A randomised trial of alendronate as prophylaxis against loss in bone mineral density following lymphoma treatment

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Abstract:
Lymphoma patients often receive high glucocorticoid doses as part of standard therapy. Observational studies have shown substantial risk of glucocorticoid-induced osteoporosis (GIO) with associated fractures. The aim of the SIESTA trial was to determine if oral alendronate (ALN) is a safe and effective prophylaxis against GIO in lymphoma. SIESTA was a single-center, randomized, double-blinded, phase 2 study of lymphoma patients planned for glucocorticoid-containing chemotherapy. After randomization, patients received weekly ALN 70mg or placebo for a total of 52 weeks. Bone mineral density (BMD) was assessed at baseline, after completion of chemotherapy (EOT, 4-6 month), and at end of study (EOS, 12 month). Vertebral fracture and biomarkers were assessed at baseline and EOS. Patients with baseline BMD assessment and at least one follow-up BMD assessment were analyzed for efficacy. Primary endpoint was change in lumbar spine T-score from baseline to EOS. Of the 59 patients enrolled, 23/30 in the ALN arm and 24/29 in the placebo arm were analyzed for efficacy. Mean change in T-score from baseline to 12 month at lumbar spine was +0.15 for ALN and -0.12 for placebo (P=0.023). The difference in ∆T_EOS between the ALN and placebo groups was larger among females (ALN 0.28; placebo -0.28) (P=0.01). Biomarker analyses confirmed reduced bone resorption in ALN treated patients. In conclusion, ALN is a safe and effective primary prophylaxis against loss in BMD following glucocorticoid-containing chemotherapy. Despite reduced BMD loss in the ALN arm, the treatment did not influence fracture risk in this small cohort of patients. This trial is registered at www.clinicaltrials.gov as 2015-005688-18.

Conflict of interest: COI declared - see note

COI notes: Funding: Paw Jensen received financial support from The Obel Family Foundation (https://obel.com/home/) for the study. The study was an investigator-initiated study, and the institution funding had no direct role in study design, patient recruitment, data collection, analysis, interpretation or writing. Conflict of interest statement: No COI related to the present study. TCEG was previously employed by Roche Ltd (Basel) (2019-2021) and has received speakers fee from Abbvie (2021). TCEG received research funding from the Danish Cancer Society (not related to this study). LHJ received honoraria from Takeda (2019) and Roche (2021). PV has received speakers fees from MSD, Amgen, Servier, and Novartis and is an investigator for Kyowa-Kirin.

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Data sharing statement

Protocol and data will be shared with qualified researcher, provided that relevant permissions can be obtained from the Danish Authorities. To request protocol and data, please e-mail corresponding author Paw Jensen at paje@rn.dk”

Key Points: Oral alendronate is a safe and effective primary prophylaxis against loss in bone mineral density in lymphoma patients.
**Introduction:**

A substantial fraction of lymphoma patients become long-term survivors, and research focus is gradually shifting from being almost exclusively focused on developing more effective therapies to a more holistic view on patient outcomes that also include focus on survivorship (1). Glucocorticoids are included in many treatment regimens for lymphoma. The typical glucocorticoid containing treatment schedule for lymphoma use short pulse therapy with high doses of prednisone repeated with each chemotherapy cycle. For example, R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone), the internationally accepted first-line therapy for diffuse large B-cell lymphoma (DLBCL), includes oral prednisone 100 mg once daily for five consecutive days in each treatment cycle (2). After standard treatment with six R-CHOP cycles, patients have received a total cumulative dose of 3000 mg of prednisone over 18 weeks, corresponding to an average of 24 mg/day during that period.

Glucocorticoids inhibit bone formation and vascularity, especially in the initial treatment phase (3), by promoting apoptosis of osteoblasts and osteocytes (4)(5) resulting in a decrease in bone mineral density (BMD). Glucocorticoids increase the lifespan of osteoclasts leading to increased bone resorption which contributes to further bone loss (6)(7). International consensus guidelines recommend use of primary prophylaxis against glucocorticoid-induced osteoporosis (GIO) provided certain criteria are fulfilled. A working group from the International Osteoporosis Foundation and the European Society of Calcified Tissues recommend GIO risk assessment for patients ≥18 years when oral glucocorticoid therapy is planned for a duration of ≥3 months (8)(9). Several recent studies have highlighted the risk of poor bone health, including risk of fractures, after glucocorticoid-containing chemotherapy for lymphoma. In a study from our institution, vertebral compression fractures were identified on surveillance CT scans in 14% of patients treated with R-CHOP after 2-years of follow-up and a significant decrease in CT-assessed BMD was seen (10). In a large observational study of 13,570 elderly non-Hodgkin lymphoma (NHL) patients with up to 11 years follow-up, those receiving chemotherapy had increased risk of osteoporosis and fractures as...
compared to patients not receiving chemotherapy (10.1% vs 8.3% and 31.1% vs 18.5%, respectively) (11).

