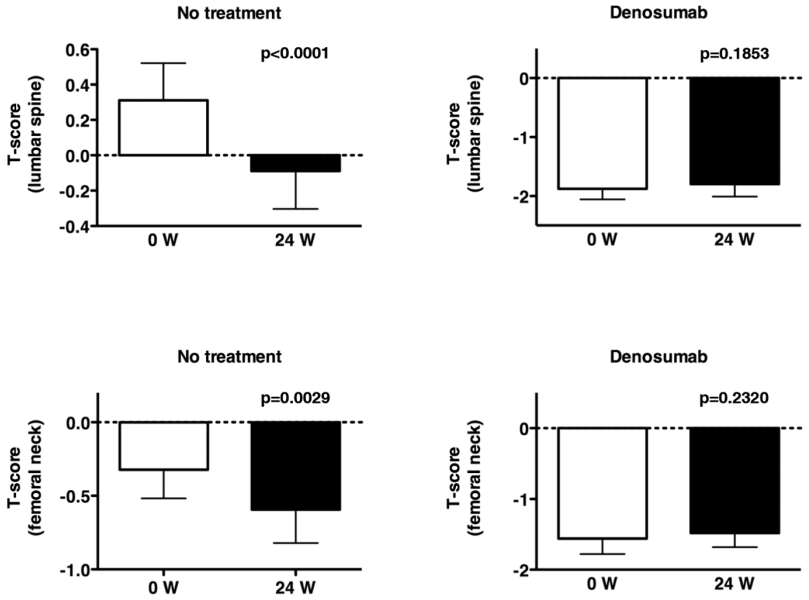

Figure 2. T-score at baseline and 24 weeks after enrollment. T-score at baseline (0 W) and 24 weeks after enrollment (24 W) of lumbar spine and femoral neck in the "No treatment" or "Denosumab" groups are shown.

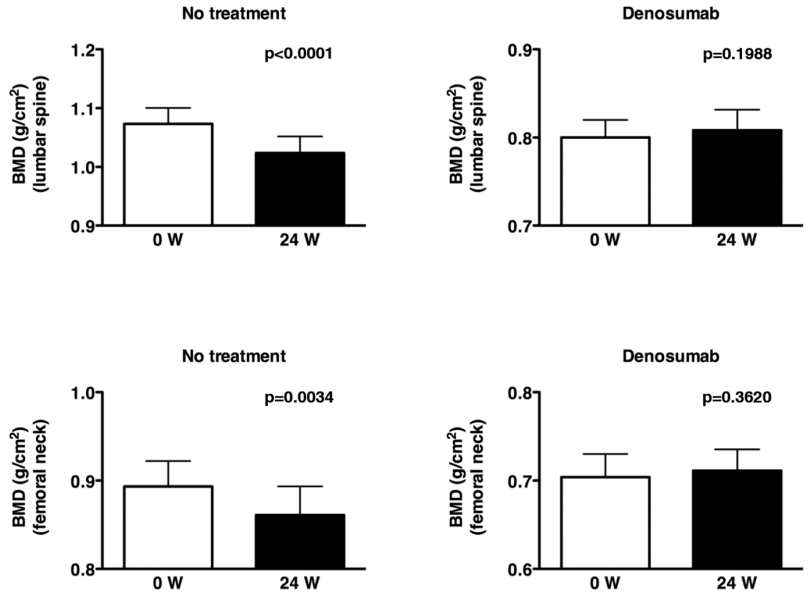

Figure 3. BMD at baseline and 24 weeks after enrollment. BMD at baseline (0 W) and 24 weeks after enrollment (24 W) of lumbar spine and femoral neck in the "No treatment" and "Denosumab" groups are shown.

and femoral neck T-score changes from the baseline (ΔT-score) for patients in the "Denosumab" group was higher than that for "No treatment" patients (P < 0.0001 or P = 0.0015, respectively) (Fig. 4). Upon assessment of the percentage BMD change from baseline (ΔBMD), patients in the "Denosumab" group had higher ΔBMD than those in the "No treatment" group for the lumbar spine (P < 0.0001) and the femoral neck (P = 0.0023).

