The Effect of Cathepsin K Inhibitor on Bone Metabolism

January 2019

Shinichi NAGASE

The Effect of Cathepsin K Inhibitor on Bone Metabolism

A Dissertation Submitted to

the Graduate School of Life and Environmental Sciences,

the University of Tsukuba

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy in Biological Science

(Doctoral Program in Biological Sciences)

Shinichi NAGASE

Table of Contents

General Abstract

Successive bone remodeling is being carried out continuously and old bone tissue is replaced with newly formed bone tissue. Osteoclasts and osteoblasts are key players for bone remodeling, which perform bone resorption and formation, respectively. Osteoclasts act on both organic and inorganic components of bone matrix. In the degradation of organic components which majority is type I collagen, cathepsin K takes a critical role. Cathepsin K is a member of cysteine protease and predominantly exists in osteoclasts. It is known that humans lacking cathepsin K exhibit pycnodysostosis, which is characterized by abnormally dense bones (osteosclerosis). Intricate interactions between osteoclasts and osteoblasts exist and maintain good balance between bone resorption and formation. The status of bone turnover is described with biochemical markers of bone turnover. C-terminal telopeptide of type I collagen (CTX) and N-terminal telopeptide of type I collagen (NTX) which are the degradation products of type-I collagen are traditionally utilized as bone resorption markers. Tartrate-resistant acid phosphatase 5b (TRAP5b) is recognized to reflect number and activity of osteoclasts. On the other hand, bone-specific alkaline phosphatase (B-ALP) and procollagen type I N-terminal propeptide (PINP) are recognized as major bone formation markers.

Osteoporosis occurs when there is an imbalance between resorption and formation of bone, with resorption predominating. The most commonly used agents are the bisphosphonates which have demonstrated significant anti-fracture efficacy, especially for vertebral fractures. Bisphosphonates suppress bone formation but not only bone resorption

through coupling mechanisms therefore there is concern that by decreasing bone turnover for many years, there may be complications such as atypical fractures of the femur. However, other than bisphosphonates, there are no anti-osteoporosis drugs with established sufficient anti-fracture efficacy along with long term safety, therefore new anti-osteoporosis drugs with new mechanisms of action is expected for long. Under such circumstance, specific inhibitors of cathepsin K such as ONO-5334 and odanacatib have been synthesized and under clinical development. ONO-5334 exhibits a potent and selective inhibitory effect on cathepsin K *in vitro* and is associated with improvements in both bone mineral density and bone strength in the ovariectomized monkey osteoporosis model. I conducted Phase I studies with healthy post-menopausal women in order to evaluate pharmacodynamic effect of ONO-5334 on biochemical markers of bone resorption and formation, in addition to primarily evaluate safety and pharmacokinetics of single and multiple dose of ONO-5334.

In Part 1, single dose of ONO-5334 displayed linear plasma pharmacokinetics over the dose range tested, 3-300 mg, with clear suppression of urinary bone resorption markers at doses 100 mg for serum markers at 24 hours. ONO-5334 was well tolerated up to 600 mg/day when administered to healthy postmenopausal women. In Part 2, plasma ONO-5334 concentration reached steady state within 2 days. ONO-5334 was well tolerated up to 600 mg/day and for up to 28 days of multiple dosing. Multiple dosing with ONO-5334 100 mg resulted in considerable suppression of bone resorption markers with no appreciable effects on bone formation markers (B-ALP, OC) or osteoclast number (TRAP5b).

Through these researches, it was demonstrated that ONO-5334, specific inhibitor for cathepsin K, shows significant reduction of bone resorption in human presented as reduction of level of peptide product of the degradation of type-I collagen, CTX and NTX. ONO-5334 did not show significant effect on bone formation markers, B-ALP and PINP. In addition to those findings, no changes in level of TRAP5b may have indicated mechanisms of ONO-5334 to show less effect on bone formation through coupling functions between osteoclasts and osteoblasts, compared with traditional anti-resorptive agents. On the other hand, CTX compared with NTX, and serum markers compared with urine markers showed more clear response on treatment with ONO-5334 however the mechanisms of these findings were not clear. Cathepsin K inhibitors are dosed for very long term in patients with osteoporosis, if become clinically available, therefore it is considered important to further investigate existence of off-target effects on other cathepsins and long-term effect on bone turnover which may be connected to anti-fracture efficacy.

Abbreviations

AE	Adverse event
ANCOVA	Analysis of covariance
AUC	Area under the plasma concentration vs. time curve
B-ALP	Bone-specific alkaline phosphatase
BCE	Bone collagen equivalent
BLQ	below the limit of quantification
BMD	Bone mineral density
BMI	Body mass index
BMU	Basic multicellular unit
CL/F	Oral clearance
C_{max}	Peak plasma concentration
CTX	C-terminal telopeptide of type I collagen
DPD	Deoxypyridinoline
ECG	Electrocardiogram
ELIZA	Enzyme-linked immunosorbent assay
FSH	Follicle stimulating hormone
LOQ	Lower limit of quantification

MRSD	Maximum recommended starting dose
NOAEL	No observed adverse effect level
NTX	N-terminal telopeptide of type I collagen
OC	Oosteocalcin
PAD	Pharmacologically active dose
PINP	Procollagen type I N-terminal propeptide
PK	Pharmacokinetics
QCT	Quantitative computed tomography
SOC	System organ class
TEAE	Treatment emergent adverse event
TRAP5b	Tartrate-resistant acid phosphatase 5b
t_{max}	Time associated with the maximal concentration
$t_{1/2}$	Half-life

General Introduction

Successive bone remodeling is being carried out continuously in healthy adult human skeleton and old bone tissue is replaced with newly formed bone tissue. Bone remodeling occurs at specific site called basic multicellular units (BMUs) [1-2] and replaces approximately 10% of skeleton each year [3]. At BMUs, osteoclasts and osteoblasts have critical role to execute bone remodeling. Osteoclasts which play a role on bone resorption are differentiated from mononuclear cells of the monocyte/macrophage lineage, while osteoblasts which practices bone formation are differentiated from mesenchymal stem cells [4-5]. Osteoclasts have a unique structure termed ruffled border and form resorption lacunae by being attached to bone surface via again the unique structure named sealing zone. In the resorption lacunae between ruffled boarder and bone surface, isolated space by sealing zone, osteoclasts carry out bone resorption. Bone matrix consists of organic and inorganic components [6]. The organic component contains approximately 20 proteins with type I collagen as the most predominant (>90%) while the inorganic part primarily includes crystalline hydroxyapatite [7]. Osteoclasts act on both organic and inorganic components of bone matrix. Bone resorption on inorganic components is induced by involvement of vacuolar H+-adenosine triphosphatase (H+-ATPase) which is predominantly present in the ruffled border membrane [8-9], and transports protons to acidify the resorption lacunae [10]. In contrast, cathepsin K takes a critical role to carry out bone resorption on organic component of bone matrix, which is secreted onto resorption lacunae and degrade type I collagen.

Cathepsin K is a papain-like cysteine protease member of the cathepsin family of lysosomal proteases which is categorized as consisting of cysteine (cathepsins B, C, F, H, K, L, O, S, V, X and W), aspartate (cathepsins D and E) and serine proteases (cathepsins A and G) [11]. Cathepsins have greatest activity in acidic environments and are ubiquitously expressed [11]. Among series of cathepsins, cathepsin K is predominantly expressed in osteoclasts. Within osteoclasts, cathepsin K has been shown to reside in lysosomes, cytoplasmic vesicles and along the osteoclast-bone resorptive interface before release into the resorption lacunae [12-13]. Collagenolytic activity of cathepsin K has a critical role in bone resorption as it degrades helical structure of type I collagen at various sites [14-15]. Humans lacking cathepsin K exhibit pycnodysostosis, which is characterized by abnormally dense bones (osteosclerosis) [16]. Cathepsin K deficient mice show osteopetrosis and display features characteristic of pycnodysostosis [17-18].

Across the mechanisms of bone remodeling, intricate interactions between osteoclasts and osteoblasts exists and it is described as (osteoclast-osteoblast) coupling. For example, several mechanisms of coupling process have been revealed that Wnt1, Wnt10b, BMP6 and S1P all released by osteoclasts enhances bone formation by osteoblast [19-21]. With the existence of proper coupling process, bone resorption by osteoclasts and bone formation by osteoblasts are well balanced and bone mass is kept maintained to support biological role of bone.