Population-based data from 2,589 DLBCL and follicular lymphoma patients treated with R-CHOP (-like) or R-CVP showed a 10-year cumulative risk of osteoporotic events of 16.3% versus 13.5% in the background population of 12,945 age and sex-matched persons (P <0.01) (12).

The aim of this randomised controlled trial was to investigate the safety and efficacy of alendronate (ALN), an easy to administer, inexpensive oral therapy against osteoporosis, as a primary prophylaxis against the observed GIO complications in lymphoma patients.

Methods

Study design

The SIESTA trial (EudraCT number: 2015-005688-18) was a randomized, double-blinded, and placebo-controlled phase 2 study of ALN as primary prophylaxis against GIO in lymphoma patients undergoing treatment with glucocorticoid-containing chemotherapy. The trial was a single center study performed at the Department of Hematology, Aalborg University Hospital, Denmark. Patients were randomized (1:1 ratio) to receive oral ALN 70mg or placebo once weekly for 52 weeks. All patients received a daily supplement of calcium (800-1200mg daily) and vitamin-D (20-40µg daily). This was given for free to all patients enrolled in the trial.

Primary endpoint of the study was change in T-score from baseline to EOS after 12 months, $\Delta T_{EOS} = T_{1y} - T_{baseline}$, measured by dual-energy X-ray absorptiometry scan (DXA) at lumbar spine L3 level. Key secondary efficacy endpoints were $\Delta T_{EOS}$ at total hip and femoral neck, incidence of new vertebral fractures, safety, and change in T-score at end of treatment (EOT, 4-6 months), $\Delta T_{EOT} = T_{EOT} - T_{baseline}$ for lumbar spine, total hip, and femoral neck.
The study was conducted in accordance with the Helsinki declaration and all patients provided written consent. The study was approved by the regional ethics committee of the North Region Denmark (N-20160004), the Danish Medicines Agency (No.2016040045), and the Danish Data protection agency (2008-58-0028). External trial monitoring was performed by the Danish Good Clinical Practice Units to ensure good clinical practice (GCP) compliance for study procedures.

Randomization in blocks of 2-8 patients was performed by the hospital. Only the pharmacy had access to the randomization key. Unblinding was performed after the last patient had last study visit and all DXA scan results had been reported. All analyses were pre-specified in the statistical analysis plan with final version signed prior to study unblinding.

Patients

Patients were eligible if they fulfilled the following inclusion criteria: 1) newly diagnosed or relapsed malignant lymphoma, 2) age ≥18, 3) planned for treatment with a glucocorticoid-containing chemotherapy regimen (such as rituximab), cyclophosphamide, vincristine, and prednisone ((R-)CVP) and all variants of (R-)CHOP, and 4) life expectancy of ≥2 years judged by the treating physician. Patients who received central nervous system prophylaxis or radiation therapy were also eligible. Additional glucocorticoid treatment for a maximum of four weeks at the time of screening was allowed. Local estrogen therapy (e.g., estradiol vaginal inserts) was permitted regardless of duration. All patients had to be able to stand or sit upright for at least 30 minutes.

Patients were excluded if they fulfilled any of the following criteria: 1) contraindications to ALN, 2) treatment with any antiresorptive or anabolic medications including hormone replacement therapy (such as bisphosphonates, denosumab, strontium ranelate, selective estrogen receptor modulators (SERMs), and estrogen used to treat symptoms associated with female menopause, teriparatide), 3) ongoing lithium or anticonvulsants treatment, 4) abnormalities of the esophagus or other conditions delaying esophageal
emptying, 5) GFR <35ml/min, 6) planned for upfront autologous stem cell transplantation consolidation, and 7) pregnancy or lactating.

Study assessments

BMD (g/cm2) was measured at lumbar spine (L1-L4), proximal femur, and femoral neck using dual-energy X-ray absorptiometry (Hologic Discovery, Marlborouh, MA, USA) (DXA). Vertebral fracture assessment (VFA) was used to detect vertebral fractures (Hologic Discovery, Marlborough, MA, USA).

