Effect of ONO-5334 on Bone Mineral Density and Biochemical Markers of Bone Turnover in Postmenopausal Osteoporosis: 2-year Results from the OCEAN Study†

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DISCLOSURE
Mr Shinichi Nagase (SN), Ms Michiyo Ohyama (MO), Ms Maria Small, Mr Tomohiro Kuwayama (TK) and Dr Steve Deacon are employees of the study sponsor, Ono Pharmaceutical Co., Ltd. (Japan) or its European office, ONO PHARMA UK LTD (UK). SN, MO and TK have stock options of Ono Pharmaceutical Co., Ltd. Professor Richard Eastell, Professor Steven Boonen and Professor Tim Spector have provided the study sponsor with scientific advice and have received consultancy fees as members of the study’s Steering Committee.
ABSTRACT

Cathepsin K inhibitors, such as ONO-5334, are being developed for the treatment of postmenopausal osteoporosis. However, their relative effects on bone resorption and formation, and how quickly the effects resolve after treatment cessation, are uncertain. The aim of this study was to examine the efficacy and safety of 24 months treatment with ONO-5334 and to assess the effect of treatment cessation over 2 months. We studied 197 postmenopausal women with osteoporosis or osteopenia with one fragility fracture. Patients were randomised to ONO-5334 50 mg twice daily, 100 mg or 300 mg once daily, alendronate 70 mg once weekly (positive control) or placebo for 24 months. After 24 months, all ONO-5334 doses were associated with increased bone mineral density (BMD) for lumbar spine, total hip and femoral neck (p<0.001). ONO-5334 300 mg significantly suppressed the bone-resorption markers urinary (u) NTX and serum and uCTX-I throughout 24 months of treatment, and to a similar extent as alendronate; other resorption marker levels remained similar to placebo (fDPD for ONO-5334 300 mg qd) or were increased (ICTP, TRAP5b, all ONO-5334 doses). Levels of B-ALP and PINP were suppressed in all groups (including placebo) for approximately 6 months, but then increased for ONO-5334 to close to baseline levels by 12-24 months. On treatment cessation, there were increases above baseline in uCTX-I, uNTX and TRAP5b, and decreases in ICTP and fDPD. There were no clinically relevant safety concerns. Cathepsin K inhibition with ONO-5334 resulted in decreases in most resorption markers over 2 years, but did not decrease most bone formation markers. This was associated with an increase in BMD; the effect on biochemical markers was rapidly reversible on treatment cessation.

Key words: DXA, Biochemical markers of bone turnover, Clinical trials, Osteoporosis, Menopause
1 INTRODUCTION

The fractures that result from osteoporosis are a major public health problem but there are now effective treatments for reducing fracture risk. The most commonly used agents are the bisphosphonates, which have been shown to reduce the risk for vertebral fractures by up to 70% compared with placebo. However, it is known that the anti-fracture efficacy of bisphosphonates on non-vertebral fractures is significantly less than that on vertebral fractures.\(^{(1)}\) In a meta-analysis based on key regulatory trials, the estimated risk reduction values for non-vertebral fractures were 0.86 (95% confidence interval [CI]: 0.76, 0.97) for alendronate and 0.81 (95% CI: 0.71, 0.92) for risedronate, respectively,\(^{(2)}\) suggesting that the magnitude of non-vertebral fracture risk reduction with antiresorptives is close to 20%. In line with this assumption, recent studies with intravenous zoledronic acid and subcutaneous denosumab yielded similar reductions in fracture risk; in the HORIZON-PFT and FREEDOM trials, zoledronic acid and denosumab reduced non-vertebral fracture risk by 25% and 20%, respectively.\(^{(3,4)}\) Furthermore, as osteoporosis requires chronic medicinal treatment, there is concern that by decreasing bone turnover for many years, there may be complications, such as atypical fractures of the femur, associated with bisphosphonates.\(^{(1)}\)

Novel targets identified as having an effect on the increased osteoclast activity of osteoporosis without affecting bone formation include enzymes such as cathepsin K.\(^{(5,6)}\) Secreted into resorption lacunae beneath the ruffled border of the osteoclast, cathepsin K is responsible for the degradation of the organic matrix of bone.\(^{(6-8)}\) The potential of inhibition of cathepsin K to reduce bone resorption and thus increase bone mineral density (BMD) has been confirmed in postmenopausal women.\(^{(9-13)}\)

ONO-5334 is a synthetic low molecular weight inhibitor of cathepsin K that is being developed as an oral therapeutic agent for bone metabolic diseases including osteoporosis. In the preclinical setting, ONO-5334 was identified as a potent and selective inhibitor of cathepsin K \textit{in vitro} and was
associated with increases in BMD and bone strength in an in vivo ovariectomised monkey osteoporosis model. Clinically, ONO-5334 was associated with marked suppression of bone resorption markers in healthy postmenopausal women.