During the bone remodeling process, several biochemical markers of bone resorption and formation are released. Biochemical markers of bone formation are produced by osteoblastic cells or derived from procollagen metabolism, whereas markers of bone

resorption are the degradation products of osteoclasts or collagen degradation. Type I collagen which consists more than 90% of organic bone matrix is degraded by cathepsin K and peptide products of the degradation include C-terminal telopeptide of type I collagen (CTX) and N-terminal telopeptide of type I collagen (NTX). These have been extensively used as biochemical markers of bone resorption to quantify treatment efficacy in bone metabolic diseases [22]. Both NTX and CTX are released in the serum then excreted in the urine therefore can be assessed in either sample. The timing of the urine sample collection is critical due to the marked circadian variation which is more pronounced for CTX. It has also been shown that fasting significantly reduces circadian variations. Thus fasting morning samples are considered optimal for clinical use [23-24]. During bone resorption, osteoclasts secrete tartrate-resistant acid phosphatase 5b (TRAP5b) which produces reactive oxygen species to digest bone degradation products in the microenvironment of the bone matrix. Therefore, TRAP5b is used as an index of osteoclast activity and numbers [25]. In contrast to resorption markers, bone-specific alkaline phosphatase (B-ALP) and procollagen type I N-terminal propeptide (PINP) are recognized as major biochemical markers of bone formation. Alkaline phosphatase is a membrane-bound enzyme and is present in almost all body tissues. Among several isoforms, B-ALP is produced by osteoblasts and its production is correlated positively with bone formation rate as measured by histomorphometry [26]. On the other hand, PINP is released as results of cleavage of procollagen, precursor, of major organic bone matrix of type I collagen [27-28]. In addition to B-ALP and PINP, osteocalcin (OC) is recognized as one of biochemical markers of bone formation. OC is a small non-collagenous protein synthesized by

osteoblasts, however the use of OC measurements in clinical practice is limited due to assay variability, sample instability and high biological variability.

Osteoporosis occurs when there is an imbalance between resorption and formation of bone, with resorption predominating. The fractures that result from osteoporosis are a major public health problem. There are now effective treatments for reducing fracture risk in osteoporosis. The most commonly used agents are the bisphosphonates. While bisphosphonates have been shown to reduce the risk for vertebral fractures by between 40% and 70%, the reduction for non-vertebral fractures is significantly less compared with placebo [29]. 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 [30], indicating that the magnitude of non-vertebral fracture risk-reduction with bisphosphonates is close to 20%. Recent studies with intravenous zoledronic acid and subcutaneous denosumab yielded similar reductions. In the HORIZON-PFT and FREEDOM trials, zoledronic acid and denosumab reduced non-vertebral fracture risk by 25% and 20%, respectively [31-32]. Furthermore, since traditional anti-resorptive agents suppress bone formation but not only bone resorption through coupling mechanisms, there is a concern that by decreasing bone turnover for many years, there may be complications such as atypical fractures of the femur [29]. It has been proposed that there may be approaches to inhibiting osteoclast activity but not reducing osteoclast number that might allow bone formation to be unaffected by the treatment [33]. There are a number of key enzymes that may be suitable targets for osteoporosis therapy, including cathepsin K [34].

Although no cathepsin K inhibitor is currently marketed, there are some challenges to develop low molecule inhibitors for osteoporosis including balicatib, relacatib and odanacatib. ONO-5334 is a low molecular weight synthetic inhibitor of cathepsin K and is being developed as an oral therapeutic agent for bone metabolic diseases including osteoporosis (Figure A[35]). ONO-5334 exhibits a potent and selective inhibitory effect on cathepsin K *in vitro* [36–38] and is associated with improvements in both bone mineral density and bone strength in the ovariectomized monkey osteoporosis model [39].

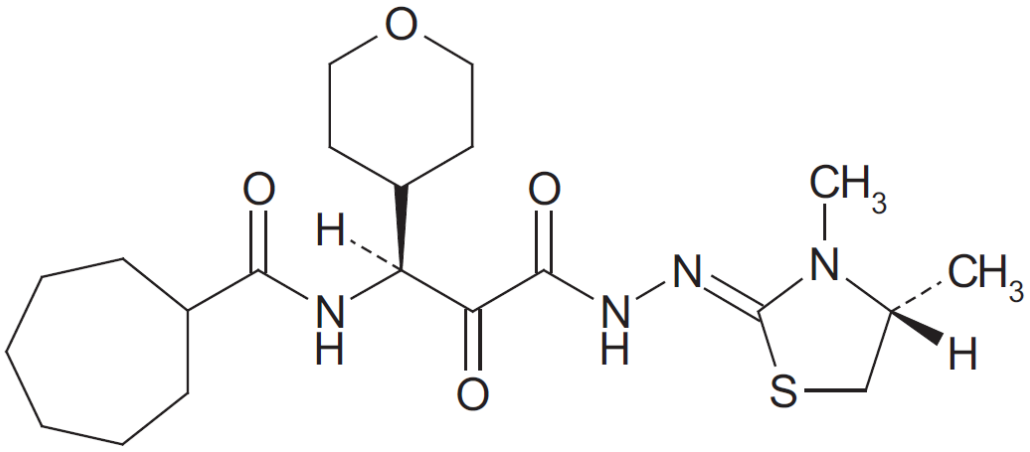

Figure A: The Chemical Structure of ONO-5334. (N-((1S)-3-{(2Z)-2-[(4R)-3,4-Dimethyl-1,3-thiazolidin-2-ylidene]hydrazino}-2,3- dioxo-1-(tetrahydro-2H-pyran-4-yl)propyl)cycloheptanecarboxamide, Ono Pharmaceutical Co. Ltd, Japan)

I conducted Phase I studies with healthy post-menopausal women in order to evaluate pharmacodynamic effect of ONO-5334, cathepsin K inhibitor, on biochemical markers of bone resorption and formation, in addition to primarily evaluate safety and pharmacokinetics of single and multiple dose of ONO-5334. ONO-5334 could suppress bone resorption without significant effects on bone formation which is different from traditional anti-resorptive agents such as bisphosphonates therefore, through the research, I aimed for exploring and clarifying effect of cathepsin K inhibitor on bone remodeling with series of biochemical markers of bone turnover.

Part I

Serum and urine bone resorption markers and pharmacokinetics of the cathepsin K inhibitor ONO-5334 after ascending single doses in postmenopausal women

Abstract

A double-blind, placebo-controlled, randomized study was carried out in 52 healthy postmenopausal females to investigate the safety, pharmacokinetics and pharmacodynamics of the new cathepsin K inhibitor, ONO-5334. Single ascending doses of ONO-5334 (3-600 mg) were evaluated in six cohorts. The effect of food was studied at ONO-5334 100 mg.

Across the doses tested, mean ONO-5334 C_{max} occurred 0.5-1.0 hours after dosing and the $t_{1/2}$ ranged from 9.1 to 22 hours. Linear increases in C_{max} and $AUC_{0-\infty}$ were observed in the 3-300 mg and 3-600 mg dose range, respectively. After food, the geometric mean ratio (95% CI) C_{max} and $AUC_{0-\infty}$ for ONO-5334 were 0.78 (0.31, 1.94) and 0.95 (0.67, 1.35)-fold greater than fasted, respectively. ONO-5334 significantly reduced serum bone resorption markers within 4 hours vs. placebo. Statistical significance was achieved for ONO-5334 doses 30 mg for C-terminal telopeptide of type I collagen (CTX) and 300 mg for N-terminal telopeptide of type I collagen (NTX). Statistical significance was still evident at 24 hours for ONO-5334 100 mg with serum CTX and 600 mg with serum NTX. The maximum suppression in serum CTX occurred at 4 hours post dose with difference compared with placebo of -32%, -59%, -60% and -66% for 30, 100, 300 and 600 mg ONO-5334, respectively. Second morning urine void 24 hours post dose showed statistically significant suppression of urinary CTX and NTX at 100 mg and above vs. placebo. ONO-5334 600 mg showed statistically significant suppression up to 72 hours for serum CTX, urinary CTX and urinary NTX and 48 hours for serum NTX vs. placebo. Adverse events were transient with no evidence of dose relationship.

In this study, ONO-5334 displayed linear plasma pharmacokinetics over the (predicted therapeutic) dose range, 3-300 mg, with clear suppression of urinary bone resorption markers at doses 100 mg for serum markers at 24 hours. ONO-5334 was well tolerated up to 600 mg/day when administered to healthy postmenopausal women.

Introduction

ONO-5334 exhibits a potent and selective inhibitory effect on cathepsin K *in vitro* [36–38] and is associated with improvements in both bone mineral density and bone strength in the ovariectomized monkey osteoporosis model [39]. This phase I clinical study was designed to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of single doses of the new cathepsin K inhibitor ONO-5334 (with/without food) in healthy postmenopausal women. Through this study as the first step in clinical trial setting, I evaluated changes in serum and urine CTX and NTX, peptide products of the degradation of type I collagen, in order to explore if ONO-5334 demonstrates suppressive effect on bone resorption in human, even with single administrative dose.