Of the 18 patients treated with denosumab, 12 had ΔT-scores ≥ 0, whereas the remaining six had ΔT-scores < 0 for the lumbar spine (Fig. 4a). Then, we analyzed the possible factors affecting T-score changes (Table 2). Comparing the two groups, 12 patients with a ΔT-score ≥ 0 had higher lumbar spine T-scores or BMDs before the start of chemotherapy than that of the six patients with a ΔT-score < 0 (P = 0.0214 or P = 0.0168, respectively). Therefore, denosumab could not easily increase the T-scores of patients with severely lowered T-scores or BMDs before chemotherapy.

Next, we analyzed whether corticosteroids dose-dependently decreased bone mineral. There was no correlation between the cumulative dose of corticosteroids and the ΔT-score or ΔBMD in the

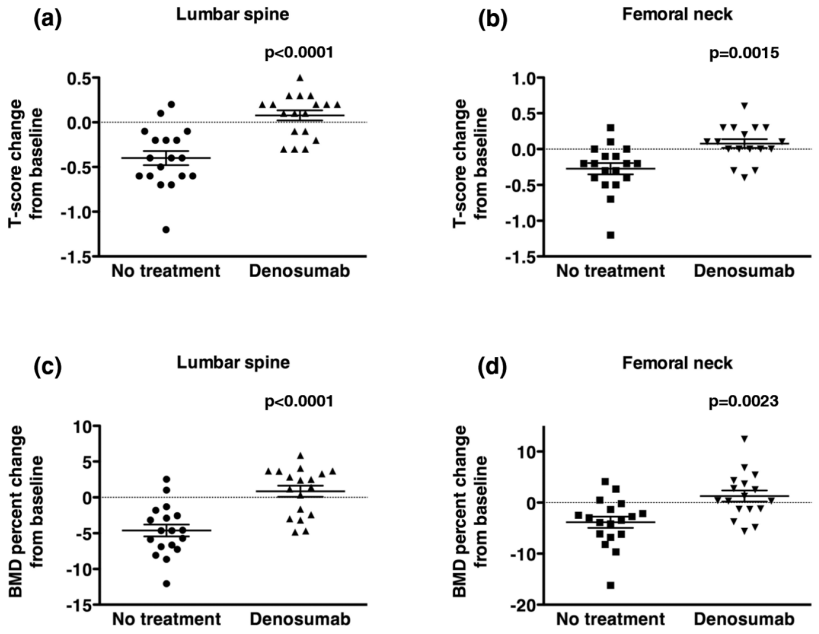

Figure 4. T-score change and BMD percent change from baseline. T-score change and BMD percent change from baseline in the "No treatment" and "Denosumab" groups of lumbar spine and femoral neck are shown.

Table 2. Factors affecting T-score change of lumbar spine in patients treated with denosumab

	ΔT-score≥0 (n=12)	ΔT-score<0 (n=6)	P value
T-score, mean (±SD)			
Lumbar spine (L1-L4)	-1.7 (±0.4)	-2.5 (±1.0)	0.0214
Femoral neck	-1.0 (±0.9)	-2.1 (±0.7)	0.0569
BMD (g/cm^2), mean (±SD)			
Lumbar spine (L1-L4)	0.83 (±0.04)	0.72 (±0.11)	0.0168
Femoral neck	0.76 (±0.11)	0.65 (±0.07)	0.0693
Age, median (range)	67 (54-81)	68 (56-75)	0.7944
BTM, mean (±SD)			
TRACP-5b (mU/dL)	446 (±312)	454 (±201)	0.9038
sNTx (nmol BCE/L)	23.2 (±10.0)	19.8 (±11.6)	0.9800
I-PINP (μg/L)	37.6 (±15.6)	45.0 (±18.2)	0.2940
OC (ng/mL)	7.4 (±2.1)	8.1 (±2.2)	0.8492
BAP (μg/L)	12.4 (±3.6)	13.0 (±3.4)	0.7674
Cumulative dose of PSL (mg)	1750 (±1100)	1500 (±1084)	0.6595
Cumulative dose of G-CSF (μg)	7725 (±5525)	8250 (±3706)	0.5776