The ONO-5334 cathepsin K inhibitor European (OCEAN) study was a proof of concept study that investigated the efficacy and safety of ONO-5334 in postmenopausal osteoporosis. The study compared three different daily doses of ONO-5334 (50 mg twice daily [bid], 100 mg once daily [qd] or 300 mg qd) with placebo and standard of care alendronate (70 mg once weekly [qw]). The treatment duration was originally set as 12 months, but then extended to a total of 24 months of treatment with a 2-month follow up assessment. By the 12-month time point, all ONO-5334 dose strengths were associated with increases in BMD for lumbar spine, total hip (except for the 100 mg qd dose group) and femoral neck bone. The primary objective of the study extension was to investigate the long-term safety of ONO-5334. The extension also provided an opportunity for the collection of additional data relating to the efficacy profile of ONO-5334 in terms of BMD, bone turnover markers and offset effects prior to investigating anti-fracture efficacy in clinical trials.
2 MATERIALS AND METHODS

This study extension was conducted from 15 December 2008 to 12 September 2010 at 13 study sites in six European countries in accordance with applicable regulations and guidelines of the International Conference on Harmonisation-Good Clinical Practice. The study was approved by local ethics committees and regulatory authorities and patients gave written informed consent for their participation in the extension phase of the study.

Participants
All women who completed the original OCEAN study were eligible to take part in the extension phase of the study. Patients were postmenopausal women aged 55 to 75 years with osteoporosis or osteopenia with one fragility fracture (at the start of the OCEAN study) but otherwise in good general health. Postmenopausal was defined as more than 5 years following onset of the menopause, with plasma estradiol less than 92 pmol/L and follicle-stimulating hormone greater than 30 IU/L. Patients were diagnosed using BMD T-score. Osteoporosis was defined as a T-score of -2.5 or less, and osteopenia was defined as a T-score of less than -1 and greater than -2.5 at the lumbar spine (LS) or total hip. Patients were excluded if the Investigator had any safety concerns and judged that continuing in the study for a further 12 months would not be in the best interest of the patient.

Study Design
This was a 12-month extension to the OCEAN study, which was originally designed as a 12-month multicentre, double-blind, randomised placebo- and active-controlled, parallel group study. The initial study design involved two phases: a 2–6 week screening phase and a 12-month treatment phase. Patients were randomised to one of five treatment arms: placebo, ONO-5334 50 mg bid, 100 mg qd, 300 mg qd, and alendronate 70 mg qw. Randomization was stratified by the following study-balancing factors: study site, diagnosis (osteopenia plus a fragility fracture or osteoporosis),
and urinary type-I collagen C-telopeptide (CTX-I) (below/above 300 mg/mmol Cr). Treatment assigned in the initial 12-month treatment phase was maintained during the 12-month extended treatment phase and dosing instructions were not changed. Daily medication, ONO-5334 or matching placebo, was given in the morning and the evening owing to one of the treatment arms being a bid regimen. Patients were instructed to take the morning dose after breakfast and the evening dose at any time before bed. Active treatment of ONO-5334 qd was given as an evening dose. Alendronate 70 mg or its matching placebo was administered qw. Throughout the study all patients received daily supplemental calcium (at least 500 mg a day) and vitamin D (at least 400 IU a day).

Study Assessments

The methods and procedures used during this 12-month extension were the same as those published previously for the OCEAN study. Timings from the OCEAN study were maintained and all procedures for the study extension are identified as starting at Month 12. Lumbar spine (L1-4), total hip and femoral neck were measured with dual-energy X-ray absorptiometry (DXA) at Month 18 and Month 24. Biochemical markers, urinary N-terminal telopeptide (NTX), serum and urinary CTX-I, urinary free deoxypyridinoline (fDPD), serum cross-linked type I collagen C-telopeptide (I-CTP), tartrate-resistant acid phosphatase isoform 5b (TRAP5b), bone specific alkaline phosphatase (B-ALP), procollagen type I intact N-terminal propeptide (PINP) and serum osteocalcin (OC) were assessed at months 18, 24, 25 and 26 (months 25 and 26 were post-treatment assessment performed as Follow-up 1 and Follow-up 2).