BMD was measured at baseline (within two weeks), at EOT (typically 4-6 months), and at EOS (12 months, +/- 4 weeks). VFA was performed at baseline and at EOS. Routine Quality Control was performed for our site in 2017, and coefficient of variation in vivo were 1.091% at lumbar spine, 1.15% at total hip, and 1.77% at femoral neck (13).

Hemoglobin, white blood cells, calcium, creatinine, N-terminal propeptides of collagen type 1 (P1NP), C-terminal telopeptide cross links (CTX were measured after overnight fast) were obtained at baseline, at EOT, and at EOS. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone/estradiogen, parathyroid hormone, and 25-hydroxy vitamin D were measured at baseline and at EOS. CTX and P1NP were measured in EDTA-plasma on an automated Cobas analyzer from Roche Diagnostics (Mannheim, Germany). The inter-assay analytical variation coefficients according to the manufacturers were CTX (<6%), P1NP (<4%).

Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. Grade 1-4 AEs were registered for the gastrointestinal canal, as these were of special interest, and registrations for other AEs were limited to Grade 3 and 4. A potential relationship of AE’s to study medication was assessed by the attending physician.

Statistical analysis
Prespecified analyses were performed according to statistical analyses plan version 1.2 (Supplementary). Differences in $\Delta T_{EOS}$ and $\Delta T_{EOY}$ between treatment groups were tested using a two-sided t-test assuming equal variance. If the assumption of equal variance was not fulfilled (based on Bartlett’s test), a t-test without the assumption of equal variance was used. Normally distribution of changes in T-scores was tested using the Shapiro-Wilk test and in cases where $\Delta T$ was judged to be non-normally distributed, the Wilcoxon rank sum test was used to test for $\Delta T$ differences between the groups. In exploratory analyses, the effect of ALN were investigated within specific patient subgroups stratified by age (<60 and ≥60), sex, BMI (<25 and ≥25), chemotherapy regiment (R-CHOP and other) and Eastern Cooperative Oncology Group performance status (0 and >0). Differences in clinicopathologic factors and adverse events were tested using Wilcoxon rank sum test (continuous variables) and Fishers’ exact test (discrete variables). Interaction tests were used to confirm differential effects of alendronate between patient subgroups in linear regression models. The efficacy population for primary and secondary endpoints was patients with baseline BMD assessment and at least one follow-up BMD assessment. The safety population was all patients who received at least one dose of study medication. All P-values ≤5% were considered statistically significant.

Results

Patient characteristics

In total, 59 (30 in the ALN arm and 29 in the placebo arm) patients were enrolled in the study during the preplanned recruitment period (December 2016 until February 2020) (Table 1a). Median age was 66 years in the ALN arm and 65 years in the placebo arm with the majority being males (75%). In total, 36 patients were diagnosed with DLBCL, 15 patients diagnosed with FL, and 8 patients with other lymphoma diagnoses (table 1a). Bone marrow involvement was found for 14 patients at baseline. Fifty-five patients (93%) received first-line treatment, whereas four patients (7%) received later lines treatment. Baseline
characteristics were generally well balanced between the ALN arm and the placebo arm, except for advanced stage disease which was observed more frequently in the ALN arm (70% vs 41%), and lower baseline T-score at lumbar spine level in the ALN arm (median T-score -0.6 vs 1.0). Importantly the cumulative doses of corticosteroids are similar between the two treatment arms. One patient was diagnosed with a pathological fracture at baseline.

Eight patients in the ALN arm and six patients in the placebo arm discontinued study treatment before final study assessments (Consort diagram, supplementary). For two patients discontinuing study treatment, DXA was performed.

Efficacy

In total, 47 patients (23 in the ALN arm and 24 in the placebo arm) were included in the primary efficacy analysis of BMD after 12 months (Table 1b). Two patients were not included in selected BMD analyses; one patient had previous lower back surgery (only femur BMD was performed), and one patient had prior hip surgery (only spine BMD was performed).

Mean change in lumbar spine T-scores from baseline to 12 months ($\Delta T_{EOS}$) was +0.15 for patients randomized to ALN and -0.12 for patients randomized to placebo ($P=0.02$). For total hip, mean $\Delta T_{EOS}$ was -0.05 and -0.10 for ALN and placebo, respectively ($P=0.18$), whereas for femoral neck, the median $\Delta T_{EOS}$ was -0.07 and -0.10 for ALN and placebo, respectively ($P=0.62$) (Table 2).