"No treatment" group (P = 0.2596 or P = 0.2209, respectively). These results indicate that corticosteroids could decrease bone mineral, even if the cumulative corticosteroid dose was not very high. We further analyzed granulocyte-colony stimulating factor (G-CSF), which was administered for the prevention of febrile neutropenia, since Asada N et al. demonstrated in a previous paper that G-CSF suppressed osteoblasts[22]. However, there was no relationship between the cumulative dose of G-CSF and the ΔT-score or ΔBMD for the lumbar spine in the "No treatment" group (P = 0.4052 or P = 0.4895, respectively).

3・3 Bone turnover marker

Two BTMs of osteoclasts, TRACP-5b and sNTx, were clearly suppressed two weeks after the administration of denosumab (P < 0.0001 and P < 0.0001, respectively) (Fig. 5). This suppression was sustained until 24 weeks after denosumab administration (P < 0.0001 and P < 0.0001, respectively). In contrast, TRACP-5b and sNTx did not fluctuate in patients not pretreated with denosumab.

Next, three BTMs of osteoblasts, I-PINP, OC, and BAP, were analyzed (Fig. 6). I-PINP was suppressed after two weeks in both the "No treatment" and "Denosumab" groups, perhaps because of the administration of corticosteroids (P = 0.0036 or P < 0.0001, respectively). After 24 weeks, I-PINP was elevated reactively in the "No treatment" group (P = 0.0005); however, it was still suppressed in the "Denosumab" group (P = 0.0014). Assessment of other BTMs for osteoblasts revealed that OC and BAP exhibited the same trend.

3・4 Vertebral fracture

New vertebral fractures were identified by comparing standard x-rays from week 0 with that from week 24. Of the 18 patients (16.7%) in the "No treatment" group, three had new fractures, whereas no patients had new fractures in the "Denosumab" group. However, the differences between the two groups were not statistically significant (P = 0.2286), possibly because of small sample size. Further, we assessed the 10-year probability of fracture using FRAX algorithms (Fig. 7). The probability of major osteoporotic (OP) fracture (of clinical spine, forearm, hip, or shoulder), or hip fracture of patients from the "No treatment" group, increased during the 24 weeks (P = 0.0195 or P = 0.0289, respectively), whereas pretreatment with denosumab prevented the increase in risk of both fractures in patients.

3・5 Adverse events (AEs)

AEs of interest are shown in Table 3. In the "No treatment" group, there was one patient with eczema.

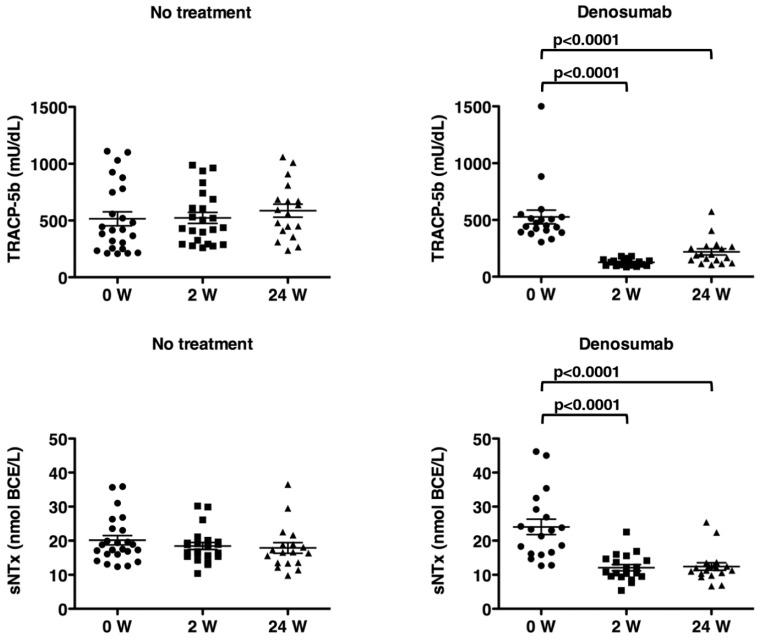

Figure 5. BTMs of osteoclastic bone resorption. TRACP-5b and sNTx at baseline (0 W), 2 weeks or 24 weeks after enrollment (2 W or 24 W) in the "No treatment" and "Denosumab" groups are shown.