An independent Data Monitoring Committee (DMC) was responsible for overseeing the safety of study participants, reviewing safety data on a regular basis. The DMC reported to a Steering Committee (SC). Standard safety assessments, including adverse event (AE) monitoring, physical examination, 12-lead electrocardiogram (Clearstone Central Laboratories, Baillet en France,
France), vital signs, haematology, biochemistry and urinalysis continued to be performed throughout the extended period of study. Treatment emergent AEs (TEAEs) were defined as events whose onset occurred, severity worsened or intensity increased after receiving double-blind study medication. All TEAEs were summarised by maximum severity for each treatment group.

Statistical Analysis

The primary efficacy variable for the study was the mean lumbar spine BMD. The primary analysis was based on the percentage change from baseline at Month 24 and was performed on the Full Analysis Set (FAS) population using a final ‘on therapy’ (last observation carried forward, LOCF) approach to account for patients who withdrew prematurely from the study.

Each dose of ONO-5334 and alendronate was compared with placebo using analysis of covariance (ANCOVA) with treatment group and country as factors in the model. Baseline values for age, body mass index (BMI), lumbar spine BMD and urinary CTX-I were used as covariates. Total hip and femoral neck BMD were key secondary endpoints and were analysed using a similar ANCOVA model. Bone mineral density data (% change from baseline) is shown as mean +/- SE in this paper.

Analysis of covariance was performed on the change from baseline in log-transformed values for each of the bone turnover markers on the FAS population using LOCF data. Changes in bone turnover markers were expressed as a ratio of geometric means compared with baseline values. Expression of the urinary markers of bone turnover are presented relative to creatinine. The baseline value of each marker was fitted as a covariate in addition to those factors fitted in the model used for primary analysis.
With a sample size of 42 patients in each group, the study had 95% power to detect a difference of 4.0% between ONO-5334 and placebo in BMD at Month 12, assuming standard deviation was 5.0% using a two-group t-test with a 5% two-sided significance level. As this was an exploratory study, no adjustment was made to control the type I error for multiple treatment groups of ONO-5334.

The primary analysis was conducted using the data from the original 12-month treatment phase. After the database had been locked for the 12-month data, some sponsor and clinical research organisation (CRO) staff were made aware of the treatment arms to which patients were assigned but not those study personnel directly involved in the management of the study. The SC, consisting of the investigators RE, SB and TS who were not employees of the study sponsor, oversaw the execution of the protocol and planned the analyses for the study before study-drug assignments were unblinded. However, the Investigators, SC and patients remained blinded.
RESULTS

Study Population Disposition and Demographics

One hundred and ninety-seven of the 265 patients who completed the original OCEAN protocol and were considered eligible to take part in the 12-month extension phase were enrolled into the study extension (Figure 1). The demographic profile and baseline characteristics of patients taking in the study extension was highly conserved from the patient population reported for the OCEAN study with no clinically relevant difference across the five treatment arms in any of the demographic or baseline characteristics. Mean patient compliance to dosing schedules (assessed by pill counting of returned tablets) during the study extension was generally high for each of the study groups, ranging from 93.3% to 100.5%. The majority of individual patients reported levels of compliance that ranged from 80% to 120%. There was no notable difference in treatment compliance across five treatment groups.

Efficacy

Bone Mineral Density

Figure 2 summarises the changes in BMD measures from baseline to the end of the 12-month study period of the original OCEAN protocol and for the following 12-month study extension (Month 12 to Month 24). During the 12-month treatment phase previously reported, all doses of ONO-5334 (with the exception of the 100 mg qd dose at the total hip site) showed significant increases in BMD at all three measurement sites. Bone mineral density continued to increase numerically with active treatments at all three sites during the study extension. All doses of ONO-5334 were associated with statistically significant (p<0.001) increases from baseline in the LS primary efficacy variable at 24 months compared with placebo (Figure 2a). At Month 24 increases in BMD from baseline (Month 0) following exposure to ONO-5334 were greatest in the 300 mg qd dose group, mean increases at the LS, total hip and femoral neck sites were 6.7 ± 0.59, 3.4 ± 0.43 and 3.7 ± 0.52%, respectively. Overall increases in BMD from baseline (Month 0) following
alendronate at LS, total hip and femoral neck (6.3 ± 0.57, 4.2 ± 0.42 and 2.9 ± 0.51%, respectively) were similar to those seen for ONO-5334 300 mg qd. In contrast to the active treatment groups, BMD at LS, total hip and femoral neck sites in the placebo group demonstrated little or no change from Month 12 to Month 24. There appeared to be a general qualitative downward trend in BMD from Month 6 to Month 24 (Figure 2).