Mean $\Delta T_{EOT}$ at lumbar spine was 0.01 for the ALN group and -0.00 for the placebo group ($P=0.90$). For total hip, mean $\Delta T_{EOT}$ was -0.05 and -0.05 for ALN and placebo, respectively ($P=1.00$), whereas for femoral neck, the mean $\Delta T_{EOT}$ was -0.11 and -0.02 for ALN and placebo, respectively ($P=0.18$) (table 2).

In the ALN arm, two patients with baseline osteopenia (T-score -1.0 to -2.5) had normal BMD at end of study and one patient with normal BMD at baseline developed osteopenia at end of study. In the placebo arm, one patient with baseline osteopenia developed osteoporosis (T-score ≤ -2.5) and one patient went
from having osteopenia to normal BMD. One new fracture was observed in the placebo group, no additional major osteoporotic fractures were identified.

The difference in $\Delta T_{EOS}$ between the ALN and placebo groups was larger among females (ALN 0.28; placebo -0.28) ($P=0.01$) compared to males (ALN 0.10; placebo -0.07) ($P=0.27$). (Fig 1).

Safety:

Serious Adverse Events (SAE) were balanced in the two treatment arms with 15 (50%) patients experiencing SAEs in the ALN arm and 14 (48%) experiencing SAEs in the placebo arm (Table 3). Nine patients experienced AEs related to the upper GI system (seven grade 1-2, two grade 3-4) with five AEs assessed as related to the study treatment (three in the ALN group and two in the placebo group). One patient (placebo) discontinued study treatment due to upper gastro-intestinal bleeding. Six patients experienced AE related to lower GI system, all assessed unrelated to study medication.

Biomarker analyses

Dynamics over time for blood biomarkers of bone turnover (CTX as marker of bone resorption and P1NP as marker for bone formation)(14) are illustrated in Figure 2. Median levels of CTX and P1NP were similar between treatment arms at baseline (Supplementary). From baseline to EOT the mean change in CTX was -0.17 in the ALN group and 0.10 in the placebo group respectively ($P<0.001$). From baseline to EOS the mean change in CTX was -0.19 in the ALN group and 0.00 in the placebo group respectively ($P=0.002$). For P1NP, EOT mean changes were 3.93 in the ALN group and 40.45 in the placebo group ($P<0.001$) and EOS mean changes were 7.76 in the ALN group, and 30.52 in the placebo group ($P = 0.045$).

Discussion
The present study of ALN as primary prophylaxis against GIO in lymphoma patients treated with glucocorticoid-containing chemotherapy regimens met its primary endpoint as ALN prevented a reduction in BMD at lumbar spine level from baseline to the 12-months assessment. In contrast, patients in the placebo arm experienced a reduction in BMD during the same period. Biomarker analyses supported a favorable effect of ALN on bone health, as CTX decreased over time among patients in the ALN arm and remained unchanged for patients in the placebo arm. However, ALN had no protective effects on BMD at total hip and left femoral neck level at any time point. One year should be sufficient to appreciate changes in T-score as glucocorticoids seem to cause marked vertebral bone loss in initial months of therapy (15).

Consistently, Svendsen et al. showed that the BMD loss primarily occurred in the first year following treatment (10). One new fracture occurred during follow-up in the placebo group, therefore no firm conclusions can be made on whether the reduced BMD loss at lumbar spine level eventually translates into a clinically relevant reduction in fractures.

Only two previous randomized clinical trials have addressed the question of prophylaxis for lymphoma patients receiving glucocorticoid-containing chemotherapy. A trial by Kim et al. from 2004 randomized between intravenous pamidronate (30 mg) every three months for one year versus placebo. Among the 50 patients randomized, patients assigned to pamidronate had a reduction in both bone loss and vertebral fractures risk (16). New vertebral fractures occurred in 6/20 patients in the placebo group versus 1/25 in the pamidronate group (p=0.01) (16). Very large doses of glucocorticoids were used in this trial, as many patients received older chemotherapy regiments (ProMACE/CytaBOM). Patients in this trial received mean 7573mg prednisone in the pamidronate group and 7527mg prednisone in the placebo group. Prednisone doses are not directly comparable to prednisone doses in R-CHOP-like regiments. Westin et al. conducted a single center study of 74 newly diagnosed lymphoma patients who on top of oral calcium and vitamin D received intravenous zoledronic acid (ZOL) or placebo. The study showed that ZOL could stabilize BMD at the lumbar spine and femoral neck level after 12 months (17). However, 21/74 patients were not evaluable at one-year follow-up due to study drop-out for financial or personal reasons.
Our results are consistent with these observations as the present study showed a clinically meaningful effect of oral ALN. The explanation for the lack of consistent results between BMD at the lumbar spine level and the total hip or femoral neck level is likely that bone loss occurs more rapidly in trabecular bone such as the lumbar spine as compared to the cortical bone of the hip and femoral neck (18).