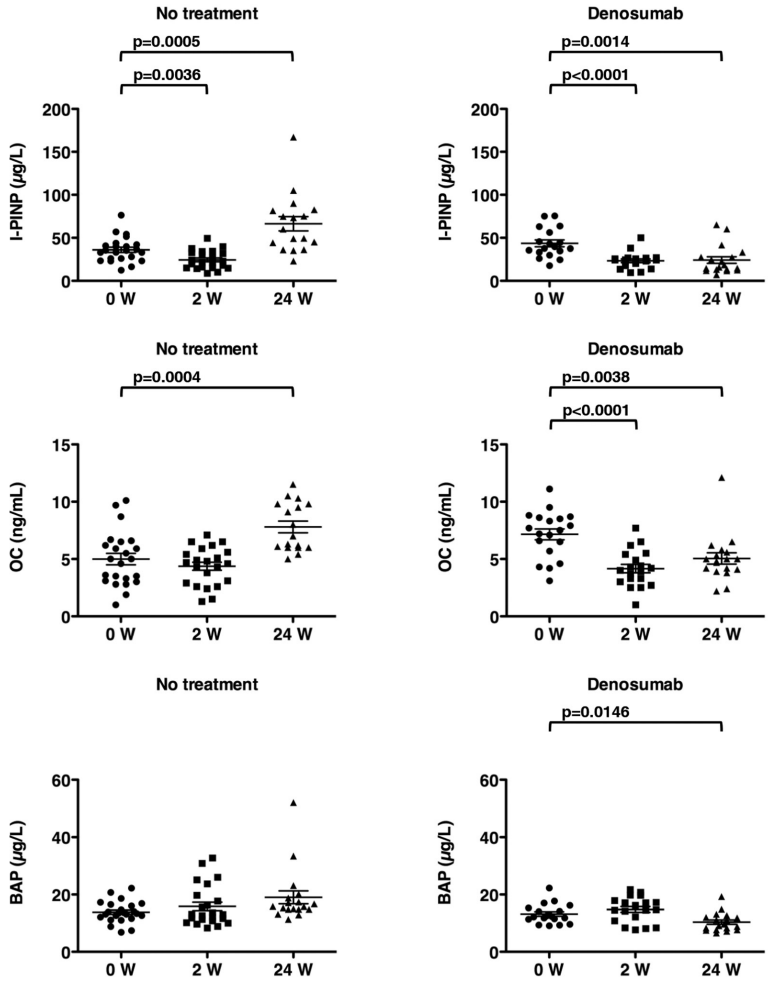

Figure 6. BTMs of osteoblastic bone formation. I-PINP, OC and BAP at baseline (0 W), 2 weeks or 24 weeks after enrollment (2 W or 24 W) in the "No treatment" and "Denosumab" groups are shown.