*Biochemical markers of bone turnover*

Figure 3 summarises the changes in urinary NTX, serum and urinary CTX-I, fDPD, I-CTP and TRAP5b from baseline to the end of the 12-month study period of the original OCEAN protocol, the following 12-month extension (Month 12 to Month 24) and the 2-month follow up period. In the ONO-5334 300 mg qd and alendronate treatment groups, maintenance of active treatment regimens from Month 12 to Month 24 was associated with levels of suppression of urinary NTX, urinary CTX-I and serum CTX-I that were similar to those seen from baseline to Month 12. However, for the ONO-5334 50 mg bid and 100 mg qd dose groups, there appeared to be a gradual return of serum CTX-I levels to those of baseline; this trend was observed from approximately 6 months onwards. This return to baseline levels was not observed for urine CTX-I or markedly for urine NTX. Cessation of ONO-5334 dosing at Month 24 was associated with a marked increase in urinary NTX, serum and urinary CTX-I beyond baseline values. Differences in the levels of NTX and CTX-I between the Month 24 and two follow up values suggest that levels were returning to baseline values. In contrast, there was only a relatively small increase (towards baseline values) in urinary NTX, serum and urinary CTX-I following cessation of alendronate therapy.

Exposure to ONO-5334 from baseline to Month 12 was associated with modest increases in the levels of fDPD, increases that continued from Month 12 to Month 24 for the 50 mg bid and 100 mg qd dose groups. In contrast, levels of fDPD in the ONO-5334 300 mg qd group remained within the range of those in the placebo group. Samples taken at the follow-up visits appear to indicate
that levels of fDPD in the ONO-5334 50 mg bid and 100 mg qd were returning to baseline values whereas those for ONO-5334 300 mg qd increased (Figure 3d). Levels of fDPD in the alendronate group were suppressed below baseline values and below values seen in the placebo group from baseline to Month 12. There was no apparent change in fDPD on withdrawal of alendronate.

At all ONO-5334 dose levels there was a marked increase in I-CTP from baseline to Month 12. Values continued to increase up to Month 24, returning to baseline values on cessation of treatment (at Follow up). In contrast, I-CTP values did not change from baseline on exposure to alendronate, remaining similar to placebo values throughout the 24-month study period (Figure 3e). Increases were observed in levels of TRAP5b in the ONO-5334 and placebo groups from baseline to Month 24. In contrast, TRAP5b levels remained close to baseline values throughout the study in the alendronate group. On finishing treatment (Follow up) there was a marked increase (approx. 2- to 2.5-fold greater than baseline values) in TRAP5b levels in the ONO-5334-treated groups that were not seen in either the placebo or alendronate groups (Figure 3f).

Figure 4 summarises the changes in bone formation markers, B-ALP, PINP and OC from baseline to the end of the 12-month study period of the original OCEAN protocol and for the following 12-month study extension (Month 12 to Month 24) and during the 2-month follow up period. A reduction in B-ALP in all study groups (including placebo) was observed during the first 6 months of the study. In all but the alendronate group, this was followed by a general trend for levels to return to baseline over the subsequent 18 months (Figure 4a). ONO-5334 was associated with a modest rebound in B-ALP on cessation of treatment. Levels of B-ALP in the alendronate group follow up samples suggest that levels were returning to baseline once dosing was completed. A reduction from baseline in all study groups was also observed in PINP levels during the first 6 months of the study. However, in all but the alendronate group this was followed by a general trend towards return to baseline over the subsequent 6 months (Figure 4b). The initial reduction in
PINP following exposure to ONO-5334 was greatest in the 300 mg qd dose group, levels had not returned to baseline by 12 months but had done so by Month 24. Modest reductions in PINP following exposure to ONO-5334 50 mg bid and 100 mg qd saw return to baseline levels by Month 12 and higher than baseline levels (up to 1.6-fold greater) by Month 24. ONO-5334 was associated with an increase in PINP on cessation of treatment, continuing to 26 weeks, that was not seen in the placebo or alendronate groups. Levels of PINP decreased in the alendronate group and plateaued at 6 months where they remained until treatment was stopped. Follow up levels of PINP in the alendronate group suggested levels were returning to baseline (Figure 4b).