Kim et al. identified six new fractures (16), but no fractures were identified in the trial by Westin et al (17) where fractures were detected clinically and not by imaging. As prednisone doses in the trial by Kim et al. are very high, the risk of fractures in the placebo group is not directly comparable with the fracture risk for patients receiving (R)-CHOP/(R)-CVP. Increased risk of fractures has been found in observational studies of GIO in lymphoma patients (19)(10)(12)(20), but causality cannot be established in this setting since cancer patients may have increased fragility and treatment-related factors such as loss of muscle mass, peripheral neuropathy and general weakness which may led to fractures independent of bone health. Possible explanations for the low fracture rate in our study is the short study duration of only 12 months and the fact that patients enrolled in clinical trials are typically healthier and often have better performance. In addition, more fractures are expected for elderly and women (21). A British study by Booth et al. based on 877 patients age ≥70 years with a follow-up of 18 months showed a fracture rate of 11.4% in patients receiving R-CHOP (20). In the present study, the median age was 67 years and only 25.4% were women (median age for women in the study was 67 years). These demographic differences may at least partially explain the differences in fracture rate observed across studies. This is supported by the fact that the analyses of BMD by sex, although not significant, show that the difference in $\Delta T_{EOS}$ between the ALN and placebo groups was larger among females, despite the fact that only 15 women, all post-menopausal, were recruited to the present study. Efficacy results by gender were not provided in the previously mentioned randomized trials by Kim et al. or Westin et al. (16)(17).

The two treatment arms were generally well balanced with respect to baseline clinical characteristics, including sex, although patients in the ALN arm had a lower baseline T-score at lumbar spine level (median
T-score -0.6 vs 1.0 for placebo arm). However, the effect of alendronate remained significant after adjusting for baseline T-scores (difference in delta T-score -0.26, \( p = 0.036 \)) in multivariable linear regression.

Most publications showing increased risk of fractures for lymphoma patients have been performed for lymphoma patients treated with standard lymphoma treatment (12)(20)(19). Although detailed information on prednisone dosing, including actual dose, was not available in these retrospective evaluations, it is likely that the patients in those studies received approximately the same dose of prednisone as in the present study. The fact that only one vertebral compression fractures were detected in the present study of 59 patients with only 12 months follow-up does not necessarily provide evidence of a clinically limited/irrelevant fracture risk. The low fracture rate may be a type II error, as the baseline fracture risk in this relatively young predominantly male study population may have been minimal. The aspect of prevention is a relevant issue for further exploration in this setting. However, with an observed difference in T-score of the lumbar spine of 0.27 between ALN and placebo, the expected effect on relative fracture risk compared to placebo would be 0.80 for spine fractures and 0.90 for any fracture according to a previous meta-analysis (22). Given that alendronate is a simple, inexpensive, and well tolerated treatment, we believe that fracture risk reduction expected from this effect on BMD is clinically meaningful.

ALN is a widely used drug with a well-established safety profile. AEs reported in the present study are likely more reflective of the side effects related to the (immuno)-chemotherapy. Only few AEs were assessed as being related to the study medicine, but there were no differences for these AEs between the ALN arm and the placebo arm.

High doses of glucocorticoids undoubtedly lead to GIO (23). The isolated effect of chemotherapy on the risk of developing osteoporosis is generally considered limited (24)(25). In the context of lymphoma treatment, where glucocorticoid-containing chemotherapy regimens are often used, the question of prevention of GIO is relevant, but for many other diseases where glucocorticoid pulse-treatments or short-term high-dose glucocorticoid treatments are given, the aspect of preventing GIO is also a relevant issue. BMD
measurements are rarely performed as a part of standard lymphoma assessment before starting
treatment, and probably should be.