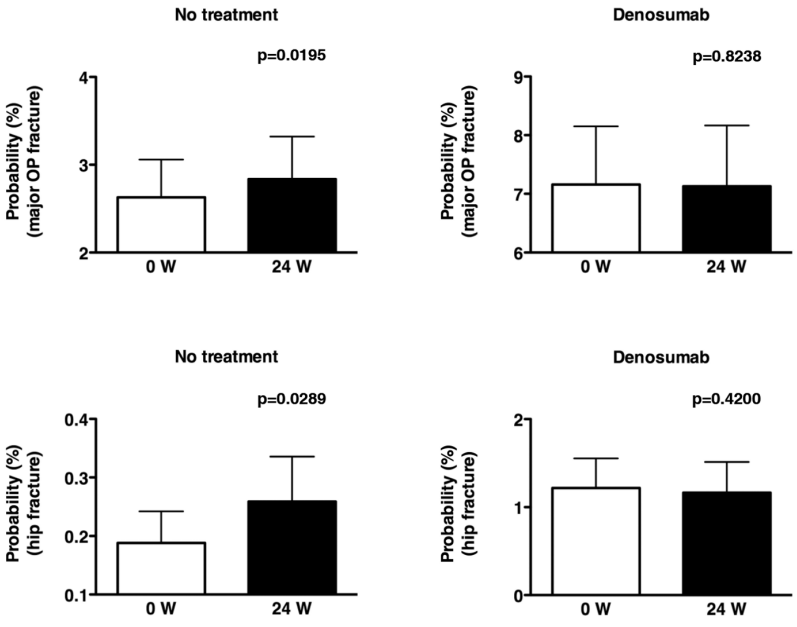

Figure 7. Probability of major OP fracture and hip fracture. Probability of major OP fracture and hip fracture at baseline (0 W) and 24 weeks after enrollment (24 W) in the "No treatment" and "Denosumab" groups are shown.

Table 3. Adverse events of interest

	No treatment (n=24)	Denosumab (n=19)
Osteonecrosis of the jaw	0	0
Hypocalcemia	0	2
Hypersensitivity	0	0
Atypical femoral fracture	0	0
Bacterial cellulitis	0	0
Eczema	1	0
cardiovascular disorder	0	1
infection	0	0
malignant or unspecified tumors	0	0
fracture healing complication	0	0

In the "Denosumab" group, there were two patients with hypocalcemia and one with cardiovascular disorder (hypertension). These AEs were not considered "serious". Osteonecrosis of the jaw was not observed in patients from either group. Two patients experienced hypocalcemia with calcium levels of > 7 and < 8 mg/dL on days 4 and 9, respectively. They had lower calcium levels on day 0 compared with that of other patients. Spontaneous recovery from hypocalcemia was achieved without any additional treatment.

4 Discussion

After the screening for eligibility to enter this trial, patients were allocated to one of two groups based on their lumbar spine T-scores. This study was designed as a non-randomized trial because it would be unethical to allocate patients with osteoporosis to the "No treatment" group. Especially because it has been previously demonstrated that denosumab is effective for fracture prevention in patients with prostate[14] or breast[15] cancer.

Patients with low BMD were allocated to the "Denosumab" group, making the "Denosumab" group female-dominated. In this group, OC, one of the osteoblastic bone formation markers, was higher than that in the "No treatment" group at the time of enrollment (Table 1). A possible explanation for this observation is that free osteocalcin is available in the blood when the bone mineralization rate is low since osteocalcin influences bone mineralization, in part, through its ability to bind with high affinity to the mineral component of bones, hydroxyapatite[23, 24]. This might also explain the increased serum osteocalcin concentration in the serum of osteoporotic postmenopausal women[23]. Another important finding of our examination regarding OS was that the serum level of OC in all patients was negatively correlated with the lumbar spine T-scores. This result suggests that the serum OC level can be used as a surrogate marker, substituting for the lumbar spine T-scores, which has to be measured by DXA examination consuming time and money.

Most patients pretreated with denosumab had increased lumbar spine T-scores after chemotherapy; however, some patients had decreased T-scores in spite of denosumab treatment. Then, we examined the factors affecting the lumbar spine T-score changes in patients treated with denosumab (Table 2). The result was that the patients with decreased T-scores after chemotherapy had severely low lumbar spine T-scores or BMDs at enrollment, unlike patients with increased T-scores after chemotherapy. Based on this finding, patients with severely low T-scores at enrollment might have to be treated with not only denosumab, but also some concomitant medication before chemotherapy. A candidate drug to co-administrate with denosumab may be teriparatide, a recombinant human parathyroid hormone[1, 24]. It has been demonstrated that two years of combined teriparatide and denosumab treatment improves bone microarchitecture and has a higher estimated strength than the individual administration for postmenopausal osteoporosis, particularly in the cortical bone[25, 26].

In this study, 24 weeks after the administration of denosumab, I-PINP, an osteoblastic BTM, was elevated reactively after the transient suppression at 2 weeks in the "No treatment" group; however, it was still suppressed in the "Denosumab" group (Fig. 6). These results indicate that decreased BMD in the "No treatment" group may recover spontaneously and increased BMD in the "Denosumab" group may gradually reduce. To address this concern, an informative

report studied postmenopausal women receiving a placebo or 60 mg of denosumab every six months for 24 months, followed by 24 months without treatment. After discontinuation, BMD declined, but the patients previously treated with denosumab maintained higher BMDs than patients previously treated with the placebo[27].