Materials and Methods

Study design

This double-blind, placebo-controlled, single dose escalation study in healthy postmenopausal female subjects was conducted at the Clinical Pharmacology Unit, Kendle International B.V., the Netherlands. Fifty-two subjects were planned for enrolment in five groups of eight subjects each and in one group of 12 subjects to determine the safety, tolerability and pharmacokinetics of ONO-5334. The pharmacodynamic effects of ONO-5334 on the bone resorption markers, serum CTX and serum NTX, were also assessed. Eligible subjects attended the unit on the morning of the day before dosing and remained for at least 72 hours post dose. In each group of eight subjects, six subjects were randomized to receive single oral doses of ONO-5334 tablets (3, 10, 30, 300 or 600 mg) and two subjects matching placebo tablets. In the group of 12 subjects, six were randomized to receive 100 mg ONO-5334 and six matching placebo. Each group of eight subjects was dosed in the fasted state during one treatment period only. The group of 12 subjects was dosed in the fasted state during the first treatment period (period 1) and received the same treatment a week later in the fed condition, after receiving a standard breakfast, consisting of three slices of bread, a cracker, diet margarine, jam, cheese, ham, semi-skimmed milk and fruit juice (period 2), as part of a preliminary investigation into the effect of food on the pharmacokinetic profile of ONO-5334. Subjects attended a follow-up visit 5 days after the final dose.

The rationale for the starting dose of ONO-5334 in this study was based upon the maximum recommended starting dose (MRSD) determined by the no observed adverse effect level (NOAEL) and the pharmacologically active dose (PAD). The MRSD determined by NOAEL was 3 mg/person, which was derived by converting the 30 mg/kg rat NOAEL dose to a value of 2.916 mg/kg for humans. A safety factor of 100 was used in the calculation instead of the standard factor of 10 to take into consideration safety concerns associated with administering new medications to postmenopausal females. The MRSD determined from the PAD was 6 mg/person, which was derived by converting the 0.6 mg/kg dose, which was the lowest pharmacologically active dose in the rat model, to a value of 0.096 mg/kg for humans. The lowest dose of 3 mg was therefore chosen as a suitable starting dose. The rationale for selecting the highest dose of ONO-5334 was based on the NOAEL of 100 mg/kg, which translated to a maximum dose (on a mg/kg basis) of 6000 mg/day for humans. The highest dose of ONO-5334 in this study was 600 mg (one tenth of the maximum dose). Dose intervals between the minimum and maximum were based on a standard pharmacokinetic-safety risk approach. The need to obtain sufficient data to evaluate pharmacokinetic linearity was balanced against risk and the number of subjects required to meet the aims of the study. No safety concerns were identified in any of the pre-clinical studies. Thus, increases of approximately three-fold were considered to be justified. Dose escalation decisions followed review of safety and tolerability data from the preceding group by the principal investigator and the sponsor.

Study subjects

Healthy postmenopausal females aged 45-75 years with a body mass index of 19 to 32 kg/m^2 were eligible to take part in the study. Postmenopausal was defined as women who had been amenorrhoeic for more than 1 year if over 50 years of age. If aged 45-50 years women were considered postmenopausal if amenorrhoeic for more than 2 years if and they demonstrated an appropriate clinical profile that was confirmed at screening by oestradiol and follicle stimulating hormone (FSH) concentrations consistent with menopause (FSH >30 IU/L, oestradiol <92 pmol/L). Subjects were excluded from the study if they had any relevant medical history including osteoporosis, history of severe allergic reaction, drug or alcohol abuse or other clinically significant abnormalities at screening. Subjects were also excluded if they smoked (even within last 6 months), drank more than 14 units of alcohol per week or received prior to dosing, prescription drugs within 28 days (St Johns'Wort within 6 weeks), nonprescription drugs or over the counter drugs within 48 hours unless deemed necessary by the principal investigator and where they did not interfere with study procedures or the subject's safety. Strenuous exercise, caffeine and alcohol were prohibited for 48 h prior to study start and during the study. All subjects gave written informed consent and the study was approved by the Stichting Therapeutische Evaluatie Geneesmiddelen Ethics Committee.

Assessments

Blood samples (7.5 mL/sample) for the determination of ONO-5334 concentration were collected prior to dosing, 10, 20 and 30 minutes,1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36,48 and 72 hours after dosing. The plasma samples obtained were divided into three sample tubes and stored at -20°C. The samples were analyzed by Sumika Chemical Analysis Service, Ltd (Japan). Plasma ONO-5334 was isolated using solid phase extraction and a validated liquid chromatography/tandem mass spectrometry method (with a lower limit of quantification (LOQ) of 0.02 ng/mL) was used to quantify concentrations of ONO-5334. The precision of the ONO-5334 assay was <15% (coefficient of variation) and the accuracy of the assay was within 15% of the actual value for ONO-5334.

Serum samples for the analysis of CTX and NTX were obtained at baseline (pre-dose), 4, 6, 12, 24, 48 and 72 hours after administration and second morning void urine samples were collected before breakfast at baseline (pre-dose), 24, 48 and 72 hours after administration. Analysis of CTX was undertaken using Serum CrossLaps® ELISA and Urine CrossLaps® EIA, Immunodiagnostic Systems Ltd, Boldon, UK and of NTX using Osteomark® NTX, Osteometrics, Decatur, GA, USA.

Standard safety assessments were performed throughout the study including vital signs, physical examinations, 12-lead electrocardiogram (ECG), continuous ECG, haematology, biochemistry, urinalysis and adverse event (AE) monitoring.

Statistical analysis

No formal power calculation was performed to estimate the appropriate number of subjects to be studied but the sample size was considered adequate to fulfil the study aims.

Pharmacokinetic parameters were calculated using WinNonlin® Ver. 4.0.1 software (Pharsight Corp., Mountain View, California). The area under the plasma concentration vs. time curve from time zero to infinity ($AUC_{0-\infty}$) was estimated using the linear trapezoidal rule. The peak plasma concentration (C_{max}) values and time associated with the maximal concentration (t_{max}) were obtained from the observed data. Oral clearance (CL/F) was calculated as dose/ $AUC_{0-\infty}$. Dose dependency of C_{max} and $AUC_{0-\infty}$ was assessed by the power model (point estimate and 95% confidence interval [95% CI] of the slope (β) of the regression formula $\log (Y) = \alpha + \beta \times \log (X)$). Dose proportionality was confirmed if the 95% CI of β included 1 and fell within the range of 0.7, 1.3. Appropriate tests were used to determine any statistically significant differences between doses for half-life ($t_{1/2}$, parametric, Tukey-Kramer test) and t_{max} (non-parametric, Steel-Dwass test) using a two-sided 5% level of significance. To evaluate the effect of food on ONO-5334 pharmacokinetics, C_{max} and $AUC_{0-\infty}$ were compared between the fasted and fed states in the 100 mg of ONO-5334 group. The ratio of the fed to the fasted state was calculated for individual subjects and the geometric mean ratios and 95% CIs determined using log transformed data. When the 95% CI of the geometric mean did not include 1, it was considered that food had affected the pharmacokinetics of ONO-5334. Half-life was analyzed in a similar way to $AUC_{0-\infty}$ and C_{max}. t_{max} was analyzed by Wilcoxon signed rank test at a two-sided 5% level of significance.

For bone resorption markers, any values below the LOQ were assigned the LOQ for the assay (serum CTX 0.173 mg/L, serum NTX 5 mg/L, urine CTX 99 mg/L and urine NTX 20 nM BCE). For urine CTX and urine NTX, values were corrected for creatinine. Percentage changes from baseline (pre-dose) could not be evaluated where a subject's bone resorption marker at baseline was below the LOQ. Any subjects with a baseline value below the LOQ were therefore excluded from the analyses. Percentage changes from baseline (pre-dose) were analyzed at each time point using analysis of covariance (ANCOVA) with log-transformed pre-dose value as a covariate and treatment as a fixed effect. ONO-5334 treatment groups were compared with placebo at each post dose time point.

There was no formal statistical analysis of safety data. Treatment emergent adverse events (TEAE) were defined as AEs that occurred or worsened after the first investigational product intake.

Results

Fifty-two subjects were randomized to the study and all completed. There were no specific differences in baseline characteristics between treatment groups (Table 1-1). No subjects were excluded from the safety or the pharmacokinetic analysis populations, although estimates for $t_{1/2}$, $AUC_{0-\infty}$ and CL/F could not be derived for one subject because concentrations of ONO-5334 were too low.

Table 1-1: Summary of baseline characteristics

		Placebo	ONO-5334					
			3mg	10mg	30mg	100mg	300mg	600mg	All
		n=16	n=6	n=6	n=6	n=6	n=6	n=6	n=52
Age (years)	mean	62.3	61.8	63.3	60.5	60.0	59.8	61.2	61.5
	SD	4.70	3.97	7.34	2.07	5.69	6.37	6.05	5.08
Height (cm)	mean	167.5	166.0	158.8	164.8	165.0	166.5	163.5	165.1
	SD	5.52	5.96	4.13	5.16	7.66	6.80	3.78	5.96
Weight (kg)	mean	72.1	71.1	65.0	69.9	75.4	75.5	71.3	71.6
	SD	9.25	5.59	6.57	6.99	6.47	10.75	5.80	8.06
BMI (kg/m^2)	mean	25.7	25.8	25.8	25.8	27.7	27.2	26.6	26.2
	SD	2.90	2.27	2.42	3.26	1.15	3.43	1.47	2.57

n, number of subjects in specified group; SD, standard deviation.

