Efficacy of 12-month romosozumab monotherapy or combination with eldecalcitol in patients with osteoporosis with and without chronic kidney disease: A real-world retrospective study

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Abstract. Evidence regarding the clinical benefits of combination therapy with romosozumab and active vitamin D analogs in the treatment of osteoporosis is lacking. The aim of the present study was to evaluate the outcome of romosozumab (ROMO) as well as ROMO + eldecalcitol (ELD) treatment in patients with osteoporosis with and without chronic kidney disease (CKD). Data from 146 patients who were administered ROMO was retrospectively investigated. Patients were classified into two groups based on estimated glomerular filtration rate (eGFR): CKD (eGFR <60 ml/min/1.73 m^2; N=64) and non-CKD (eGFR ≥60 ml/min/1.73 m^2; N=82) groups. A total of 15 patients in the CKD and 18 in the non-CKD group received ROMO + ELD treatment. The primary outcomes were bone mineral density (BMD) responses in the lumbar spine (LS) and femoral neck (FN). The combined impact of ROMO + ELD treatment was also assessed. The LS BMD was significantly increased in the both groups after 12 months of treatment compared with that at baseline. The FN BMD value was not significantly altered from baseline in both groups, and there were no significant differences in the LS and FN BMD increases between the groups. In the CKD group, the percentage changes in the FN BMD and serum procollagen type I N-terminal propeptide levels observed with ROMO + ELD treatment were greater than those with ROMO monotherapy. ROMO demonstrated efficacy in the treatment of osteoporosis, irrespective of renal function. ROMO + ELD treatment may be beneficial for patients with CKD.

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Introduction

Osteoporosis is estimated to affect 200 million women globally (1). Osteoporosis causes significant disability, surpassing most cancers (except lung cancer) and numerous chronic diseases. It has substantial economic implications, with fragility fractures costing Europe €37.5 billion in 2017, a figure projected to increase by 27% in 2030 (2).

Chronic kidney disease (CKD) is associated with an increased incidence of fractures due to CKD-mineral bone disorder and uremic osteoporosis is exacerbated by CKD-specific factors, including uremic toxins. Consequently, osteoporosis and CKD are prevalent comorbidities in the elderly population and frequently coexist.

In Japan, recent data suggest that >14 million individuals are estimated to have CKD, with the number of chronic dialysis patients reaching 2,754 per million in 2020 and continuing to rise annually (3). Globally, ~700 million individuals are estimated to have CKD (4).

Nickolas *et al* (5) reported a significantly increased incidence of hip fractures in patients with an estimated glomerular filtration rate (eGFR) of <60 ml/min/1.73 m^2 (odds ratio, 2.12; 95% confidence interval, 1.18-3.80). In the Dialysis Outcomes and Practice Patterns Study report, the incidence of fractures was significantly higher in dialysis patients than in the general population (6). Therefore, treatment for osteoporosis among patients with CKD is an important issue. However, there is currently no gold standard for osteoporosis treatment among patients with CKD or a history of kidney transplantation. In a recent systematic review and meta-analysis by Chen *et al* (7), teriparatide and denosumab exhibited the highest efficacy in improving bone mineral density (BMD) in the vertebrae and femoral neck (FN) of patients with CKD. Conversely, the use of teriparatide or denosumab in patients with end-stage CKD has been reported to elevate the risk of transient hypotension or severe hypocalcemia (8,9).

Romosozumab (ROMO) is a humanized monoclonal antibody that binds to sclerostin, which is a key inhibitor of bone formation, thereby increasing bone formation and reducing bone resorption. ROMO was approved in March 2019 in Japan for patients with osteoporosis and a high fracture risk. Previous research has demonstrated that ROMO significantly

increases BMD in the lumbar spine (LS), total hip, and FN, while reducing the risk of both vertebral and non-vertebral fractures (10). For example, in the FRAME study, it reduced the risk of new vertebral fractures by 73% compared with placebo over 12 months (P<0.001) (10).

ROMO is contraindicated in patients with hypocalcemia. No dose adjustment is required in patients with renal impairment; however, careful prescription is needed for those with eGFR <60 ml/min/1.73 m^2 or those undergoing dialysis, as hypocalcemia appears to be more common. Post hoc analysis of results from the placebo-controlled phase 3 FRAME study and the active-controlled phase 3 ARCH showed the efficacy and safety of ROMO in postmenopausal women with osteoporosis and reduction in kidney function (eGFR of 30-90 ml/min/1.73 m^2) (11,12). Excluding these post hoc analyses from clinical trials, there is limited evidence for the efficacy and safety of 12 months of ROMO treatment for patients with osteoporosis and reduction in kidney function in actual clinical practice.

Notably, serum 25(OH)D levels are reportedly low in patients with CKD (13,14). Vitamin D and calcium play an essential role in calcium and phosphate homeostasis, as well as bone formation and mineralization. The Kidney Disease: Improving Global Outcomes clinical guidelines recommend monitoring 25(OH)D levels and correcting vitamin D insufficiency and deficiency using treatment strategies recommended for the general population (15).

Most osteoporotic fractures occur due to falls (16). One-third of people >65 fall each year, with the risk of falling increasing with age. The global population of people aged 65 and older is growing at a faster rate than younger age groups, with their share projected to increase from 10% in 2022 to 16% by 2050 (17). It is reported that vitamin D supplementations may help prevent falls by enhancing dynamic physical function (18,19).

Data are lacking on the benefits of ROMO and active vitamin D analog combination treatment on clinical outcomes. It is hypothesized that ROMO is effective and safe in patients with osteoporosis regardless of renal function, and the addition of active vitamin D analog [eldecalcitol (ELD)] may provide additional benefits.

The primary objective of the present study was to examine the safety and efficacy of ROMO in patients with and without CKD. The secondary objective was to elucidate the influence of ELD on the effects of ROMO treatment in patients with and without CKD.

Patients and methods

Study subjects. The present retrospective observational cohort study was conducted at Tomidahama Hospital (Yokkaichi, Japan) from January 2019 to December 2023. The total number of included patients was 146 (14 men and 132 women), with an average age of 84±8 years (mean ± SD).

The inclusion criteria for this study were patients aged ≥55 years who underwent 12 months of ROMO therapy for osteoporosis and had a high risk of fractures. The requirement for institutional review board approval from our institute was waived owing to the anonymized and retrospective nature of this study; however, written informed consent was obtained from the patients to perform further research.

Our indication for ROMO treatment was patients at a high risk of fracture. A high risk of fracture was defined as: i) a BMD of <70% of the young adult mean (YAM) of all participants reported in the Japanese Normative Female Database, with a minimum of one prevalent fragility fracture; ii) a BMD of the LS (L1-4) of <60% of the YAM; or iii) >2 previous fragility fractures.

The exclusion criteria were bone metabolic disorders or conditions affecting bone and calcium metabolism, untreated thyroid dysfunction, current parathyroid disorders, abnormal calcium levels, significant cardiopulmonary or liver disease, major psychiatric conditions, excessive alcohol intake, and prior glucocorticoid use.

The study participants were divided into two groups according to their eGFR value at baseline (non-CKD group, eGFR ≥60 ml/min/1.73 m^2; and CKD group, eGFR <60 ml/min/1.73 m^2). The eGFR value was calculated using the Japanese Modification of Diet in Renal Disease guidelines (20). The primary outcomes were percentage changes in BMD of the LS and FN from baseline to 12 months, as well as the percentage changes in serum total procollagen type I N-terminal propeptide (PINP) from baseline to 1, 4, 8, and 12 months with respect to baseline eGFR. The incidence of new fractures and adverse events were also evaluated at 12 months. In addition, as a secondary outcome, the efficacy of ROMO with or without ELD according to baseline eGFR was evaluated.