There was an increase from baseline in the levels of OC in all groups following the start of the study. The magnitude of these changes were similar in the placebo and ONO-5334 50 mg bid and 100 mg qd groups and appeared to plateau at 18 months. Increases were not as marked in the ONO-5334 300 mg qd group, although levels continued increase throughout the study and were similar to the other ONO-5334 and placebo groups at Month 24. Increases in OC in the alendronate group were smaller than those in the ONO-5334 300 mg qd group. There were no notable changes in OC for any group on cessation of treatment.

**Safety and Tolerability**

There were no deaths reported during the extension phase of the study. The profiles of the most frequently reported (occurring in ≥5% of patients in any treatment group) TEAEs and serious TEAEs are summarised by treatment group in Table 1. A total of 14 patients experienced their first serious TEAEs with onset during the extension phase (placebo, n = 2; ONO-5334 50 mg bid n = 4; ONO-5334 100 mg qd, n = 2; ONO-5334 300 mg qd, n = 4; alendronate 70 mg, n = 2). The majority of TEAEs were of mild or moderate intensity; severe TEAEs were experienced by 14 patients in total (placebo, n = 2; ONO-5334 50 mg bid, n = 5; ONO-5334 100 mg qd, n = 3; ONO-5334 300 mg qd, n = 2; alendronate 70 mg, n = 2). There was no single TEAE that occurred most
frequently in all groups. For the placebo group, the most frequent TEAEs were nasopharyngitis (nine patients; 15.8%), back pain (13 patients; 22.8%) and headache (seven patients; 12.3%). For the ONO-5334 groups, the most frequent TEAEs were arthralgia, affecting a maximum of eight patients (14.0%) in the 50 mg bid group, and hypertension, affecting a maximum of ten patients (17.5%) in the 100 mg qd group. There was no apparent relationship between dose and reporting of any particular adverse event. The most frequently reported TEAE for the alendronate group was nasopharyngitis, affecting nine patients (15.8%).

Rash was reported as a TEAE in one patient (1.8%) in the ONO-5334 100 mg qd group, three patients (5.3%) in the ONO-5334 300 mg qd group, and one patient (1.8%) in the alendronate 70 mg group. None of these AEs was considered drug-related. The patient in the ONO-5334 100 mg qd group had a mild lichenoid rash that began at 2 years, was treated with hydrocortisone and was ongoing at follow-up. One patient in the ONO-5334 300 mg qd group had a mild rash on the chest that began on Day 14 of the study, led to the discontinuation of study medication and resolved after a duration of 58 days. A second patient in the ONO-5334 300 mg qd group had an exanthema on the bottom that began on Day 420, was of moderate intensity, was treated with topical hydrocortisone and resolved after a duration of 3 days. A third patient in the ONO-5334 300 mg qd group had a mild skin rash that began on Day 417 and resolved without treatment after a duration of 3 days. One patient in the alendronate 70 mg group had a mild exanthema on the right foot that began on Day 6 and resolved without treatment after a duration of 216 days.

All other safety parameters were generally unremarkable with no clinically relevant differences occurring across the treatments.
DISCUSSION

In reporting 12 months of dosing with ONO-5334 in the OCEAN study we showed how treatment with the three different doses resulted in statistically significant and clinically relevant increases in LS BMD at Month 12 compared with placebo in women at moderate risk of fracture.\(^\text{(16)}\) In extending the length of the study to 2 years we have been able to confirm that the influence of ONO-5334 on BMD is both persistent and progressive. In fact, the study extension demonstrated statistically significant improvements at all bone density sites and at all doses at 24 months compared with placebo, whereas the effect of the 100 mg daily dose level on BMD at 12 months failed to achieve statistical significance versus placebo. Similarities in the magnitude and profile of changes in BMD over 2 years between ONO-5334 and those reported previously for odanacatib provide initial indications of the pharmacodynamic effects of cathepsin K inhibition.\(^\text{(10,16,18)}\) Unlike the study with odanacatib, the present study included an alendronate dosing arm as a positive control.