Pharmacokinetic assessments

Within the tested dose range (3-600 mg), fasted plasma ONO-5334 concentrations reached a mean C_{max} (5.69 to 941 ng/mL) within 0.33 to 1.5 hours of dosing and thereafter decreased in a biphasic fashion (Table 1-2, Figure 1-1). In the initial phase, ONO-5334 concentrations fell rapidly, decreasing to less than one-tenth of the mean C_{max} 4 hours after dosing. Mean $t_{1/2}$ ranged from 9.1 to 22 hours and mean $AUC_{0-\infty}$ from 7.70 to 2400 ng h/mL over the dose range. In the latter phase of elimination in the 600 mg dose group, plasma ONO-5334 concentrations decreased at a slower rate relative to other dose groups.

Table 1-2: Pharmacokinetic parameters after administration of single oral doses of ONO-5334 to post-menopausal women (fasted and both fed/fasted for 100 mg).

Dose (mg)	C_{max} (ng/ml)	T_{max} (h)	AUC(0,∞) (ng/ml h)	$t_{1/2}$ (h)	CL/F (ml/min)	CL/F (ml/min/kg)
3	5.69 (1.84)	0.50 (0.33–0.50)	7.70[a)] (3.44)	11[a)] (3)	7270[a)] (2230)	102[a)] (32)
10	24.0 (13.9)	0.50 (0.33–0.50)	27.0 (8.9)	17 (12)	6690 (1980)	102 (26)
30	90.6 (68.3)	0.50 (0.50–1.5)	96.4 (43.4)	22 (14)	6020 (2370)	86.4 (33.2)
100 (gasted)	339 (259)	1.0 (0.50–1.0)	422 (118)	17 (3)	4210 (1150)	55.7 (13.1)
100 (fed)	255 (165)	1.5 (1.0–3.0)	419 (196)	18 (2)	4530 (1480)	59.8 (16.0)
300	832 (738)	0.75 (0.33–1.5)	1480 (860)	13 (5)	4390 (2520)	59.5 (36.3)
600	941 (500)	1.0 (0.50–1.5)	2400 (530)	9.1 (1.8)	4320 (850)	60.9 (10.6)

[n = 6, [a)] n = 5]. C_{max}, AUC, $t_{1/2}$, CL/F: mean (SD). t_{max}: median (range).

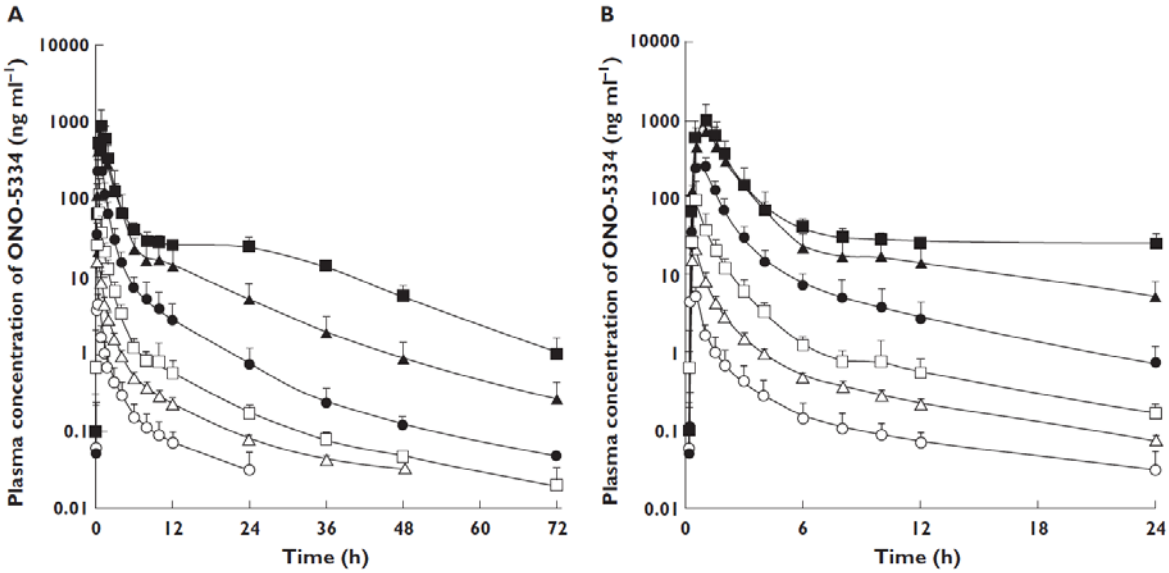

Figure 1-1: Log-transformed mean (± SE) change in plasma concentration of ONO-5334 with time after single oral administration at doses of 3–600 mg in a fasted condition. Changes in plasma concentration are presented for 0-72 h (A) and 0-24 h (B) with different time scale. n = 5–6. ○ ONO-5334 3 mg; △ ONO-5334 10 mg; □ ONO-5334 30 mg; ● ONO-5334 100 mg; ▲ ONO-5334 300 mg; ■ ONO-5334 600 mg

Mean $AUC_{0-\infty}$ increased linearly with dose from 3 to 600 mg and mean C_{max} increased linearly with dose from 3 to 300 mg, but not from 300 to 600 mg. Dose dependency of C_{max} (3-300 mg) and $AUC_{0-\infty}$ (3-600 mg) were assessed and the point estimate (95% CI) of β was calculated to be 1.04 (0.89, 1.20) for C_{max} and 1.12 (1.05, 1.19) for $AUC_{0-\infty}$. Maximum plasma concentration met the dose proportionality criteria. The parameter $AUC_{0-\infty}$ did not meet the dose proportionality criteria, although the point estimate for β was close to 1 and its 95% CI fell within 0.70, 1.30.

At 600 mg, the t_{max} was slightly delayed compared with that in the 3 and 10 mg dose groups (1.0 hour vs. 0.5 hours and 0.5 hours, respectively). These differences achieved statistical significance (both $P < 0.05$). For mean $t_{1/2}$, differences between ONO-5334 doses from 3 to 600 mg did not achieve statistical significance.

The geometric means (95% CIs) for C_{max} and $AUC_{0-\infty}$ after post prandial administration were 0.78 (0.31, 1.94) and 0.95 (0.67, 1.35) fold those obtained in fasting subjects, respectively. Median t_{max} after post prandial administration was 1.5 hours (range 1.0-3.0), which was slightly but significantly delayed (by ~0.5 hours) compared with the fasted condition ($P = 0.039$). Mean $t_{1/2}$ was similar for the fasted and fed states.

Pharmacodynamic assessments

One subject in the 600 mg dose group for serum CTX and one subject in the 100 mg dose group for urine NTX had pre-dose (baseline) concentrations below the LOQ. Analyses of serum CTX and urine NTX were produced excluding these subjects.

There was a notable variation in concentrations of both serum CTX and NTX throughout the day (placebo group) with a minimum concentration occurring at 6 hours post dose followed by a return to baseline values at 24 hours post dose. The pre-dose sample was collected at approximately 09.00 hours.

After dosing with ONO-5334, serum CTX was suppressed within 4 hours (first time point measured post dose) but only consistently (in all subjects) for 30 mg. The exception was one subject in the 600 mg dose group who showed no apparent suppression for the

reasons explained above. The maximum suppression (% change from baseline) in serum CTX for each dose occurred 6 hours post dose (-64% for 30mg, -68% for 100 mg, -66% for 300 mg, -72% for 600 mg compared with -50% for placebo). A number of subjects had values below the LOQ post dose (Table 1-3) indicating the actual magnitude of effect may be slightly greater than can be measured by the assay. This was notable from the 100 mg dose level where five, six and two (of the six subjects) had values below LOQ at 4, 6 and 12 hours post dose, respectively. In the 600 mg group, all five evaluable subjects had values below the LOQ at 4, 6, 12 and 24 hours post dose. When taking into account the normal variation in serum CTX concentrations across the day (as observed under placebo treatment), the maximum suppression occurred earlier at 4 hours post dose (difference to placebo: -32% for 30mg, -59% for 100 mg, -60% for 300 mg, -66% for 600 mg; 6 hours post dose: -14% for 30mg, -18% for 100 mg, -16% for 300 mg, -22% for 600 mg). This difference from placebo achieved statistical significance at the 4 and 6 hours post dose time points for doses of 30 mg and above. Serum CTX suppression continued at 12 and 24 hours post dose for doses above 100 mg, and for 600 mg serum CTX was statistically significantly suppressed vs. placebo at 48 and 72 hours post dose.