Measurements. The BMD of the LS and FN was measured using dual-energy X-ray absorptiometry with a DPX-BRAVO instrument (GE Healthcare) at baseline and at the end of the 12-month treatment. The interobserver percentage coefficient of variability (%CV) was 0.6% for the LS and 0.9% for the FN. The baseline PINP level (normal range, 26.4-98.2 mg/l in postmenopausal women and 18.1-74.1 mg/l in men) was measured using a radioimmunoassay (Orion Diagnostica, Ltd.; Aidian). The intra-assay and inter-assay %CV values for PINP were 3.5 and 4.2%, respectively.

Statistical analysis. For repeated measures data, repeated measures analysis of variance (ANOVA) with Bonferroni correction was applied. Between-group comparisons of the measurements were analyzed using the Mann-Whitney U test.

Differences in categorical variables were assessed using Fisher's exact test. Differences in fracture incidence were assessed using the Kaplan-Meier analysis and log-rank test. All data are expressed as the mean ± standard deviation, unless otherwise indicated. P<0.05 was considered to indicate a statistically significant difference.

In a previous study, the BMD response within each subgroup was found to be normally distributed with standard deviation of 1.1 (21). If the true difference in the ROMO and ROMO with ELD group is 3, a sample size of 3 patients per group will be required to reject the null hypothesis that the population means are equal, with probability (power) of 0.8. The type I error probability associated with this test of the null hypothesis is set at 0.05.

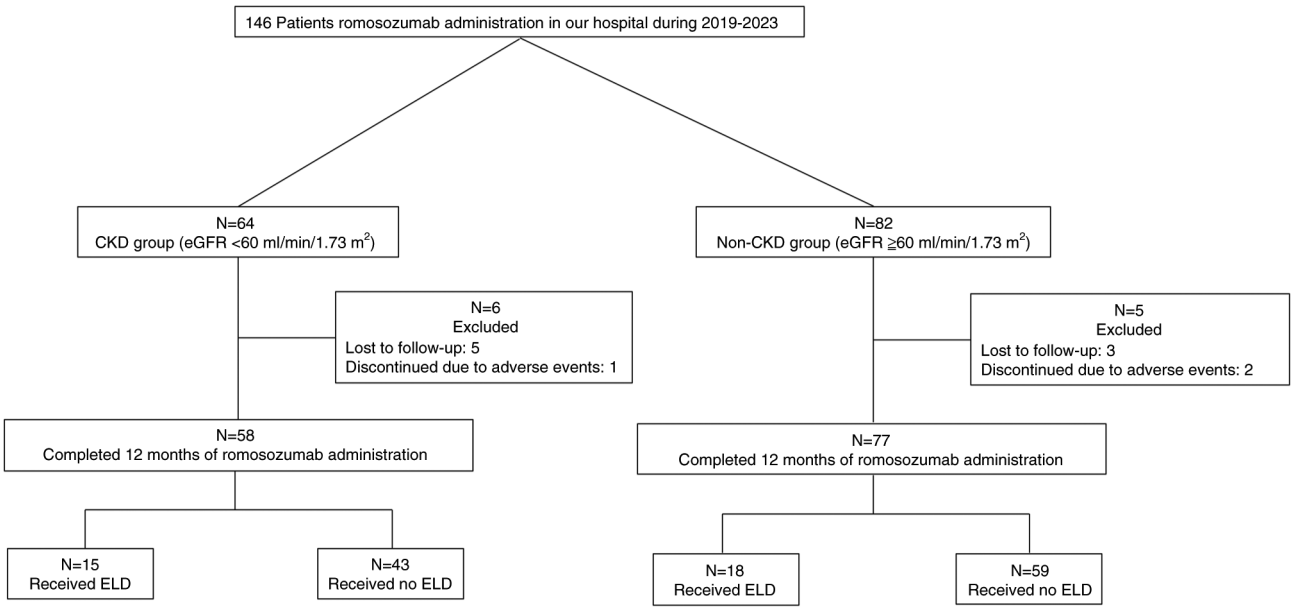

Figure 1. Flow chart of the study population. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ELD, eldecalcitol.

All statistical analyses were performed using the EZR graphical user interface (Saitama Medical Center, Jichi Medical University, Saitama, Japan) for the R version 4.4.1 software package (R Foundation for Statistical Computing), which is a modified version of the R Commander designed to add statistical functions commonly used in biostatistics.

Results

Study participants and groups. A flow diagram of the study participants is presented in Fig. 1. This retrospective observational cohort study was conducted at Tomidahama Hospital (Yokkaichi, Japan) from January 2019 to December 2023. A total of 146 patients ranging in age from 56 to 98 years who underwent ROMO therapy were eligible for the present study.

The 146 patients were classified into 2 groups according to their baseline eGFR values (CKD group, N=64 or non-CKD group, N=82). Of the 64 patients in the CKD group, 6 (9.3%) were excluded, as well as 5 (6.1%) of the 82 patients in the non-CKD group, as a result of treatment discontinuation and loss to follow-up, leaving 135 patients selected for analysis. There were no significant differences in completion rates between the two subgroups (P=0.53; Fisher's exact test).

ELD was prescribed at the discretion of the attending clinician, with 33 patients receiving ELD at a fixed dose of 0.75 μg, initiated concurrently with ROMO treatment. A total of 15 (25.9%) patients in the CKD and 18 (23.4%) patients in the non-CKD group underwent ROMO + ELD treatment.

Baseline characteristics. Data including age, sex, height, weight, body mass index, rates of previous vertebral or non-vertebral fractures, baseline mean percentage of YAM at the LS and FN, PINP levels, serum albumin, serum calcium, serum creatinine, baseline serum eGFR, presence of diabetes, serum 25(OH)D levels, and history of osteoporosis treatment are presented in Tables I and II. There were significant differences in age between the CKD and non-CKD groups (P<0.05). In the CKD group, baseline serum PINP levels were significantly higher with ROMO monotherapy than with combination treatment.

Primary outcomes

Changes from baseline BMD. In the CKD group, the LS BMD percentage significantly increased by 10.2±8.3% (P<0.001) after 12 months of ROMO therapy (Fig. 2A) compared with the baseline value. The FN BMD percentage decreased by-0.22±6.1% after 12 months of ROMO therapy, but this change was not statistically significant (P=0.86) compared with the baseline value (Fig. 2B). In the non-CKD group, the LS BMD percentage significantly increased by 12.1±9.8% (P<0.001) after 12 months of ROMO therapy compared with the baseline value (Fig. 2A). The FN BMD percentage increased significantly by 1.7±6.0% (P=0.042) after 12 months of ROMO therapy compared with the baseline value (Fig. 2B). The percentage change in LS and FN BMD values was statistically comparable between the various groups after 12 months of ROMO therapy (P=0.59 and P=0.14, respectively).

Changes in serum PINP levels in response to treatment. In the CKD group, the serum PINP change rate from baseline was 42, -12, -22, and -49% at 1, 4, 8, and 12 months of ROMO treatment, respectively (Fig. 2C). In the non-CKD group, the serum PINP change rate from baseline was 69, 21, -11, and -20% at 1, 4, 8, and 12 months of ROMO treatment, respectively (Fig. 2C). There were no significant differences between either parameter and baseline values at any time point in both groups. There were significant differences at 12 months (P=0.013) between the groups after ROMO treatment.

Adverse events and new fractures. There was one fracture (FN fracture) in the CKD group and no fractures in the non-CKD group. There were no significant differences in fracture incidence between the groups (P=0.25; Table III).

Table I. Patient demographics and clinical characteristics of primary analysis.