The study was designed as an intention-to-treat with expected balanced drop-out rate of 30% in each
treatment arm. The observed drop-out rates was 27% in the ALN and 24% in the placebo arm. The patients
discontinuing study treatment were encouraged to accept performance of remaining DXA scans, which was
done for two of 14 patients. As missing data are considered unrelated to the treatment effects and as the
number of patients discontinuing study treatment are equally distributed in the two treatment groups, the
risk of bias due to missing data is considered low.

The number of patients in the study was relatively low making the trial underpowered for secondary
analysis. The primary endpoint was reached, and despite non-significant results for the secondary
endpoints, the positive effect on T-score for lumbar spine at 12 months is supported by the biomarker
analysis indicating reduced bone resorption.

In conclusion, oral weekly ALN appears to be an effective and safe prophylaxis against loss in BMD for
lymphoma patients treated with glucocorticoid-containing chemotherapy regimens. The beneficial effect
appears to be stronger in female patients. Biomarker analyses supported a biological effect of ALN in this
patient setting. Larger trials with longer follow-up are needed to determine if ALN improve patient
outcome by lowering the risk of fractures.
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Authors contributions

PJ, TE, MB, MTS, PV contributed to conception and design of the study. PJ performed the management and coordination responsibilities. All authors contributed to acquisition of data. LHJ, MB, TE and PJ performed analysis of data. PJ drafted the manuscript. All authors contributed to interpretation of the results and revised the manuscript. All authors read and approved the final manuscript.

Conflict of interest statement

No COI related to the present study. TCEG was previously employed by Roche Ltd (Basel) (2019-2021) and has received speakers fee from Abbvie (2021). TCEG received research funding from the Danish Cancer Society (not related to this study). LHJ received honoraria from Takeda (2019) and Roche (2021). PV has received speakers fees from MSD, Amgen, Servier, and Novartis and is an investigator for Kyowa-Kirin.

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Paw Jensen received financial support from The Obel Family Foundation (https://obel.com/home/) for the study. The study was an investigator-initiated study, and the institution funding had no direct role in study design, patient recruitment, data collection, analysis, interpretation or writing.

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Figure 1: The difference in mean ΔT at lumbar spine (L1-L4) between patients in the efficacy group (n=47) allocated to alendronate and placebo groups. The displayed 95% confidence intervals indicate whether alendronate has a significant effect in each of the subgroups. Test for differential effect of alendronate between clinical subgroups did not reveal any significant differences: female vs male (P=0.145), age <60 years vs. age >=60 years (P=0.913), bone narrow involvement vs. no involvement (P=0.995), BMI <25 vs. BMI >=25 (P=0.655), R-CHOP vs other chemotherapy (P=0.572).

Figure 2: Boxplot of CTX and P1NP at baseline, end of treatment, and 12 months stratified by randomization arm. CTX = C-terminal telopeptide cross link. P1NP= N-terminal propeptides of collagen type 1.
Table 1a: Baseline characteristics of 59 adult lymphoma patients enrolled in the Siesta trial.

Performance status = Eastern Cooperative Oncology Group performance status (ECOG). BMI = body mass index. DLBCL = diffuse large B-cell lymphoma. FL = follicular lymphoma. LDH = lactate dehydrogenase. R-CHOP = Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone. R-CVP = Rituximab, Cyclophosphamide, Vincristine, Prednisone. CNS = central nervous system. MTX = high-dose Methotrexate.