Although the only one denosumab dose was administered to patients in this study, it was enough to prevent a decrease in T-scores and BMDs, unlike patients without denosumab treatment (Fig. 2 and 3). Nevertheless, whether continuous administration of denosumab after chemotherapy is necessary is unclear. Appropriate number of times that denosumab should be administered is still under evaluation; however, we recommend continuous dosing, if the patients are postmenopausal women, provided circumstances permit it. This recommendation is based on a case of multiple vertebral fractures soon after discontinuation of six doses of denosumab in a breast cancer patient treated with AIs[28].

Further, application of denosumab to patients with a lumbar spine T-score of > -1, allocated to the "No treatment" group in this study, should be examined. We believe that denosumab protects any patients with lymphoma undergoing corticosteroid-containing chemotherapy from bone loss; however, a large-scale clinical trial may be necessary to prove statistical significance in the incidence of vertebral fracture.

5 Conclusions

We demonstrated that denosumab prior to corticosteroid-containing chemotherapy protects NHL patients with a T-score < -1 from decrease in T-score or BMD. Our findings suggest that evaluating BMD at diagnosis of NHL should be considered. This would enable better bone health in an increasing number of lymphoma survivors by simply administering denosumab.

Ethics approval and consent to participate

This study was conducted according to the Declaration of Helsinki and was approved by the ethics committees of Sapporo Medical University (reference number 25-79). Written informed consent was obtained from all patients before participating in this study.

Consent for publication

All participants in the study signed a written consent form to permit publication of the individual data.

Availability of data and materials

Data and materials are included in the manscript.

Competing interests

The authors declare that they have no competing interests.

Funding

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Authors' contributions

TS designed the research. AT, TS, SIyama, KT, KMurase, MY, SIbata, AH, YK, HH, KMiyanishi, MK, and JK performed the research. TS and AT collected the data and performed the statistical analysis. TS analyzed and interpreted the data. TS and AT wrote the manuscript. All authors reviewed the draft and approved the final version of the manuscript for submission.

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Not applicable.

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副腎皮質ステロイドを用いる化学療法に伴う悪性リンパ腫患者の骨喪失をデノスマブで予防する前向き非無作為化臨床試験

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悪性リンパ腫患者は，副腎皮質ステロイドを用いる化学療法のため，骨密度減少のリスクが高い．ビスホスホネートは骨量減少の予防に用いられてきたが，一方で破骨細胞に対する完全人化モノクローナル抗体であるデノスマブの効果は明らかではない．

今回我々は，デノスマブが副腎皮質ステロイドを用いる化学療法を行う悪性リンパ腫患者の骨密度低下を予防するか検討した．主要評価項目は，骨密度と骨代謝マーカーの変化，副次評価項目は椎体骨折と有害事象とした．腰椎 T スコア≦ -1 の骨塩減少群をデノスマブ投与群，腰椎 T スコア＞ -1 の骨塩正常群は非投与群とした．デノスマブ群は，化学療法開始時にデノスマブ 60mg を皮下投与された．43 人が登録された（デノスマブ 19 人，無治療 24 人）．

骨密度は，非投与群では有意に低下したが（P<0.0001），デノスマブ群では骨密度は保たれた．また破骨細胞の骨吸収マーカーは，非投与群では変化しなかったものの，デノスマブ群においては有意に低下した（P<0.0001）．

非投与群における，骨粗鬆症関連骨折または大腿骨頸部骨折の確率は 24 週間で有意に増加した（P=0.0195, P=0.0289），一方でデノスマブ群では骨折のリスク増加は認めなかった．

以上より，悪性リンパ腫患者の T スコア≦ -1 骨塩減少群において，デノスマブは骨密度低下を予防することが示された．悪性リンパ腫診断時に，骨密度のスクリーニングは全例で検討されるべきである．