Variables	CKD group (n=64)	Non-CKD group (n=82)	P-value
Age (years)	85.66±7.42	81.91±8.59	0.006[a]
Female sex	58 (91%)	74 (90%)	>0.999
Height (cm)	149.92±7.16	150.14±6.86	0.869
Weight (kg)	45.92±8.13	44.92±8.85	0.522
BMI	20.40±3.56	19.92±3.69	0.466
Previous vertebral fracture, n (%)	28 (43.8%)	39 (47.6%)	0.738
Previous non-vertebral fracture, n (%)	14 (21.9%)	23 (28%)	0.447
Mean percentage of BMD (YAM ± SD) at the lumbar spine (%)	77.95±14.87	73.69±15.06	0.087
Mean percentage of BMD (YAM ± SD) at the femoral neck (%)	64.3±9.69	63.37±8.47	0.537
Median serum PINP (IQR)	79.05 (81-363)	67.3 (48-274)	0.174
Serum albumin	3.81±0.48	3.93±0.38	0.120
Serum Ca	9.46±0.72	9.34±0.48	0.406
Serum Cre	0.97±0.33	0.59±0.1	<0.001[a]
Serum eGFR	45.61±10.62	77.90±14.71	<0.001[a]
Diabetes	7 (10.9%)	9 (10.9%)	>0.999
Serum 25(OH)D	15.15±5.97	14.07±5.89	0.337
History of prior osteoporosis treatment, n (%)			
Naïve	27 (42.2%)	38 (46.3%)	0.737
Switched medications			
Bisphosphonates	9 (14.1%)	15 (18.3%)	0.653
Teriparatide	25 (39.1%)	20 (24.4%)	0.071
Denosumab	0 (0)	3 (3.7%)	0.256
SERMs	3 (4.7%)	3 (3.7%)	>0.999
Vitamin D analog	0 (0)	3 (3.7%)	0.256

Data are expressed as the mean ± standard deviation or number (%) of subjects. PINP is expressed as a median value. Differences between the groups were analyzed using Fisher's exact test and Mann-Whitney U test. [a]Significant difference between the groups. ROMO, romosozumab; ELD, eldecalcitol; BMI, body mass index; BMD, bone mineral density; YAM, young adult mean; PINP, procollagen type I N-terminal propeptide; IQR, interquartile range; eGFR, estimated glomerular filtration rate; Ca, calcium; Cr, creatinine; 25(OH)D, 25-hydroxy vitamin D; SERMs, selective estrogen receptor modulators.

Adverse events that led to treatment discontinuation were reported in 1 patient (hyponatremia) in the CKD group and 2 patients (1 injection site pain and 1 renal dysfunction) in the non-CKD subgroup; there were no significant differences between the groups (P=0.71). No fatal events were reported in either group. No cases of clinical hypocalcemia, defined as calcium levels <7.0 mg/dl and Common Terminology Criteria for Adverse Events v5.0 (22) grade 3 or higher, were reported.

Secondary outcomes

Change from baseline BMD. ROMO treatment with and without ELD in the non-CKD and CKD groups, respectively, was compared. The percentage change in BMD after 12 months of ROMO treatment with and without ELD, at two sites in the CKD group is illustrated in Fig. 3A and B. At the LS, the BMD increased by 9.7±3.7% (P<0.001) and 10.4±9.5% (P<0.001), respectively, compared to baseline. At the FN, the BMD changes were 1.02±6.2% (P=0.73) and -0.64±6.1% (P=0.65), respectively, compared to baseline. There were no significant differences between treatment with and without ELD (P=0.45 in the LS BMD; and P=0.60 in the FN BMD). The percentage change in BMD after 12 months of ROMO treatment, with and without ELD, at two sites in the non-CKD group is presented in Fig. 4A and B. At the LS, the BMD increased by 9.0±7.9% (P<0.001) and 13.1±10.3% (P<0.001), respectively, compared to baseline, while at the FN, the BMD changes were 1.9±5.6% (P=0.18) and 1.7±6.2% (P=0.13), respectively, compared to baseline. There were no significant differences between treatment with and without ELD (P=0.26 in the LS BMD; and P=0.56 in the FN BMD).

Changes in serum PINP levels in response to treatment. The serum PINP change rates from baseline after 1, 4, 8, and 12 months of ROMO treatment, with and without ELD are shown in Figs. 3C and 4C. In the CKD group, the changes were 101, 25, 15, -24% with ELD and 51, 25, -7, -25% without ELD, respectively (Fig. 3C). In the non-CKD group, the corresponding changes were 82, 38, 5, -6% with ELD and 80, 27, -2, -14% without ELD, respectively (Fig. 4C).

Table II. Patient demographics and clinical characteristics in the secondary analysis of subjects.

	CKD group			Non-CKD group		
Variables	ROMO + ELD (n=17)	ROMO monotherapy (n=47)	P-value	ROMO + ELD (n=18)	ROMO monotherapy (n=64)	P-value
Age (years)	86.53±4.84	84.70±7.66	0.390	85.61±7.06	80.61±8.91	0.033[a]
Female sex	14 (93.3%)	39 (90.7%)	>0.999	18 (100%)	51 (86.4%)	0.190
Height (cm)	148.82±6.88	149.62±7.22	0.745	149.58±6.4	150.57±7.2	0.618
Weight (kg)	45.19±6.48	45.89±8.37	0.792	42.52±5.65	45.79±9.75	0.197
BMI	20.31±2.47	20.49±3.85	0.881	19.02±2.17	20.17±4.05	0.271
Previous vertebral fracture, n (%)	4 (57.1)	20 (64.5)	0.230	7 (53.8)	29 (64.4)	0.593
Previous non-vertebral fracture, n (%)	3 (42.9)	11 (35.5)	>0.999	6 (46.2)	16 (35.6)	0.771
Mean percentage of BMD (YAM ± SD) at the lumbar spine (%)	77.87±14.98	76.93±14.17	0.829	73.83±11.14	73.10±16.48	0.861
Mean percentage of BMD (YAM ± SD) at the femoral neck (%)	68.67±10.16	66.99±8.56	0.550	64±9.26	63.14±8.54	0.714
Median serum PINP (IQR)	47.20 (15-147)	90.70 (19-363)	0.007[a]	59.50 (10.8-226)	(20.7-274) 67.70	0.764
Serum albumin	3.93±0.35	3.79±0.52	0.328	4.03±0.37	3.90±0.39	0.211
Serum Ca	9.41±0.66	9.49±0.75	0.713	9.32±0.46	9.34±0.49	0.845
Serum Cre	0.93±0.23	0.98±0.37	0.645	0.55±0.1	0.60±0.1	0.064
Serum eGFR	46.19±10.22	45.94±10.73	0.938	80.91±19.53	76.83±13.29	0.313
Diabetes, n (%)	3 (20)	3 (6.9)	0.172	0	9 (15.2)	0.106
Serum 25(OH)D	15.38±6.57	15.43±5.92	0.980	13.86±6.71	14.22±5.99	0.862
History of prior osteoporosis treatment, n (%)						
Naïve	3 (20)	19 (44.2)	0.122	6 (33.3)	30 (50.8)	0.283
Switch medications						
Bisphosphonates	5 (33.3)	5 (11.6)	0.114	2 (11.1)	13 (22)	0.493
Teriparatide	7 (46.7)	16 (37.2)	0.552	6 (33.3)	11 (18.6)	0.200
Denosumab	0 (0)	0 (0)		2 (11.1)	1 (1.7)	0.133
SERMs	0 (0)	3 (7.0)	0.563	1 (5.6)	2 (3.4)	0.552
Vitamin D analog	0 (0)	0 (0)	N/A	1 (5.6)	2 (3.4)	0.551

Data are expressed as the mean ± standard deviation or number (%) of subjects. PINP is expressed as a median value. Differences between the groups were analyzed using Fisher's exact test and Mann-Whitney U test. [a]Significant difference between the groups. ROMO, romosozumab; ELD, eldecalcitol; BMI, body mass index; BMD, bone mineral density; YAM, young adult mean; PINP, procollagen type I N-terminal propeptide; IQR, interquartile range; eGFR, estimated glomerular filtration rate; Ca, calcium; Cr, creatinine; 25(OH)D, 25-hydroxy vitamin D; SERMs, selective estrogen receptor modulators; N/A, not applicable.