Table 1-3: Analysis of percentage changes in serum CTX compared with baseline

		Placebo	ONO-5334					
			3mg	10mg	30mg	100mg	300mg	600mg
		n=16	n=6	n=6	n=6	n=6	n=6	n=5
Pre-dose (Absolute	LSMean	0.7	0.73	0.66	0.71	0.53	0.61	0.65
CTX value) (mg/l)	95% CI for LSMean	(0.58, 0.81)	(0.55, 0.92)	(0.47, 0.85)	(0.52, 0.89)	(0.34, 0.72)	(0.42, 0.8)	(0.45, 0.86)
4 h (% CFB)	Difference vs. Placebo	NC	-3.34	-10.18	-31.64	-58.95	-60.37	-66.07
	95% CI for difference	NC	(-13.73, 7.06)	(-20.55, 0.19)	(-42.02, -21.25)	(-69.56, -48.35)	(-70.84, -49.9)	(-77.17, -54.97)
6 h (% CFB)	Difference vs. Placebo	NC	18.83	-11.64	-14.08	-18.32	-16.42	-22.47
	95% CI for difference	NC	(6.32, 31.34)	(-24.12, 0.84)	(-26.58, -1.58)	(-31.08, -5.56)	(-29.02, -3.82)	(-35.83, -9.11)
12 h (% CFB)	Difference vs. Placebo	NC	8.86	-5.6	-8.71	-31.65	-29.18	-37.76
	95% CI for difference	NC	(-2.87, 20.58)	(-17.3, 6.11)	(-20.43, 3.01)	(-43.62, -19.68)	(-40.99, -17.36)	(-50.29, -25.24)
24 h (% CFB)	Difference vs. Placebo	NC	8.18	-6.43	8.62	-24.54	-25.45	-74.18
	95% CI for difference	NC	(-14.03, 30.38)	(-28.58, 15.73)	(-13.56, 30.81)	(-47.2, -1.88)	(-47.82, -3.08)	(-97.89, -50.46)
48 h (% CFB)	Difference vs. Placebo	NC	1.1	-7	9.07	-9.26	-0.44	-56.03
	95% CI for difference	NC	(-19.07, 21.28)	(-27.13, 13.13)	(-11.09, 29.22)	(-29.85, 11.33)	(-20.76, 19.88)	(-77.58, -34.49)
72 h (% CFB)	Difference vs. Placebo	NC	-14.8	-7.29	5.34	-11.37	11.6	-25.77
	95% CI for difference	NC	(-32.88, 3.28)	(-25.33, 10.75)	(-12.72, 23.4)	(-29.82, 7.08)	(-6.62, 29.81)	(-45.08, -6.46)

CFB, change from baseline; n, number of subjects with evaluable data within group. Model = log(pre-dose) + Treatment, except for pre-dose where Model = Tteatment. NC, Not calculated.

After dosing with ONO-5334, serum NTX was suppressed within 4 hours (first time point measured post dose) and significantly vs. placebo for 300 mg (Figure 1-2). One subject in the 100 mg dose group showed greater than 200% increases relative to baseline at 4, 6 and 12 hours post dose (Table 1-4). The next highest within the 100 mg group was 28% (at 12 hours). The maximum suppression compared with baseline in serum NTX for each dose generally occurred 6 hours post dose (notwithstanding 100 mg) (-40% for 30mg,65% for 300 mg and -62% for 600 mg compared with -23% for placebo). The mean suppression for 100 mg with and without data from the subject with the outstanding increase was 14% and -24%, respectively. There was no apparent reason (e.g. procedural or AEs) why the 100 mg subject group or the one outstanding subject in particular failed to show results expected to be more consistent with a dose response effect. Serum NTX was significantly suppressed compared with placebo at 12 to 48 hours post dose, inclusive, only

for 600 mg although there was a tendency (P< 0.1) for 300 mg to stay suppressed at 12 and 24 hours.

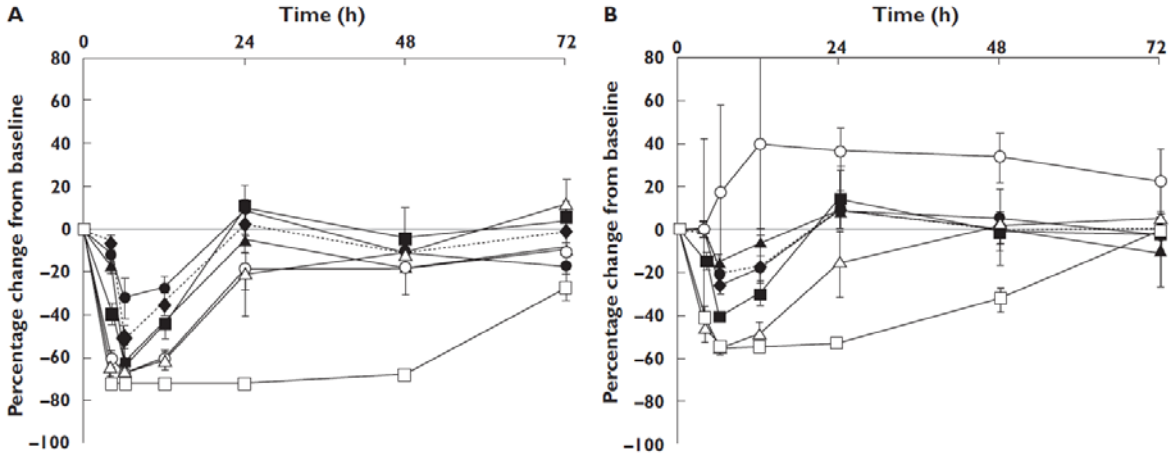

Figure 1-2: Effects of single ascending doses of ONO-5334 on A) serum CTX and B) serum NTX throughout 72 hours post dose. Mean (SE) percentage change from baseline, n=6 for all treatment groups (n=5 for 600 mg xCTX), n=16 for placebo. ◆ placebo; ● ONO-5334 3 mg; ▲ ONO-5334 10 mg; ■ ONO-5334 30 mg; ○ ONO-5334 100 mg; △ ONO-5334 300 mg; □ ONO-5334 600 mg.

Table 1-4: Analysis of percentage changes in serum NTX compared with baseline

		Placebo	ONO-5334					
			3mg	10mg	30mg	100mg	300mg	600mg
		n=16	n=6	n=6	n=6	n=6	n=6	n=6
Pre-dose (Absolute	LSMean	18.02	20.23	22.58	16.62	14.48	12.57	12.88
NTX value) (nM BCE)	95% CI for LSMean	(15.11, 20.93)	(15.48, 24.99)	(17.83, 27.34)	(11.86, 21.37)	(9.73, 19.24)	(7.81, 17.32)	(8.13, 17.64)
4 h (% CFB)	Difference vs. Placebo	NC	6.76	9.39	-15	-6.15	-57.24	-51.32
	95% CI for difference	NC	(-27.86, 41.38)	(-25.31, 44.09)	(-49.54, 19.53)	(-41.44, 29.14)	(-94.06, -20.42)	(-87.54, -15.11)
6 h (% CFB)	Difference vs. Placebo	NC	8.42	14.12	-17.1	36.92	-41.54	-38.91
	95% CI for difference	NC	(-25.25, 42.09)	(-19.63, 47.86)	(-50.68, 16.49)	(2.6, 71.24)	(-77.35, -5.73)	(-74.13, -3.69)
12 h (% CFB)	Difference vs. Placebo	NC	3.03	14.37	-12.85	53	-38.84	-44.55
	95% CI for difference	NC	(-34.47, 40.52)	(-23.21, 51.94)	(-50.25, 24.54)	(14.79, 91.21)	(-78.71, 1.03)	(-83.77, -5.34)
24 h (% CFB)	Difference vs. Placebo	NC	1.31	0.86	4.97	26.32	-26.63	-63.88
	95% CI for difference	NC	(-26.01, 28.64)	(-26.52, 28.25)	(-22.29, 32.23)	(-1.53, 54.17)	(-55.69, 2.43)	(-92.46, -35.29)
48 h (% CFB)	Difference vs. Placebo	NC	5.4	1.87	-3.75	28.73	-4.45	-39.79
	95% CI for difference	NC	(-19.73, 30.54)	(-23.32, 27.07)	(-28.82, 21.32)	(3.11, 54.35)	(-31.18, 22.29)	(-66.08, -13.5)
72 h (% CFB)	Difference vs. Placebo	NC	0.28	-6.38	-4.3	14.28	-9.59	-13.7
	95% CI for difference	NC	(-22.86, 23.42)	(-29.57, 16.81)	(-27.38, 18.78)	(-9.3, 37.87)	(-34.19, 15.02)	(-37.9, 10.5)

CFB, change from baseline; n, number of subjects with evaluable data within group. Model = log(pre-dose) + Treatment, except for pre-dose where Model = Treatment. NC, Not calculated.

Secondary void urine CTX at 24 hours showed statistically significant suppression compared with placebo at doses 100 mg (-38% for 100 mg, -96% for 300 mg and -104% for 600 mg; Table 1-5, Figure 1-3). For the 600 mg dose group, there was clear, statistically significant suppression of urine CTX compared with placebo that continued for 3 days (-52% at 72 hours).