|                          | Alendronate (n=30) | Placebo (n=29) | p  |
|--------------------------|--------------------|----------------|----|
| Age, mean(SD)            | 66(9.2)            | 65(11.2)       | 1.000 |
| Sex, n(%)                |                    |                | 1.000 |
| -Male                    | 22(73.3)           | 22(75.9)       |     |
| -Female                  | 8(26.7)            | 7(24.1)        |     |
| --Postmenopausal, n(%)   | 8(100.0)           | 7(100.0)       | 1.000 |
| Performance status, n(%) |                    |                | 0.212 |
| -0                       | 21(70.0)           | 24(85.7)       |     |
| ->0                      | 9(30.0)            | 4(14.3)        |     |
| BMI, mean(range)         | 29(19-43)          | 27(22-37)      | 0.088 |
| Subtype, (%)             |                    |                | 0.301 |
| -DLBCL                   | 18(60.0)           | 18(62.1)       |     |
| -FL                      | 6(20.0)            | 9(31.0)        |     |
| -Other (Marginal zone lymphoma, Hodgkin lymphoma, T-cell lymphoma, unspecified low grade lymphoma) | 6(20.0) | 2(6.9) |     |
| Bone marrow involvement, n(%) | 9(30.0) | 5(17.2) | 0.360 |
| Ann Arbor stage, n(%)    |                    |                | 0.037 |
| -1-2                     | 9(30.0)            | 17(58.6)       |     |
| -3-4                     | 21(70.0)           | 12(41.4)       |     |
| LDH, n(%)                |                    |                | 0.438 |
| -Normal                  | 18(60.0)           | 14(48.3)       |     |
| -Elevated                | 12(40.0)           | 15(51.7)       |     |
| Treatment line, n(%)     |                    |                | 1.000 |
| -First line              | 28(93.3)           | 27(93.1)       |     |
| -Second or later lines   | 2(6.7)             | 2(6.9)         |     |
| Chemotherapy, n(%)       |                    |                | 1.000 |
| -R-CHOP                  | 15(50.0)           | 14(48.3)       |     |
| -R-CVP                   | 10(33.3)           | 10(34.5)       |     |
Table 1b: Baseline characteristics efficacy group. Efficacy group of 47 patients. Performance status =

Eastern Cooperative Oncology Group performance status (ECOG). BMI = body mass index, DLBCL = diffuse large B-cell lymphoma. FL = follicular lymphoma. LDH = lactate dehydrogenase. R-CHOP = Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone. R-CVP = Rituximab, Cyclophosphamide, Vincristine, Prednisone. CNS = central nervous system. MTX = high-dose Methotrexate.
|                                | Alendronate  | Placebo  | P   |
|--------------------------------|--------------|----------|-----|
|                                | (n=23)       | (n=24)   |     |
| Age, mean(SD)                  | 66(7.8)      | 64(10.3) | 0.617 |
| Sex, n(%)                      |              |          | 1.000 |
| -Male                          | 17(73.9)     | 18(75.0) |     |
| -Female                        | 6(26.1)      | 6(25.0)  |     |
| --Postmenopausal, n(%)         | 6(100.0)     | 6(100.0) | 1.000 |
| Performance status, n(%)       |              |          | 0.188 |
| -0                             | 19(82.6)     | 23(95.8) |     |
| ->0                            | 4(17.4)      | 1(4.2)   |     |
| BMI, mean(range)               | 28(19-38)    | 27(22-37)| 0.441 |
| Subtype, (%)                   |              |          | 0.311 |
| -DLBCL                         | 13(56.5)     | 13(54.2) |     |
| -FL                            | 5(21.7)      | 9(37.5)  |     |
| -Other (Marginal zone lymphoma, Hodgkin lymphoma, T-cell lymphoma) | 5(21.7) | 2(8.3) |     |
| Bone marrow involvement, n(%)  | 7(30.4)      | 5(20.8)  | 0.517 |
| Ann Arbor stage, n(%)          |              |          | 0.041 |
| -1-2                           | 7(30.4)      | 15(62.5) |     |
| -3-4                           | 16(69.6)     | 9(37.5)  |     |
| LDH, n(%)                      |              |          | 0.147 |
| -Normal                        | 15(65.2)     | 10(41.7) |     |
| -Elevated                      | 8(34.8)      | 14(58.3) |     |
| Treatment line, n(%)           |              |          | 1.000 |
| -First line                    | 21(91.3)     | 22(91.7) |     |
| -Second or later lines         | 2(8.7)       | 2(8.3)   |     |
| Chemotherapy, n(%)             |              |          | 1.000 |
| -R-CHOP                        | 11(47.8)     | 10(41.7) |     |
| -R-CVP                         | 9(39.1)      | 10(41.7) |     |
| -Other                         | 3(13.1)      | 4(16.6)  |     |
| CNS prophylaxis with high doses MTX, n(%) |              |          | 0.287 |
| -No                            | 17(73.9)     | 21(87.5) |     |
| -Yes                           | 6(26.1)      | 3(12.5)  |     |
| Total prednisolone doses, mean(range) | 3291(2400-4400) | 3398(2000-4000) | 0.400 |
| T-score (lumbar spine)         |              |          |     |
| -Available, n(%)               | 22(95.7)     | 24(100.0)| 0.489 |
| -Lumbar spine, median(range)   | -0.8(-3.1;3.8)| 1.0(-3.2;2.7)| 0.097 |
| T-score (hip/femoral neck)     |              |          |     |
| -Available, n(%)               | 23(100.0)    | 23(95.8) | 1.000 |
|                      | Alendronate (n=23) | Placebo (n=24) | P     |
|----------------------|-------------------|---------------|-------|
| Total hip, median(range) | -0.2(-2.5;1.5)   | 0.1(-2.3;1.6) | 0.322 |
| Femoral neck, median(range) | -0.9(-3.0;1.5)   | -0.4(-3.1;1.0) | 0.475 |