In both groups, no significant change from baseline was observed, regardless of ELD administration. Additionally, no significant differences were found between treatments with and without ELD in either group.

Adverse events and new fractures. In the CKD group, there was one fracture (FN fracture) in the ROMO + ELD treatment group and no fractures in the ROMO treatment group. There were no significant differences in fracture incidence between treatment with and without ELD (P=0.090) (Table IV).

In the CKD group, adverse events that led to treatment discontinuation were reported in 1 patient (hyponatremia) who underwent ROMO treatment without ELD. There were no significant differences between treatments with or without ELD (P=0.55). In the non-CKD group, no fractures were observed after treatment with or without ELD.

Table III. Incidence of adverse events and new fractures in the primary analysis of subjects.

Parameters	CKD group (n=64)	Non-CKD group (n=82)	P-value
AEs leading to discontinuation of medication			0.712
Hyponatremia	1 (1.56) at 6 mo	0	
Injection site pain	0	1 (1.22) at 5 mo	
Renal dysfunction	0	1 (1.22) at 8 mo	
New fractures during the therapy			0.249
FN fracture	1 (1.56) at 10 mo	0	

Data are expressed as n (%). CKD, chronic kidney disease; AEs, adverse events; FN, femoral neck; mo, months.

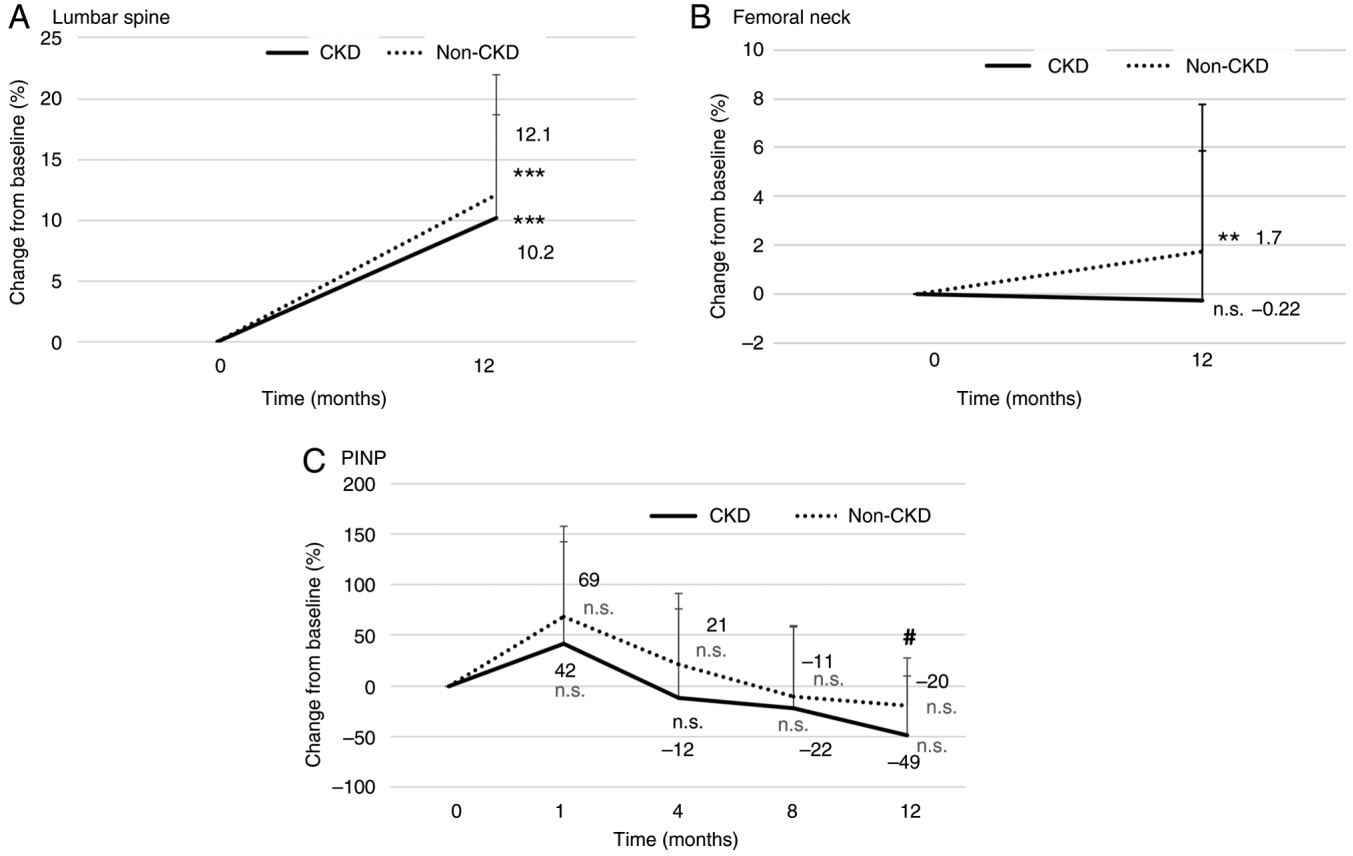

Figure 2. Mean percent changes from month 0 to month 12 in (A) LS BMD, (B) FN BMD, and (C) PINP. Bars indicate (A and B) the mean ± standard error, and (C) the median ± standard error. **P<0.05, ***P<0.001 and n.s. compared to baseline (repeated measures ANOVA); $^{\#}$P<0.05 between the groups (Mann-Whitney U test). BMD, bone mineral density; LS, lumbar spine; FN, femoral neck; PINP, procollagen type I N-terminal propeptide; CKD, chronic kidney disease; n.s., not significant.

Adverse events leading to treatment discontinuation were reported in 1 patient (renal dysfunction) receiving treatment with ELD and in another patient (injection site pain) receiving treatment without ELD. There were no significant differences between treatments with and without ELD (P=0.36).

Discussion

In the present study, the safety and efficacy of a 12-month ROMO treatment regimen in Japanese patients with osteoporosis grouped into CKD and non-CKD groups based on their baseline eGFR values (≥60 or <60 ml/min/1.73 m^2), were investigated. In addition, the ROMO + ELD treatment with respect to baseline eGFR was evaluated.