Table 1-5: Analysis of percentage changes in urine CTX compared with baseline

		Placebo	ONO-5334					
			3mg	10mg	30mg	100mg	300mg	600mg
		n=16	n=6	n=6	n=6	n=6	n=6*	n=6
Pre-dose (Absolute CTX value)	LSMean	344.88	298.67	308.83	328.33	378	200	280
(mg/mmol creatinine)	95% CI for LSMean	(295.08, 394.67)	(217.35, 379.98)	(227.52, 390.15)	(247.02, 409.65)	(296.68, 459.32)	(110.92, 289.08)	(198.68, 361.32)
24 h (% CFB)	Difference vs. Placebo	NC	4.6	-8.58	0.58	-38.2	-96.38	-103.7
	95% CI for difference	NC	(-16.95, 26.16)	(-29.81, 12.66)	(-20.61, 21.77)	(-59.59, -16.82)	(-121.6, -71.16)	(-125.3, -82.13)
48 h (% CFB)	Difference vs. Placebo	NC	12.19	-5.38	17.94	1.42	13.76	-73.38
	95% CI for difference	NC	(-21.42, 45.8)	(-38.5, 27.74)	(-15.11, 50.99)	(-31.93, 34.78)	(-25.57, 53.09)	(-107.1, -39.68)
72 h (% CFB)	Difference vs. Placebo	NC	-1.85	-11.58	2.98	25.56	21.17	-52.01
	95% CI for difference	NC	(-36.84, 33.15)	(-46.05, 22.9)	(-31.42, 37.39)	(-9.16, 60.28)	(-19.77, 62.11)	(-87.09, -16.93)

n, number of subjects with evaluable data within group. Model = log(pre-dose) + Treatment, except for pre-dose where Model = Tteatment. NC, Not calculated.

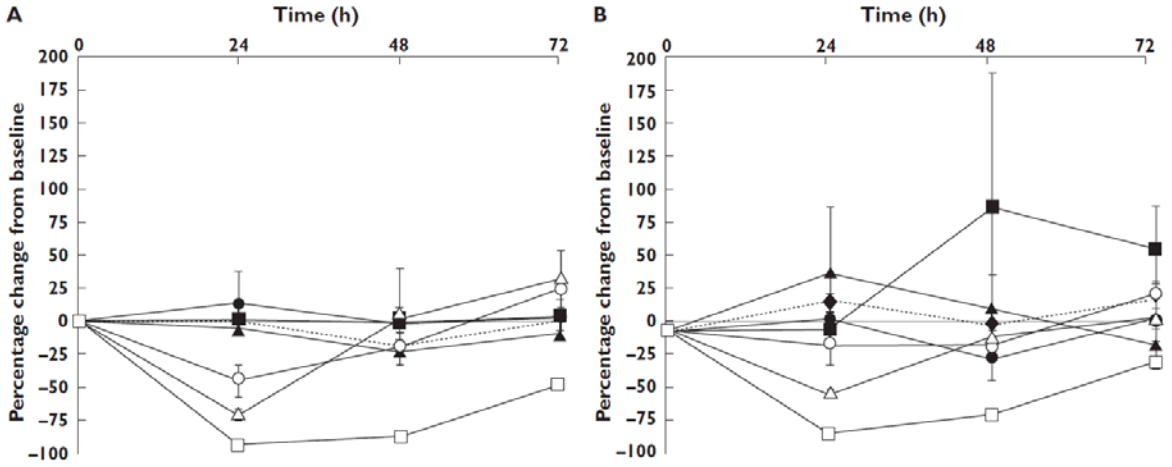

Figure 1-3: Effects of single ascending doses of ONO-5334 on (A) urinary CTX and (B) urinary NTX throughout 72 hours post dose. Second morning urine void collected. Mean (SE) percentage change from baseline, n=5-6 for all treatment groups and n=16 for placebo. ◆ placebo; ● ONO-5334 3 mg; ▲ ONO-5334 10 mg; ■ ONO-5334 30 mg; ○ ONO-5334 100 mg; △ ONO-5334 300 mg; □ ONO-5334 600 mg.

For urinary NTX, one 100 mg subject had pre-dose (baseline) concentration below the LOQ and so these results exclude this subject. Statistically significant suppression in urine NTX at 24 hours compared with placebo was observed for100 mg (-63% for 100 mg,-75% for 300 mg and -113% for 600 mg; Table 1-6, Figure 1-3). For 600 mg, significant suppression continued for 3 days (-57% at 72 hours, P <0.01).

Table 1-6: Analysis of percentage changes in urine NTX compared with baseline

		Placebo	ONO-5334					
			3mg	10mg	30mg	100mg	300mg	600mg
		n=16	n=6	n=6*	n=6	n=5+	n=6	n=6
Pre-dose (Absolute NTX value)	LSMean	63.5	72.83	66.67	62.67	32.2	59.17	50.5
(nm BCE/mmol creatinine)	95% CI for LSMean	(48.69, 78.31)	(48.65, 97.02)	(42.48, 90.85)	(38.48, 86.85)	(5.71, 58.69)	(34.98, 83.35)	(26.32, 74.68)
24 h (% CFB)	Difference vs. Placebo	NC	-2.93	24.34	-25.08	-62.81	-75.23	-112.6
	95% CI for difference	NC	(-46.81, 40.95)	(-19.06, 67.75)	(-68.51, 18.34)	(-115.7, -9.97)	(-118.6, -31.83)	(-156.2, -69.08)
48 h (% CFB)	Difference vs. Placebo	NC	-5.68	17.09	92.26	-65.59	-10.31	-85.24
	95% CI for difference	NC	(-88.72, 77.35)	(-70.82, 105)	(10.09, 174.44)	(-165.1, 33.97)	(-92.43, 71.82)	(-167.6, -2.84)
72 h (% CFB)	Difference vs. Placebo	NC	-3.02	-35.07	38.3	-20.85	-12.95	-56.92
	95% CI for difference	NC	(-45.51, 39.46)	(-77.09, 6.95)	(-3.74, 80.35)	(-67.99, 26.28)	(-54.97, 29.07)	(-99.08, -14.76)

n, number of subjects with evaluable data within group. Model = log(pre-dose) + Treatment, except for pre-dose where Model = Treatment. NC, Not calculated. * one subject had missing value at 48 hours. +, two subjects had missing values, one at 24 hours and one at 48 hours.

Safety assessments

There were no serious adverse events or withdrawals/discontinuations from the study treatment due to adverse events (AEs). Fifty-five TEAEs were reported by 30 of the 52 subjects (57.7%) when dosed under fasted conditions. Ten out of 16 subjects (62.5%) reported TEAEs after placebo treatment whereas 20 of 36 (55.5%) subjects reported TEAEs after ONO-5334 (fasted states; Table 1-7). Eight TEAEs were reported by six out of 12 subjects who were dosed after receiving a standard breakfast. One subject on placebo treatment had one TEAE, whereas five subjects dosed with 100 mg ONO-5334 had seven TEAEs. All TEAEs were transient. Thirty-two of 55 TEAEs occurred within the first day of dosing. The duration of the TEAEs did not appear to be related to treatment or dose. Four subjects experienced TEAEs that were graded as severe in intensity: vomiting and back pain in the placebo group, renal colic after ONO-5334 300 mg and headache after ONO-5334 600 mg. All these events resolved within 1 day. The most frequently reported TEAEs according to System Organ Class were gastro-intestinal disorders and nervous

system disorders (Table 1-7). There were no other clinically relevant changes in ECG, laboratory assessments, vital signs or physical examinations.

Table 1-7: Summary of adverse events

	Placebo	ONO-5334							
	Fasted	3mg	10mg	30mg	100mg	300mg	600mg	100mg fed	Placebo
	n=16	n=6	n=6	n=6	n=6	n=6	n=6	n=6	n=6
At least one AE	10	6	2	2	4	4	2	5	1
At least one drug-related AE*	6	4	1	-	1	3	2	3	1
AEs of moderate intensity	3	-	-	-	-	1	-	-	-
AEs of severe intensity	-	-	-	-	-	-	-	-	-
Serious AEs	-	-	-	-	-	-	-	-	-
Gastrointestinal disorders	4	3	2	2	4	4	2	5	1
Hard faeces	0	0	0	0	0	0	0	0	0
Flatulence	0	0	0	0	0	2	0	0	0
Nausea	2	1	0	1	0	1	0	1	0
Vomiting	2	0	0	0	0	0	0	0	0
Nervous system disorders	5	4	1	2	1	1	1	3	0
Jeadacje	4	2	1	2	0	1	0	2	0
Somnolence	2	2	0	0	1	0	1	0	0

n, number of subjects in specific group. *, Relationship to study medication according to the investigator: related = probably, possibly or definitely related.