Since changes in BMD following treatment with alendronate for 2 years were generally consistent with those reported for alendronate in other studies it is possible to confirm the integrity and sensitivity of the study design.\(^\text{(10, 19-21)}\) Care should be taken when comparing the two treatments, as the study was not powered to look at differences between ONO-5334 and alendronate, however, the findings suggest that inhibition of cathepsin K can deliver improvements in BMD similar to those seen with current standard of care agents. Although anti-fracture efficacy is the ultimate therapeutic target and true measure of clinical utility, the findings of increased BMD and suppression of several resorption markers following treatment with ONO-5334 are comparable with the bisphosphonate alendronate, yet achieved through an alternative mode of action. The increase in BMD with ONO-5334 may result from the apparent imbalance between bone resorption and bone formation.
Plasma ONO-5334 concentrations have been suggested to be a good surrogate for its pharmacodynamic effects,\(^{15,16}\) and the quantitative differences in BMD for the three different ONO-5334 dosing schedules in the present study appear to support a dose-response relationship. At 12 months, ONO-5334 50 mg bid and 300 mg qd was associated with significant increases in BMD in all regions measured whereas the 100 mg qd did not, only achieving statistically significant effects on BMD by 24 months. Furthermore, increases in BMD with 300 mg qd were consistently larger than those with 100 mg qd. Dose-response relationships with BMD effects have also been suggested for odanacatib and when this is considered in conjunction with the consistently greater increases in BMD associated with ONO-5334 50 mg bid over 100 mg qd it seems reasonable to propose that higher trough levels or sustained inhibition rather than a higher maximum plasma concentrations are more important for a more potent increase in BMD. It therefore seems reasonable to speculate that additional clinical benefits could be derived through manipulation of the plasma profile of ONO-5334, perhaps via a sustained release formulation\(^{22}\).

ONO-5334 and alendronate significantly suppressed the bone-resorption markers urinary NTX and serum and urinary CTX-I throughout 24 months of treatment, with the majority of the effect occurring within 1.5 months of initiating treatment. There appeared to be some differences between the markers in the degree of suppression by ONO-5334, whereby urinary CTX-I and NTX appeared to be similar in all treatments whereas serum CTX-I appeared to be sensitive to dose. An interesting observation not previously recorded in studies with cathepsin K inhibitors was that suppression of levels of serum CTX-I started to return to the levels of the placebo group prior to cessation of dosing with ONO-5334 50 mg bid and 100 mg qd. This was in contrast to the levels of suppression observed for alendronate and the 300 mg qd dose of ONO-5334. Bone mineral density increased for the 50 mg bid and 100 mg qd doses of ONO-5334 despite the reduced levels of suppression of serum CTX-I that were observed. Levels of TRAP5b showed increases during treatment with ONO-5334. In comparison, there was clear suppression of TRAP5b levels with
alendronate. Such an increase was also reported for odanacatib.\(^{(23)}\) It has previously been proposed that this pattern may indicate that the number of osteoclasts actually increases (which is generally said to be reflected with TRAP5b as the change in TRAP5b during treatment period suggests), but the work of each cell decreases (and hence the decrease in CTX).\(^{(16)}\) After cessation of treatment, because bone resorption was suppressed during treatment, there may be a compensation effect after suppression of bone resorption for so long and this may result in facilitating a quick increase in the number of osteoclasts. Interestingly, there were similar re-bound effects on bone formation markers after cessation of treatment except for OC. The pattern of such off-set effects on bone turnover markers was similar to those with odanacatib.\(^{(18)}\) The underlying mechanisms responsible for these effects are unknown. The increase in osteoclasts may stimulate bone formation via unknown coupling factors. As OC exists mainly in cortical bone, where the rate of turnover is relatively low,\(^{(24)}\) this may explain why no immediate rebound effects were observable.

Levels of I-CTP and fDPD increased on initiation of dosing with ONO-5334 and continued to increase throughout the 24-month treatment period. The influence of ONO-5334 on fDPD appeared to be dependent on dose whereas those on I-CTP appeared to be independent. Similar patterns of effect have also been reported for odanacatib.\(^{(10,18)}\) In contrast, alendronate was associated with suppression of fDPD that plateaued within 3 months of starting dosing. Alendronate appeared to have no effect in I-CTP. Released from type 1 collagen by matrix metalloproteinase, I-CTP is usually further degraded by cathepsin K to fragments, such as CTX-I. Thus an increase might be predicted following cathepsin K inhibition.\(^{(25)}\) Altered degradation pathways also may explain the increase in fDPD with cathepsin K inhibitor.