At the end of the 12-month ROMO treatment regimen, the LS BMD significantly increased regardless of baseline eGFR. The FN BMD slightly increased in the non-CKD group, whereas it decreased slightly in the CKD group after 12 months of ROMO treatment. Serum PINP levels were significantly elevated after 1 month and significantly decreased after 8 months and 12 months of ROMO treatment in both subgroups. The incidence of new fractures and adverse events was low in both subgroups, and the differences were not statistically significant between the subgroups. There

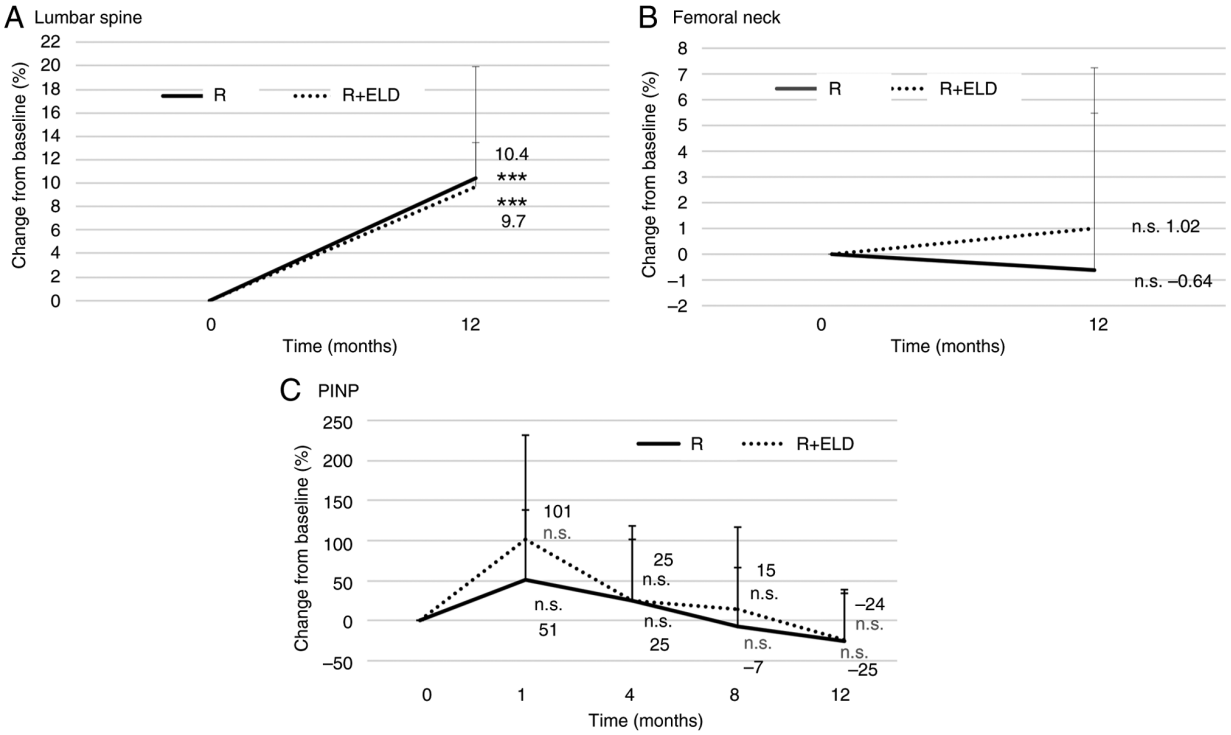

Figure 3. Mean percent changes from month 0 to month 12 in (A) LS BMD, (B) FN BMD, and (C) PINP in the CKD group. Bars indicate (A and B) the mean ± standard error, and (C) the median ± standard error. ***P<0.001 and n.s. compared to baseline (repeated measures ANOVA). BMD, bone mineral density; LS, lumbar spine; FN, femoral neck; PINP, procollagen type I N-terminal propeptide; R, romosozumab; ELD, eldecalcitol; n.s., not significant.

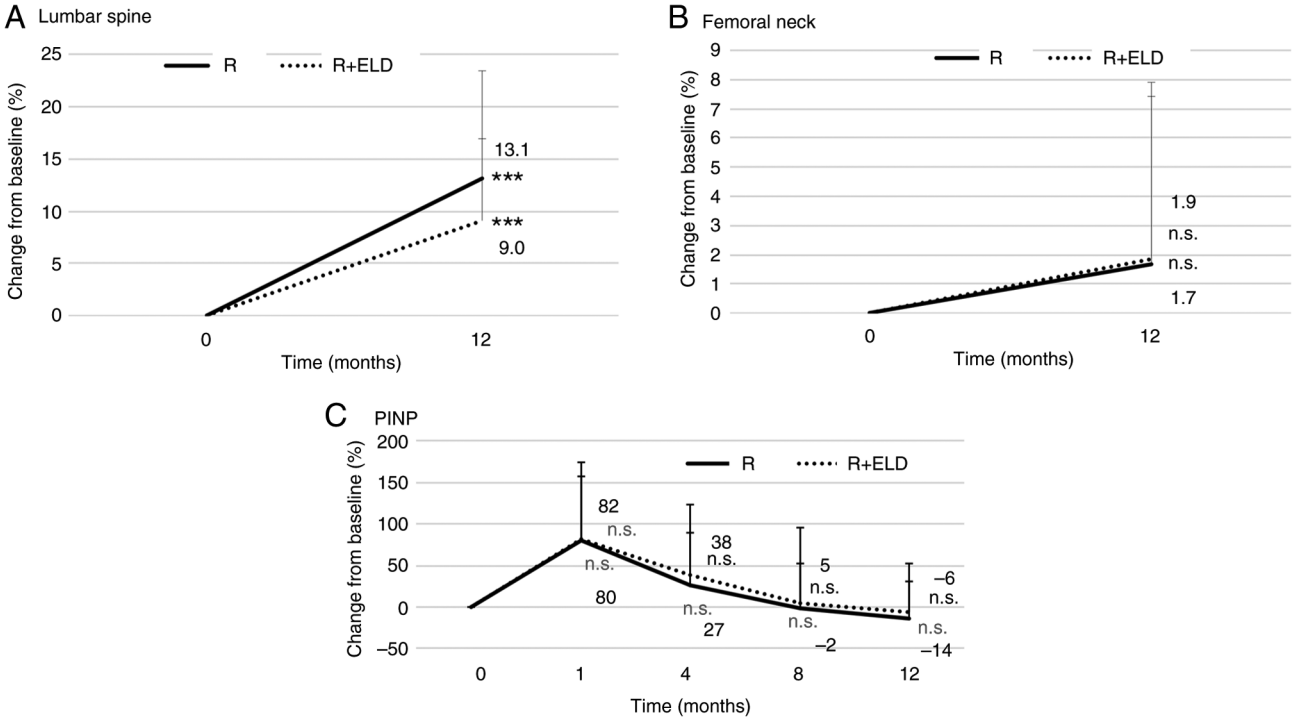

Figure 4. Mean percent changes from month 0 to month 12 in (A) LS BMD, (B) FN BMD, and (C) PINP in the non-CKD group. Bars indicate (A and B) the mean ± standard error, and (C) the median ± standard error. ***P<0.001 and n.s. compared to baseline (repeated measures ANOVA). BMD, bone mineral density; LS, lumbar spine; FN, femoral neck; PINP, procollagen type I N-terminal propeptide; R, romosozumab; ELD, eldecalcitol; n.s., not significant.

were no significant differences in BMD, fracture incidence, or adverse events between the combination treatment and ROMO monotherapy groups.

In the CKD group, the percentage change in the serum PINP levels and the FN BMD values observed were greater in the combination treatment group than in the ROMO

Table IV. Incidence of adverse events and new fractures in the secondary analysis of subjects.