Completetion status, n(%)  
- Completed  
  22(95.7)  
- Dead  
  0(0.0)  
- Withdrawal  
  0(0.0)  
- Drop-out  
  1(4.3)  

Table 2: Mean (range) T-score difference from baseline to EOS and baseline to EOT at lumbar spine, total hip, and femoral neck respectively. Efficacy group; 47 patients.

|                      | $\Delta T_{EOS}$ (Alendronate) | $\Delta T_{EOS}$ (Placebo) | Difference | P     |
|----------------------|--------------------------------|----------------------------|------------|-------|
| EOS (12 months)      |                                |                            |            |       |
| Lumbar spine*        | 0.15 (-0.70;0.90)              | -0.12 (-0.80;0.80)         | 0.28(0.04;0.51) | 0.023 |
| Total hipJ            | -0.05 (-0.70;0.30)             | -0.10 (-0.40;0.30)         | 0.05       | 0.175 |
| Femoral neck*        | -0.07 (-0.60;0.40)             | -0.10 (-0.50;0.30)         | 0.03(-0.11;0.18) | 0.624 |
| EOT (4-6 months)     |                                |                            |            |       |
| Lumbar spine*        | 0.01 (-0.60;0.70)              | -0.00 (-0.60;1.00)         | 0.01(-0.19;0.22) | 0.896 |
| Total hipJ            | -0.05 (-0.70;0.50)             | -0.05 (-0.30;0.20)         | 0.00(-0.12;0.12) | 1.000 |
| Femoral neckJ        | -0.11 (-0.40;0.40)             | -0.02 (-0.40;0.30)         | -0.10      | 0.175 |

*T-test with equal variance; † t-test with unequal variance; J Wilcoxon rank-sum test (no confidence intervals are provided from this test)
Table 3: Number of patients with adverse events among 59 lymphoma patients stratified by randomization.

| Event                                      | Alendronate (n=30) | Placebo (n=29) |
|--------------------------------------------|-------------------|----------------|
| Any adverse events, n(%)                  | 19(63.3)          | 15(51.7)       |
| Any serious adverse event, n(%)           | 15(50.0)          | 14(48.3)       |
| AE related to infection, n(%)              | 6(20.0)           | 9(31.0)        |
| AE related to upper gastrointestinal tract, n(%) | 7(23.3)          | 2(6.9)         |
| AE related to lower gastrointestinal tract, n(%) | 3(10.0)          | 3(10.3)        |
| AE related to renal toxicity, n(%)         | 1(3.3)            | 2(6.9)         |
| Other AEs, n(%)                            | 12(40.0)          | 8(27.6)        |
| AE grade 1-2 related to study drug, n(%)   | 3(10.0)           | 1(3.4)         |
| AE grade 3-4 related to study drug, n(%)   | 0(0.0)            | 1(3.4)         |
| AE resulting in termination of study drug, n(%) | 1(3.3)          | 2(6.9)         |
Figure 1 revised version

All patients (N=47)
- Female (N=12)
- Male (N=35)
- Age <60 years (N=13)
- Age ≥60 (N=34)
- No bone marrow involvement (N=35)
- Bone marrow involvement (N=12)
- BMI <25 (N=13)
- BMI ≥25 (N=34)
- R-CHOP (N=21)
- Other chemotherapy (N=26)

Difference in mean ΔT (lumbar spine)
Figure 2

The figure shows box plots comparing CTX and P1NP levels between Alendronate and Placebo groups at Baseline, End of treatment, and 12 months.

**CTX**
- Baseline: Alendronate (black) and Placebo (green)
- End of treatment: Alendronate (black) and Placebo (green)
- 12 months: Alendronate (black) and Placebo (green)

**P1NP**
- Baseline: Alendronate (black) and Placebo (green)
- End of treatment: Alendronate (black) and Placebo (green)
- 12 months: Alendronate (black) and Placebo (green)

The y-axis represents Absolute blood measure (microgram/L).