Discussion

When ONO-5334 was orally administered to postmenopausal Caucasian women in a fasted state at doses of 3 to 600 mg, its C_{max} was proportional to dose within the range of 3 to 300 mg. However, it was less than proportional between 300 and 600 mg. Although the point estimate of β for $AUC_{0-\infty}$ (3-600 mg) did not meet pre-defined criteria which define dose-proportionality, the point estimate of β was close to 1 and the 95% CI was within pre-defined levels. Therefore, it was considered that $AUC_{0-\infty}$ of ONO-5334 was nearly proportional to dose from 3 to 600 mg. The t_{max} value for ONO-5334 at 600 mg was slightly delayed compared with those at 3 and 10 mg but there was no difference among the doses 3-300 mg. $t_{1/2}$ was not significantly different between any doses. Taking into account the available plasma pharmacokinetic data, it appears that ONO-5334 demonstrated linear pharmacokinetics over the expected therapeutic dose range 3 to 300 mg after single dosing.

The delayed t_{max} at 600 mg and the less than proportional C_{max} between 300 and 600 mg may have been a consequence of the saturated dissolution rate of the ONO-5334 tablet. Consequently, the absorption rate decreased with increasing dose. However, as $AUC_{0-\infty}$ was almost proportional to dose even between 300 and 600 mg, the total absorption ratio may not be affected by the increase in dose. This suggests that the gastrointestinal region involved in ONO-5334 absorption may not be limited just to the upper tract, and that the lower tract may play some role in absorption. Hence controlling the dissolution rate of the ONO-5334 tablet may achieve a more sustained ONO-5334 plasma profile.

When comparing the pharmacokinetic profile between the fasted and fed conditions at a dose of 100 mg, C_{max} after post prandial administration was slightly lower (0.78-fold) than in the fasted state. However, changes in C_{max} were not consistent across all subjects tested and the ratio of the geometric mean for C_{max} after post prandial administration compared with fasting was also wide (0.31 to 1.94). Hence, C_{max} may be more under the influence of intra-individual variability than food. As the ratio of the geometric mean for $AUC_{0-\infty}$ after post prandial administration compared with the fasted state was 0.95 and its 95% CI was 0.67, 1.35, it was concluded that total exposure to ONO-5334 was unaffected by food, although it should be noted that t_{max} was slightly delayed. Given the nature of the target disease (osteoporosis) and the mechanism of action of antiresorptive agents (cathepsin K inhibitors), we conclude that this small effect of food on t_{max} will be of little clinical significance.

ONO-5334 decreased serum CTX in a dose-dependent manner. As serum CTX values of several subjects decreased below the limit of detection, the observed dose effect of ONO-5334 on serum CTX is slightly underestimated. The serum NTX concentrations in the 100 mg dose group changed in a different manner over time to other dose groups. The difference in percentage change was affected by a subject in the 100 mg group who had a low pre-dose (baseline) value and relatively high post dose values up to 12 hours (200%). However, even without this subject the 100 mg results were not consistent in following the expected dose-response effect. These findings cannot be explained by changes in study conduct (e.g. sample viability or assay procedures) in this dose group compared with others and may have been due to the limited number of subjects in the current study. As the

impact of natural variability among subjects on study endpoints increases with decreasing sample size, effects of natural subject variability were a possibility inherent in the sample size planned for the current study.

Serum and urine CTX and NTX are recognized markers of bone resorption. After collagen is degraded by proteases such as cathepsin K and matrix metalloproteases, these peptides of collagen are released into the bloodstream and metabolized in the kidney and liver. These peptides are then excreted into urine. However, the preference for which of the two markers provides the most useful indicator of ONO-5334 activity is unclear. It appeared that serum CTX concentrations were more sensitive to single doses of ONO-5334 than serum NTX even after natural diurnal variation had been taken into account. Compared with placebo, mean maximum values of suppression were -66% for serum CTX and -57% for NTX.

Placebo data suggest that the diurnal variation in serum NTX is less marked than that of serum CTX. The suppressive effect of ONO-5334, with the diurnal variation taken into account, was more easily detected for serum CTX than for NTX at lower dose levels due to a greater magnitude of decrease. Suppression at doses of 100 mg and above was detectable for serum CTX and 300 mg for serum NTX. However, at dose levels of 100 mg and above, accurate serum CTX values could not be measured due to the quantification limit of the assay. This occurred much less frequently for serum NTX and, therefore, serum NTX may be more reliable as a marker of bone turnover in evaluating the pharmacodynamics of ONO-5334 (notwithstanding the issues with the 100 mg dose group). At lower dose levels,

however, more data points may be required for serum NTX than CTX to detect significant suppression due to the smaller magnitude of the suppression for serum NTX.

When considering the administration of a once daily dosing schedule, it is important that suppression of bone markers is maintained for 24 hours and that suppression is observed in both serum and urine samples. In the current study, suppression of serum CTX appeared to be ongoing at 24 hours at ONO-5334 doses of 100 mg and above. Suppression of serum NTX was observed at doses of ONO-5334 300 mg and above, although statistical significance was achieved only at the 600 mg dose. Statistically significant suppression of urine markers was observed at 24 hours for both CTX and NTX at doses of ONO-5334 100 mg and above. The CTX marker appeared to show slightly greater sensitivity to ONO-5334 than NTX, thus highlighting the importance of selecting the correct bone turnover marker, especially when making decisions on dose selection.

In a fashion similar to that of serum CTX, urinary CTX appeared to exhibit greater suppression than NTX. It is not known whether such differences in these bone resorption markers are due to the mechanism of action of ONO-5334 or some impact on metabolism. Since the cleavage sites of cathepsin K are located close to the sequence of type I collagen, low molecular weight CTX/NTX may be considered to be a more sensitive marker for cathepsin K inhibitors than total CTX/NTX. Interestingly, Kawada et al. measured plasma CTX in rats before and after centrifugation with a filter to remove molecules with a molecular weight of 30 000 or more [40]. The results showed a maximum suppression of about 80% in change from baseline using the filter compared with 40% without the filter. Unfortunately, the authors did not measure serum NTX or urinary CTX. Large molecular

weight fragments are not filtered through the kidney. It remains to be confirmed whether this means the assays are likely to detect predominantly low molecular weight CTX in urine and thus magnify changes in low molecular weight CTX in urine compared with that in serum.

The immunoassays employed in this study used different antibodies to detect CTX in serum and urine. In serum, the antibody was specific for the β (isomerized) form of CTX only, whereas, in urine the antibody was specific for both the α (non-isomerized) and β forms of CTX. In postmenopausal women α-CTX represents a smaller proportion of total urinary CTX than β-CTX [41-43]. Differences in the detection of the α and β forms may provide an explanation for the observed difference in apparent increased suppression of urine CTX compared with serum CTX following administration of ONO-5334, although this is not certain. Differences were observed between serum and urinary NTX even though NTX does not exist as isomers. Therefore, it is prudent to use more than one marker of bone resorption and further evaluation is warranted to identify the most appropriate marker of bone turnover for ONO-5334 and cathepsin K inhibitors.

With regards to the safety and tolerability of ONO-5334, no serious adverse events were reported in the study. TEAEs were reported in all treatment groups and the nature of AEs with active treatment was not clearly different compared with placebo. There was no clear dose dependent relationship in the timing or duration of AEs. In fact, the lowest dose group (3 mg) reported the greatest number of AEs. Increasing the dose did not result in an increase in the frequency, incidence or intensity of the reported AEs. All AEs were transient and predominantly mild in nature. There were no other clinically significant

findings in any other safety assessment. Therefore, ONO-5334 was considered to be well-tolerated after single administration in postmenopausal women.

In summary, ONO-5334 was well tolerated in postmenopausal women at doses up to 600 mg per day. ONO-5334 pharmacokinetics were linear up to 300 mg. Food may have delayed the absorption of, but not the exposure to, ONO-5334. At doses of ONO-5334 100 mg and above, decreases were observed in serum CTX, urine CTX and urine NTX for at least 24 hours. At the 600 mg dose, suppression of serum NTX was observed for 48 hours and suppression of serum CTX, urine CTX and urine NTX continued until 72 hours post dose. Further investigation into the pharmacodynamics effect on bone turnover markers and pharmacokinetic effects of repeated dosing would be a justified next step in the development of ONO-5334 for osteoporosis.

Part II

Pharmacodynamic Effects on Biochemical Markers of Bone Turnover and Pharmacokinetics of the Cathepsin K Inhibitor, ONO-5334, in an Ascending Multiple-Dose, Phase 1 Study

Abstract

The pharmacokinetics and the pharmacodynamic effects on biochemical markers of bone turnover of the new cathepsin K inhibitor, ONO-5334, were investigated in a multiple ascending dose, phase 1 study. A total of 120 healthy postmenopausal women were enrolled, and doses of 10 to 600 mg once daily and 50 and 300 mg twice daily were evaluated in 15- and 28-day multiple-dosing cohorts. Plasma ONO-5334 concentration reached steady state within 2 days. Twenty-four hours after the last dose in the 15-day multiple-dose cohort, 100, 300 and 600 mg once daily reduced urinary C-terminal telopeptide of type I collagen (CTX) by a mean (± standard deviation) 44.9% ± 13.6%, 84.5% ± 4.4% and 92.5% ± 1.3%, respectively. The 28-day cohort showed similar effects. There were far smaller effects on bone-specific alkaline phosphatase (B-ALP), tartrate-resistant acid phosphatase 5b (TRAP5b), or osteocalcin (OC) (measured after 28 days). ONO-5334 was well tolerated up to 600 mg/day and for up to 28 days of multiple dosing. Multiple dosing with ONO-5334 100 mg resulted in considerable suppression of bone resorption markers with no appreciable effects on bone formation markers (B-ALP, OC) or osteoclast number (TRAP5b).