	CKD group			Non-CKD group		
Parameters	ROMO + ELD (n=17)	ROMO monotherapy (n=47)	P-value	ROMO + ELD (n=18)	ROMO monotherapy (n=64)	P-value
AEs leading to discontinuation of drug			0.555			0.361
Hyponatremia	0	1 (2.12) at 6 mo		0	0	
Injection site pain	0	0		0	1 (1.56) at 5 mo	
Renal dysfunction	0	0		1 (5.55) at 8 mo	0	
New fractures during the therapy			0.090			N/A
FN fracture	1 (5.88) at 10 mo	0		0	0	

Data are expressed as n (%). CKD, chronic kidney disease; AEs, adverse events; FN, femoral neck; mo, months; N/A, not applicable.

monotherapy. Bone metabolism is essential for maintaining bone tissue homeostasis and function. Disruptions in this balance can lead to osteoporosis, osteoarthritis, bone defects, bone tumors, and other bone diseases (23,24). Osteoporosis is categorized as primary or secondary. Primary osteoporosis results from the natural aging process, whereas secondary osteoporosis is caused by various systemic diseases and organ disfunction, such as CKD and endocrine disorders. Glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis. Currently, the most widely used drugs for treating osteoporosis include anabolic agents, anti-resorptive agents, and medications with alternative mechanisms of action. If the underlying disease is unrecognized and left untreated, the response of secondary osteoporosis to conventional anti-osteoporotic therapy may be inadequate (25,26). In addition, numerous osteoporosis treatment drugs are associated with undesirable adverse effects or are unsuitable for long-term use. Thus, there is a critical need for the development of targeted molecules capable of safely preserving bone homeostasis. Recent research suggests that gut hormones such as GIP and GLP-1, may influence bone remodeling, with GIP inhibiting bone resorption and GLP-1 promoting bone formation (27). Flavonoids have been attracting attention as potential therapeutic targets (28-33). Recently, Yu *et al* (29) reported on osteoclast-targeting nanoparticles, named OAPLG, which were developed by integrating oroxylin A, a natural flavonoid, with amorphous calcium carbonate and coating them with glutamic acid hexapeptide-modified phospholipids. These smart nanoparticles can neutralize acid and release oroxylin A in the osteoclast microenvironment, synergistically inhibiting osteoclast formation and activity. This approach effectively reversed systemic bone loss in an ovariectomized mouse model (29).

Mesenchymal stem cells (MSCs) are mesoderm-derived adult stem cells with self-renewal and multilineage differentiation potential, capable of forming osteocytes, chondrocytes, adipocytes, and fibroblasts. They offer clinical advantages due to the ease of their isolation and secretion of growth factors. However, challenges such as low survival rates, immune rejection, and environmental sensitivity limit their direct therapeutic use. Extracellular vesicles (EVs), which are nano-sized vesicles released by cells, play a key role in intercellular communication and immune response (34). MSC-derived EVs (MSC-EVs) mimic MSC functions but are less immunogenic and more stable due to lipid protection. By combining the advantages of MSCs and EVs, MSC-EVs present a promising therapeutic option for bone disorders (35).

The kidneys play a critical role in bone development and metabolism by regulating calcium and phosphate homeostasis and the production of key substances such as $1,25(OH)_2D3$, klotho, and erythropoietin (36). Renal dysfunction causes phosphate retention, reduces calcitriol production, and leads to hypocalcemia. Reduced 1-α-hydroxylase activity and underexpression of parathyroid calcium-sensing and vitamin D receptors further contribute to low calcitriol levels, stimulating parathyroid hormone (PTH) secretion and resulting in secondary hyperparathyroidism. Excessive PTH levels lead to the release of calcium from bone. The mechanism of action of ELD is poorly characterized; however, ELD has demonstrated the ability to control the migration of osteoclast precursor monocytes and limit osteoclastic bone resorption (37). ROMO is a bone-forming agent that inhibits sclerostin, exerting a dual effect by increasing bone formation and decreasing bone resorption (38). Global sclerostin inhibition is linked to cardiovascular side effects. The β-1,4-N-acetyl-galactosaminyltransferase 3 (B4GALNT3) gene has been identified as a key regulator of circulating sclerostin

levels. Recent research has shown that B4GALNT3-mediated glycosylation of sclerostin may be a promising bone-specific osteoporosis target, separating anti-benefits from cardiovascular side effects (39).

The effect of ROMO on bone formation was revealed to be characterized by a rapid increase, which subsequently returns to baseline despite continued administration, whereas the reduction in circulating markers of bone resorption was sustained throughout the 12-month dosing period (38). Unfortunately, in the present study the markers of bone resorption were not assessed, limiting the present analysis to PINP. In the present study, the rate of increase in PINP levels peaked at month 1, followed by a decline below baseline values over time, which is consistent with previous findings (38).

The present study revealed several important findings. First, the study demonstrated that, in actual clinical practice, ROMO is an effective and safe treatment option for patients with osteoporosis and impaired kidney function. The incidence of serious adverse events was similarly low in both eGFR subgroups, which may be pharmacologically plausible given that ROMO is a humanized monoclonal IgG2 antibody with a large molecular size. The glomerular filtration of monoclonal antibodies is limited by the size of the protein molecule; molecules >70 kDa are not filtered in glomeruli (40). Thus, there may have been minimal renal filtration of ROMO (molecular weight, 145 kDa). Therefore, ROMO may be an effective and safe treatment option for patients with CKD.

Second, the combination of active vitamin D analog with ROMO did not significantly affect the rate of increase in BMD with and without CKD. Currently, several studies have reported that combination treatment with active vitamin D and bisphosphonate or denosumab increased BMD gains compared with monotherapy; however, the superiority of combination treatment is controversial (41-43). In regard to the combination treatment of ROMO with active vitamin D analog in previous studies, Kobayakawa *et al* (21) compared the efficacy of 12-month ROMO in the presence or absence of an active vitamin D analog and reported that such combination treatment may not be essential in terms of BMD improvement in postmenopausal patients with osteoporosis (21). In the course of CKD, there is a slow and progressive reduction in the levels of 1,25-dihydroxyvitamin D as kidney function declines. A 2023 systematic review found that vitamin D receptor activators effectively suppress PTH levels but carry an increased risk of hypercalcemia (36). In particular, ELD slightly increased the risk of hypercalcemia in patients with stage 3B CKD (44). The effects of active vitamin D analog among people with CKD are uncertain. There are no reports published regarding the combination treatment of ROMO and active vitamin D analogs across different levels of renal function. In the present present study, it was revealed that the use of an active vitamin D analog did not significantly influence the increase in BMD, regardless of baseline eGFR.

Third, the results of the present study revealed a greater increase in serum PINP levels and the FN BMD with combination treatment in the CKD group. Additionally, baseline serum PINP levels in the CKD group were higher in the ROMO monotherapy group than in the combination treatment group. Baseline serum PINP levels are reportedly associated with elevated hip bone BMD after ROMO treatment (45). Conversely, in the CKD group, the percentage change in the FN BMD observed in the combination treatment group was greater than that in the ROMO monotherapy group.

There is a well-documented association between an early increase in PINP levels after ROMO induction and ROMO treatment response (46,47). A previous study showed that patients with a greater increase in PINP levels at 1 month had improved BMD gains over 12 months (46).

In the CKD group, the combination treatment group showed substantial increase in serum PINP levels at 1 month. The serum $1,25(OH)_2D$ levels were significantly affected by the degree of renal failure. A reduced renal mass and uremic factors are considered to lower the activity of 25(OH)D3 1-α-hydroxylase. A significant positive correlation between $1,25(OH)_2D$ and estimated creatinine clearance has been reported (48) Therefore, ROMO + ELD treatment may have a greater impact by restoring the active form of vitamin D, which is often depleted in patients with CKD.

These results indicate that ROMO + ELD treatment may be beneficial for patients with an eGFR of <60 ml/min/1.73 m^2.

Several limitations of this study should be acknowledged. Firstly, only a small number of patients received combination treatment with ROMO and an active vitamin D analog, which limited the assessment of the efficacy and safety of this combination treatment. In particular, the number of patients with severe renal insufficiency (eGFR ≤29 ml/min/1.73 m^2) was extremely limited (ROMO, 5 patients; ROMO + ELD, 1 patient), preventing a detailed subgroup analysis based on renal function from being conducted.

Although it was not possible in this study due to the small sample size, the effectiveness of ROMO + ELD treatment on BMD may potentially be demonstrated by collecting data on detailed renal function parameters such as eGFR ranges of 0-29, 30-59, 60-89, and ≥90 ml/min/1.73 m^2.