In terms of evidence of actions on bone formation, alendronate showed clear and characteristic suppression of B-ALP and P1NP throughout the treatment period. There seemed to be a dose-dependent effect of ONO-5334, changes in these markers with the two lower doses were
comparable with those for placebo during the initial 12-month treatment period. However, levels of markers increased during the second year and the difference between ONO-5334 and placebo became clearer. Even the highest dose of ONO-5334, 300 mg qd, which suppressed these markers during early treatment phase, ended with similar levels at Month 24 compared with placebo. The changes in bone formation markers may be reflecting different changes in the different bone envelopes. In the ovariectomy monkey model, cathepsin K inhibition with odanacatib\(^{(26)}\) or balicatib\(^{(27)}\) for 18-21 months results in a decrease in the rate of bone formation in trabecular and intra-cortical bone, but in a 3 to 6-fold increase in periosteal bone formation at the femoral neck and consequent increase in cortical thickness. The suppressive effect of odanacatib 50 mg qw treatment on bone formation markers, B-ALP and PINP, remained at approximately 20% compared with placebo at Month 24.\(^{(10)}\) Effect of ONO-5334 on OC is difficult to interpret as there was large increase in OC with placebo treatment; this may indicate degradation of the OC during sample storage.

It has been suggested that the long-term use of bisphosphonates potentially may affect the quality and structural integrity of bone. Whether this relates to an increase in atypical fractures seen in certain vulnerable patients treated with bisphosphonates has yet to be confirmed.\(^{(23,28)}\) Nevertheless, concern has also been raised over the possible increased risk of atypical fracture of the femur that could be a consequence of chronic suppression of turnover.\(^{(1)}\) It was clear from the present data that higher doses of ONO-5334 tended to have more marked BMD increases than alendronate in femoral neck where even the ONO-5334 50 mg bid dose appeared comparable. Clear dose-response between 100 mg and 300 mg qd of ONO-5334 observed during initial 12-month treatment remained in the second year. Whether these differences translate into better clinical outcomes in any particular patient group has yet to be confirmed. However, the reports of stimulation of periosteal bone formation in the hip of ovariectomised monkeys (above) provides promising results. If these effects are confirmed they might be expected to provide long term
benefits to patients. Further long-term observational and prospective intervention studies are needed to address the clinical significance of osteoporosis treatment on bone quality over time.

ONO-5334 was generally well tolerated during the study with most TEAEs being rated as mild or moderate in severity. Reporting of AEs with ONO-5334 was similar in frequency and profile to that for placebo and alendronate. There were no clinically relevant signs or symptoms of concern and no overt signs of the gastrointestinal events associated with the oral bisphosphonates\(^{(29)}\). Three patients reported a rash at the highest dose level of ONO-5334, 300 mg, but those were considered not related to study drug and none of the rashes in the present study resembled the morphea reported with the cathepsin k inhibitor, balicatib\(^{(9,12,30)}\).

In conclusion, daily exposure to ONO-5334 at doses of up to 300 mg was generally well tolerated with no signs or signals of safety concern emerging during the 24 months of treatment. ONO-5334 was associated with clinically relevant, progressive and consistent increases in BMD and those observed at the 300 mg qd dose level were comparable with those seen with alendronate. The difference in biomarker profile between ONO-5334 and alendronate confirms a clear difference in mechanism of action. Further clinical studies are warranted to investigate the long-term efficacy on fracture rate and longer term safety profile of ONO-5334 as a potential treatment for osteoporosis.
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The SC members approved the manuscript for publication and vouch for the completeness and accuracy of the data. The data was transferred to the first author (RE) and the analyses checked. The sponsor was involved in the design, conduct, analysis and reporting of the study. The manuscript authors’ employed by the sponsor were specifically involved in the design, data review, analysis and interpretation of the study and in the writing of the manuscript.
REFERENCES


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Table 1: Summary of treatment emergent adverse events (preferred terms) experienced by ≥5% of patients in any treatment group up to Month 24 (safety population)