Secondly, there were significant differences in baseline age between the CKD and non-CKD groups. Notably, changes in bone metabolism associated with aging result in a net bone loss, with some studies indicating a more pronounced relative loss and thinning with age for all trabeculae (49-51). Changes in bone metabolism associated with aging may affect the increase in BMD. Therefore, there is a possibility that the present findings may change when comparing individuals with different kidney functions within the same age group. Thirdly, the effects of prior osteoporosis treatments were not considered in the present study.

Fourthly, the present study did not consider populations with conditions affecting vitamin D metabolism, such as liver disease. Cirrhosis leads to vitamin D deficiency due to impaired metabolism and malabsorption, contributing to osteoporosis and osteomalacia. Liver disease disrupts calcium and vitamin D balance, reducing bone formation and increasing bone loss, with alcohol-related liver disease and primary biliary cirrhosis further elevating the risk of osteoporosis (52,53). In such populations, combining ROMO with ELD may enhance BMD more effectively than ROMO monotherapy. Thus, additional large-scale randomized controlled trials are required to address these limitations.

Lastly, only serum PINP levels were evaluated among the various bone turnover markers. There is a possibility that other bone turnover markers are more strongly associated with the BMD response.

In summary, the results of the present study revealed that ROMO was an effective and safe treatment option for osteoporosis regardless of renal function. Comination treatment with ROMO and ELD may be beneficial for patients with an eGFR of <60 ml/min/1.73 m^2.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

TU contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data, as well as manuscript preparation. TU and TK confirm the authenticity of all the raw data. TK and KM contributed to the design of the study, and acquisition of data. MH contributed to the design of the study. All authors read and approved the manuscript and agree to be accountable for all aspects of the research, ensuring that any issues related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Ethics approval and consent to participate

The requirement for institutional review board approval from our institute was waived owing to the anonymized and retrospective nature of this study; however, written informed consent was obtained from the patients to perform further research.

Patient consent to publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. de Villiers TJ and Goldstein SR: Bone health 2022: An update. Climacteric 25: 1-3, 2022.
2. Borgström F, Karlsson L, Ortsäter G, Norton N, Halbout P, Cooper C, Lorentzon M, McCloskey EV, Harvey NC, Javaid MK, *et al*: Fragility fractures in Europe: Burden, management and opportunities. Arch Osteoporos 15: 59, 2020.
3. Hanafusa N, Abe M, Joki N, Hoshino J, Kikuchi K, Goto S, Kanda E, Taniguchi M, Nakai S, Naganuma T, *et al*: Annual dialysis data report 2020, JSDT Renal Data Registry. Ren Replace Ther 10: 14, 2024.
4. GBD Chronic Kidney Disease Collaboration: Global, regional, and national burden of chronic kidney disease, 1990-2017: A systematic analysis for the global burden of disease study 2017. Lancet 395: 709-733, 2020.
5. Nickolas TL, McMahon DJ and Shane E: Relationship between moderate to severe kidney disease and hip fracture in the United States. J Am Soc Nephrol 17: 3223-3232, 2006.
6. Tentori F, McCullough K, Kilpatrick RD, Bradbury BD, Robinson BM, Kerr PG and Pisoni RL: High rates of death and hospitalization follow bone fracture among hemodialysis patients. Kidney Int 85: 166-173, 2014.
7. Chen CH, Lo WC, Hu PJ, Chan HC, Shen WC, Wu MS and Wu MY: Efficacy of osteoporosis medications for patients with chronic kidney disease: An updated systematic review and network meta-analysis. Front Pharmacol 13: 822178, 2022.
8. Thongprayoon C, Acharya P, Acharya C, Chenbhanich J, Bathini T, Boonpheng B, Sharma K, Wijarnpreecha K, Ungprasert P, Gonzalez Suarez ML and Cheungpasitporn W: Hypocalcemia and bone mineral density changes following denosumab treatment in end-stage renal disease patients: A meta-analysis of observational studies. Osteoporos Int 29: 1737-1745, 2018.
9. Sumida K, Ubara Y, Hoshino J, Mise K, Hayami N, Suwabe T, Kawada M, Imafuku A, Hiramatsu R, Hasegawa E, *et al*: Once-weekly teriparatide in hemodialysis patients with hypoparathyroidism and low bone mass: A prospective study. Osteoporos Int 27: 1441-1450, 2016.
10. Cosman F, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, Hofbauer LC, Lau E, Lewiecki EM, Miyauchi A, *et al*: Romosozumab treatment in postmenopausal women with osteoporosis. N Engl J Med 375: 1532-1543, 2016.
11. Miyauchi A, Hamaya E, Nishi K, Tolman C and Shimauchi J: Efficacy and safety of romosozumab among Japanese postmenopausal women with osteoporosis and mild-to-moderate chronic kidney disease. J Bone Miner Metab 40: 677-687, 2022.
12. Miller PD, Adachi JD, Albergaria BH, Cheung AM, Chines AA, Gielen E, Langdahl BL, Miyauchi A, Oates M, Reid IR, *et al*: Efficacy and safety of romosozumab among postmenopausal women with osteoporosis and mild-to-moderate chronic kidney disease. J Bone Miner Res 37: 1437-1445, 2022.
13. LaClair RE, Hellman RN, Karp SL, Kraus M, Ofner S, Li Q, Graves KL and Moe SM: Prevalence of calcidiol deficiency in CKD: A cross-sectional study across latitudes in the United States. Am J Kidney Dis 45: 1026-1033, 2005.
14. Craver L, Marco MP, Martínez I, Rue M, Borràs M, Martín ML, Sarró F, Valdivielso JM and Fernández E: Mineral metabolism parameters throughout chronic kidney disease stages 1-5-achievement of K/DOQI target ranges. Nephrol Dial Transplant 22: 1171-1176, 2007.
15. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group: KDIGO 2017 clinical practice guideline update for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). Kidney Int Suppl (2011) 7: 1-59, 2017.
16. Pluskiewicz W, Adamczyk P, Czekajło A, Grzeszczak W, Burak W and Drozdzowska B: Epidemiological data on osteoporosis in women from the RAC-OST-POL study. J Clin Densitom 15: 308-314, 2012.
17. United Nations Department of Economic and Social Affairs, Population Division. World Population Prospects 2022: Summary of Results. UN DESA/POP/2022/TR/NO. 3, 2022.
18. Saito K, Miyakoshi N, Matsunaga T, Hongo M, Kasukawa Y and Shimada Y: Eldecalcitol improves muscle strength and dynamic balance in postmenopausal women with osteoporosis: An open-label randomized controlled study. J Bone Miner Metab 34: 547-554, 2016.
19. Ling Y, Xu F, Xia X, Dai D, Xiong A, Sun R, Qiu L and Xie Z: Vitamin D supplementation reduces the risk of fall in the vitamin D deficient elderly: An updated meta-analysis. Clin Nutr 40: 5531-5537, 2021.
20. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H and Hishida A; Collaborators developing the Japanese equation for estimated GFR: Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 53: 982-992, 2009.
21. Kobayakawa T, Miyazaki A, Takahashi J and Nakamura Y: Effects of romosozumab with and without active vitamin D analog supplementation for postmenopausal osteoporosis. Clin Nutr Espen 48: 267-274, 2022.
22. U.S. Department of Health and Human Services. Common terminology criteria for. adverse events (CTCAE) version 5.0. National Institutes of Health, National Cancer. Institute, 2017. https: //ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. Accessed February 27, 2025.

23. Faber BG, Frysz M, Boer CG, Evans DS, Ebsim R, Flynn KA, Lundberg M, Southam L, Hartley A, Saunders FR, *et al*: The identification of distinct protective and susceptibility mechanisms for hip osteoarthritis: Findings from a genome-wide association study meta-analysis of minimum joint space width and Mendelian randomisation cluster analyses. EBioMedicine 95: 104759, 2023.
24. Nakao H, Yokomoto-Umakoshi M, Nakatani K, Umakoshi H, Ogata M, Fukumoto T, Kaneko H, Iwahashi N, Fujita M, Ogasawara T, *et al*: Adrenal steroid metabolites and bone status in patients with adrenal incidentalomas and hypercortisolism. EBioMedicine 95: 104733, 2023.
25. Ebeling PR, Nguyen HH, Aleksova J, Vincent AJ, Wong P and Milat F: Secondary osteoporosis. Endocr Rev 43: 240-313, 2022.
26. Dong L, Jiang L, Xu Z and Zhang X: Denosumab, teriparatide and bisphosphonates for glucocorticoid-induced osteoporosis: A Bayesian network meta-analysis. Front Pharmacol 15: 1336075, 2024.
27. Liu H, Xiao H, Lin S, Zhou H, Cheng Y, Xie B and Xu D: Effect of gut hormones on bone metabolism and their possible mechanisms in the treatment of osteoporosis. Front Pharmacol 15: 1372399, 2024.
28. Rodríguez V, Rivoira M, Picotto G, de Barboza GD, Collin A and Tolosa de Talamoni N: Analysis of the molecular mechanisms by flavonoids with potential use for osteoporosis prevention or therapy. Curr Med Chem 29: 2913-2936, 2022.
29. Yu B, Gao Q, Sheng S, Zhou F, Geng Z, Wei Y, Zhang H, Hu Y, Wang S, Huang J, *et al*: Smart osteoclasts targeted nanomedicine based on amorphous CaCO3 for effective osteoporosis reversal. J Nanobiotechnology 22: 153, 2024.
30. Zhao L, Cai C, Wang J, Zhao L, Li W, Liu C, Guan H, Zhu Y and Xiao J: Dihydromyricetin. protects against bone loss in ovariectomized mice by suppressing osteoclast activity. Front Pharmacol 8: 928, 2017.
31. Xu L, Sun X, Han X, Li H, Li X, Zhu L, Wang X, Li J and Sun H: Dihydromyricetin ameliorate postmenopausal osteoporosis in ovariectomized mice: Integrative microbiomic and metabolomic analysis. Front Pharmacol 15: 1452921, 2024.
32. Chen YJ, Jia LH, Han TH, Zhao ZH, Yang J, Xiao JP, Yang HJ and Yang K: Osteoporosis treatment: Current drugs and future developments. Front Pharmacol 15: 1456796, 2024.
33. Deng TT, Ding WY, Lu XX, Zhang QH, Du JX, Wang LJ, Yang MN, Yin Y and Liu FJ: Pharmacological and mechanistic aspects of quercetin in osteoporosis. Front Pharmacol 15: 1338951, 2024.
34. Wu Y, Song P, Wang M, Liu H, Jing Y and Su J: Extracellular derivatives for bone metabolism. J Adv Res 66: 329-347, 2024.
35. Zeng ZL and Xie H: Mesenchymal stem cell-derived extracellular vesicles: A possible therapeutic strategy for orthopaedic diseases: A narrative review. Biomater Transl 3: 175-187, 2022.
36. Yeung WG, Palmer SC, Strippoli GFM, Talbot B, Shah N, Hawley CM, Toussaint ND and Badve SV: Vitamin D therapy in adults with CKD: A systematic review and meta-analysis. Am J Kidney Dis 82: 543-558, 2023.
37. Kikuta J, Kawamura S, Okiji F, Shirazaki M, Sakai S, Saito H and Ishii M: Sphingosine-1-phosphate-mediated osteoclast precursor monocyte migration is a critical point of control in antibone-resorptive action of active vitamin D. Proc Natl Acad Sci USA 110: 7009-7013, 2013.
38. McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A, Langdahl BL, Reginster JY, Zanchetta JR, Wasserman SM, *et al*: Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med 370: 412-420, 2014.
39. Movérare-Skrtic S, Voelkl J, Nilsson KH, Nethander M, Luong TTD, Alesutan I, Li L, Wu J, Horkeby K, Lagerquist MK, *et al*: B4GALNT3 regulates glycosylation of sclerostin and bone mass. EBioMedicine 91: 104546, 2023.
40. Valverde MG, Mille LS, Figler KP, Cervantes E, Li VY, Bonventre JV, Masereeuw R and Zhang YS: Biomimetic models of the glomerulus. Nat Rev Nephrol 18: 241-257, 2022.
41. Sakai S, Endo K, Takeda S, Mihara M and Shiraishi A: Combination therapy with eldecalcitol and alendronate has therapeutic advantages over monotherapy by improving bone strength. Bone 50: 1054-1063, 2012.
42. Nakamura Y, Suzuki T, Kamimura M, Murakami K, Ikegami S, Uchiyama S and Kato H: Vitamin D and calcium are required at the time of denosumab administration during osteoporosis treatment. Bone Res 5: 17021, 2017.
43. Suzuki T, Nakamura Y and Kato H: Effects of monthly minodronate with or without eldecalcitol addition in osteoporosis patients with rheumatoid arthritis: An 18-month prospective study. Osteoporos Sarcopenia 5: 122-127, 2019.
44. Kondo S, Takano T, Ono Y, Saito H and Matsumoto T: Eldecalcitol reduces osteoporotic fractures by unique mechanisms. J Steroid Biochem Mol Biol 148: 232-238, 2015.
45. Kashii M, Kamatani T, Nagayama Y, Miyama A, Tsuboi H and Ebina K: Baseline serum PINP level is associated with the increase in hip bone mineral density seen with romosozumab treatment in previously untreated women with osteoporosis. Osteoporos Int 34: 563-572, 2023.
46. Takada J, Dinavahi R, Miyauchi A, Hamaya E, Hirama T, Libanati C, Nakamura Y, Milmont CE and Grauer A: Relationship between P1NP, a biochemical marker of bone turnover, and bone mineral density in patients transitioned from alendronate to romosozumab or teriparatide: A post hoc analysis of the STRUCTURE trial. J Bone Miner Metab 38: 310-315, 2020.
47. Hattori K and Kanayama Y: Association between P1NP value and increases in bone mineral density in patients with postmenopausal osteoporosis treated with romosozumab. Mod Rheumatol 34: 1047-1055, 2024.
48. Ishimura E, Nishizawa Y, Inaba M, Matsumoto N, Emoto M, Kawagishi T, Shoji S, Okuno S, Kim M, Miki T and Morii H: Serum levels of 1,25-dihydroxyvitamin D, 24,25-dihydroxyvitamin D, and 25-hydroxyvitamin D in nondialyzed patients with chronic renal failure. Kidney Int 55: 1019-1027, 1999.
49. Pignolo RJ: Aging and bone metabolism. Compr Physiol 13: 4355-4386, 2023.
50. McCalden RW, McGeough JA and Court-Brown CM: Age-related changes in the compressive strength of cancellous bone. The relative importance of changes in density and trabecular architecture. J Bone Joint Surg Am 79: 421-427, 1997.
51. Chen H, Zhou X, Fujita H, Onozuka M and Kubo KY: Age-related changes in trabecular and cortical bone microstructure. Int J Endocrinol 2013: 213234, 2013.
52. Ravaioli F, Pivetti A, Di Macro L, Chrysanthi C, Frassanito G, Pambianco M, Sicuro C, Gualandi N, Guasconi T, Pecchini M and Colecchia A: Role of vitamin D in liver disease and complications of advanced chronic liver disease. Int J Mol Sci 23: 9016, 2022.
53. Konstantakis C, Tselekouni P, Kalafateli M and Triantos C: Vitamin D deficiency in patients with liver cirrhosis. Ann Gastroenterol 29: 297-306, 2016.