<table>
<thead>
<tr>
<th>Preferred Term</th>
<th>Placebo (N=57)</th>
<th>ONO-5334 50 mg bid (N=57)</th>
<th>ONO-5334 100 mg qd (N=57)</th>
<th>ONO-5334 300 mg qd (N=57)</th>
<th>Alendronate 70 mg qw (N=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%), TEAEs</td>
<td>n (%), TEAEs</td>
<td>n (%), TEAEs</td>
<td>n (%), TEAEs</td>
<td>n (%), TEAEs</td>
<td>n (%), TEAEs</td>
</tr>
<tr>
<td>Abdominal pain upper</td>
<td>2 (3.5)</td>
<td>5 (8.8)</td>
<td>3 (5.3)</td>
<td>3 (5.3)</td>
<td>8 (14.0)</td>
</tr>
<tr>
<td>Constipation</td>
<td>2 (3.5)</td>
<td>6 (10.5)</td>
<td>2 (3.5)</td>
<td>1 (1.8)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>6 (10.5)</td>
<td>8 (14.0)</td>
<td>2 (3.5)</td>
<td>7 (12.3)</td>
<td>3 (5.3)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>9 (15.8)</td>
<td>7 (12.3)</td>
<td>6 (10.5)</td>
<td>3 (5.3)</td>
<td>9 (15.8)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>6 (10.5)</td>
<td>4 (7.0)</td>
<td>4 (7.0)</td>
<td>5 (8.8)</td>
<td>2 (3.5)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>6 (10.5)</td>
<td>8 (14.0)</td>
<td>8 (14.0)</td>
<td>8 (14.0)</td>
<td>10 (17.5)</td>
</tr>
<tr>
<td>Back pain</td>
<td>13 (22.8)</td>
<td>2 (3.5)</td>
<td>4 (7.0)</td>
<td>4 (7.0)</td>
<td>7 (12.3)</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>2 (3.5)</td>
<td>3 (5.3)</td>
<td>4 (7.0)</td>
<td>5 (8.8)</td>
<td>7 (12.3)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>4 (7.0)</td>
<td>5 (8.8)</td>
<td>6 (10.5)</td>
<td>4 (7.0)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Headache</td>
<td>7 (12.3)</td>
<td>3 (5.3)</td>
<td>2 (3.5)</td>
<td>5 (8.8)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>6 (10.5)</td>
<td>8 (14.0)</td>
<td>10 (17.5)</td>
<td>6 (10.5)</td>
<td>7 (12.3)</td>
</tr>
<tr>
<td>Rash</td>
<td>0</td>
<td>0</td>
<td>1 (1.8)</td>
<td>3 (5.3)</td>
<td>1 (1.8)</td>
</tr>
</tbody>
</table>

TEAEs are AEs whose onset occurred or severity worsened on or after the first dose of double-blind study medication. n = number of patients reporting a TEAE; if a patient experienced more than one episode of an AE, it was counted only once within a preferred term.
FIGURE LEGENDS

Figure 1: Summary of patient disposition

Figure 2: Mean percentage changes (± standard error) from baseline in a) lumbar spine, b) total hip and c) femoral neck BMD during 24 months treatment are shown. Closed circle (●), open circle (○), open triangle (▲), open square (□) and cross (×) indicate placebo, ONO-5334 50 mg bid, 100 mg qd, 300 mg qd and alendronate 70 mg qw, respectively. Statistical analyses were performed with t-tests from the ANCOVA model, difference to placebo treatment arm, *** p<0.001 (shown for Month 24 only) (FAS, LOCF).

Figure 3: Changes from baseline in serum and urinary biochemical markers of bone turnover, a) urinary NTX, b) serum CTX-I, c) urinary CTX-I, d) urinary free DPD, e) serum I-CTP and f) serum TRAP5b, are shown as geometric means ± 95% CI. Closed circle (●), open circle (○), open triangle (▲), open square (□) and cross (×) indicate placebo, ONO-5334 50 mg bid, 100 mg qd, 300 mg qd and alendronate 70 mg qw, respectively. FAS, observed data.

Figure 4: Changes from baseline in serum and urinary biochemical markers of bone turnover, a) serum B-ALP, b) serum PINP and c) serum OC, are shown as geometric means ± 95% CI. Closed circle (●), open circle (○), open triangle (▲), open square (□) and cross (×) indicate placebo, ONO-5334 50 mg bid, 100 mg qd, 300 mg qd and alendronate 70 mg qw, respectively. FAS, observed data.
Figure 1
a: Lumbar spine

Figure 2a
b: Total hip
Figure 2c

**c: Femoral neck**

![Graph showing percentage change from baseline over months for femoral neck.](image)
Figure 3a

a: Urinary NTX

Change from Baseline (Geo means)

Months

discontinuation of study drug (2 M)
Figure 3b
Figure 3c: Urinary CTX-I
Figure 3d

d: fDPD

Change from Baseline (Geo means)

0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2

0 6 12 18 24

Months

discontinuation of study drug (2 M)

24 25 26
Figure 3e
Figure 3f

f: TRAP5b

Change from Baseline (Geo means)

Months

discontinuation of study drug (2 M)
a: B-ALP

Figure 4a
b: PINP

Figure 4b
Figure 4c