Introduction

It is expected that cathepsin K inhibitor may have no or less effect on bone formation not likewise traditional anti-resorptive agents such as bisphosphonates. Cathepsin K inhibitor shows an anti-resorptive effect based on its collagenolytic activity. This means treatment may not affect osteoclast activity or numbers which could result in no or lesser suppression on bone formation via coupling mechanisms between osteoclasts and osteoblasts. Under this assumption, I conducted Phase I multiple-dose study to investigate the effect of ONO-5334 on a variety of established biochemical markers of bone turnover as well as evaluating its pharmacokinetics, safety, and tolerability in healthy postmenopausal women. In the study, I aimed for confirming sustained suppression on bone resorption by evaluating CTX and N-terminal telopeptide of type I collagen (NTX), bone resorption markers. In addition to those, I also evaluated a couple of biochemical markers of bone formation including B-ALP and PINP in order to confirm that ONO-5334 does not suppress bone formation. I also evaluated TRAP5b in order to determine if osteoclast activity or numbers are not affected which could explain mechanisms of ONO-5334 not to suppress bone formation.

Materials and Methods

Study Design

A total of 120 postmenopausal females were enrolled in the study, having given written informed consent. The study was approved by the local ethics committee. Subjects had to be aged 45 to 75 years with body mass index (BMI) between 19 and 32 kg/m^2. Subjects were excluded if they had any relevant medical history, including osteoporosis, severe allergic reaction, or drug or alcohol abuse, or if they had other clinically significant abnormalities at screening. Subjects were also excluded if they had smoked within the last 6 months, drank more than 14 units of alcohol per week, or had received prescription drugs within 28 days prior to dosing (St John's wort within 6 weeks), nonprescription drugs within 28 days prior to dosing, or over-the-counter drugs within 48 hours unless such drugs were deemed necessary by the principal investigator and did not interfere with study procedures or subjects' safety. Strenuous exercise, caffeine, and alcohol were prohibited for 48 hours prior to study start and during the study.

At screening, health status was determined by pre-study medical history, physical examination, vital signs, 12-lead electrocardiogram (ECG), continuous ECG monitoring, BMI assessment, and routine clinical laboratory tests, including tests for drugs of abuse. Postmenopausal was defined as being amenorrheic for more than 1 year if older than 50 years and more than 2 years if 45 to 50 years with an appropriate clinical profile and confirmed at screening by estradiol and follicle-stimulating hormone (FSH) levels consistent with menopause (FSH >30 IU/L, estradiol <92 pmol/L).

Following confirmation of eligibility and on entry into the study on admission, subjects were randomly assigned to ONO-5334 or placebo. There were 8 dose groups in the 15-day cohort and 2 dose groups in the 28-day cohort. Each dose group had a total of 12 subjects, 9 for ONO-5334 and 3 for placebo.

As a rationale for the doses used, in a single ascending dose study, ONO-5334 was generally well tolerated and found to be safe when administered in single oral doses from 3 to 600 mg to healthy postmenopausal women [44]. The current study was undertaken to evaluate the effect of repeated dosing of ONO-5334 on pharmacokinetics (PK), pharmacodynamics, safety, and tolerability up to a total daily dose of 600 mg. The twice-daily treatment groups were chosen to compare the efficacy and safety profile of 2 different dose regimens (with 100 mg and 600 mg total daily dose) because the outcome may suggest development of a sustained-release formulation. For an effect to be observed on bone formation, longer treatment is expected; therefore, the study duration was extended to 28 days in 2 dose groups, 100 and 600 mg once daily.

Standard safety assessments were performed throughout the study including vital signs, physical examination, 12-lead ECG, continuous ECG Holter monitoring, hematology, biochemistry, urinalysis, and adverse event monitoring.

Fifteen-day multiple-dosing cohort

Once-daily treatments of 10, 30, 50, 100, 300 and 600 mg, and twice-daily treatments of 50 and 300 mg were assessed. Subjects received ONO-5334 or placebo 30 minutes after

a standardized breakfast with approximately 200 mL of water. In the twice-daily groups, the evening dose was provided 30 minutes after dinner, 12 hours after a morning dose. In all dose groups, subjects were followed for 48 hours after the first morning administration (day 1) without further dosing in order to assess the PK. After this follow-up, subjects were dosed for 14 consecutive days (days 3-16) according to the treatment assigned. In the twice-daily group, dosing was completed with the morning dose on the last day (day 16). This study design was intended to investigate possible effect of repeated dosing on PK by comparing those profiles between the first and the last dosing. Subjects were admitted to the study unit on the day before the first dosing and were followed for 72 hours after the last dosing before being discharged. A follow-up visit was arranged for each subject 5 to 7 days after the last dose.

PK blood sampling was performed on the first (day 1) and last (day 16) day of administration at baseline (before dosing) and at 15 minutes, 30 minutes, and 1, 1.5, 2, 4, 6, 8, 12, 24, 36 and 48 hours after dosing (48 hours: before morning administration on day 3). To evaluate steady-state PK, pre-dose (trough) PK samples were obtained on days 5, 7 and 9 (before the morning administration) and days 3, 5, 7 and 9 (before the evening administration for the twice-daily regimen group).

To assess the effect of ONO-5334 on bone resorption, serum and urine were collected. Serum CTX and serum NTX were assessed at baseline and at 4, 8, 11 and 24 hours after administration on days 1 and 16. Pre-dose (trough) levels were assessed on days 3, 5, 7 and 9 and again at the follow-up visit (5-7 days post treatment). Urine from the second

morning void was obtained prior to morning administration on days 1, 2, 3, 5, 7, 9, 16, 17 and 18 and at the follow-up visit (5-7 days post treatment).

Twenty-eight-day multiple-dosing cohort

Once-daily treatments of 100 and 600 mg were assessed. Subjects received ONO-5334 or placebo 30 minutes after a standardized breakfast with approximately 200 mL of water. A dosing design similar to that received by the 15-day cohort was selected, that is, 1 morning dose (day 1) with 48-hour assessment followed by 27 days of consecutive daily doses (days 3-29). Subjects were admitted to the study unit on the day before the first dosing and remained until day 16, after which time they were discharged. Subjects were readmitted to the unit on the day before the last dosing. After the last dosing, subjects were followed up for 72 hours in the unit. A follow-up visit was arranged for each subject 5 to 7 days after the last dose.

Blood samples (7.5 mL) were collected for PK analysis on the first day (day 1) and the last day (day 29) of administration at the same times as described for the 15-day cohort. Trough (pre-dose) samples were also obtained on days 9, 16, 23 and 29 (baseline for the last dosing). The plasma samples obtained were divided into 3 sample tubes and stored at -20°C.

Serum markers of bone turnover were assessed at baseline and days 9, 16, 23 and 29 (pre-dose levels). Urine from the second morning void was obtained prior to morning administration on days 1, 2, 3, 9, 16, 23 and 29.

This study was conducted at Kendle International B.V., Clinical Pharmacology Unit (the Netherlands).

Analysis

Clinical laboratory tests were conducted at Eurofins Medinet BV (the Netherlands). Plasma ONO-5334 assays were performed by Sumika Chemical Analysis Service, Ltd (Sumika, Japan).

Plasma concentrations of ONO-5334 were determined by a validated liquid chromatography-tandem mass spectroscopy method with a lower limit of quantification of 0.02 ng/mL. ONO-5334 was extracted from plasma samples using a solid-phase extraction column followed by quantification. The precision of the ONO-5334 assay was less than 15% (coefficient of variation), and the accuracy of the assay was within 15% of the actual value for ONO-5334.

To investigate the effect of ONO-5334 on biochemical markers of bone turnover, serum and urinary CTX (CrossLaps enzyme-linked immunosorbent assay [ELISA], Nordic Bioscience Diagnostics A/S, Herlev, Denmark), serum and urinary NTX (Osteomark NTX diagnostic kit OsteoMark Wampole Laboratories, Princeton, USA), and deoxypyridinoline (DPD) (ELISA Quidel UK Limited, Hannover, Germany) were assessed in both cohorts (15 and 28 days). In the 28-day cohort, procollagen type I intact N-terminal propeptide (PINP) (Orion Diagnostica UniQ PINP RIA Orion Diagnostica, Espoo, Finland), bone-specific alkaline phosphatase (B-ALP) (Metra BAP immunoassay kit Quidel UK Limited, Marburg,

Germany), osteocalcine (OC) (Metra Osteocalcin EIA kit Quidel UK Limited, Hannover, Germany), and tartrate-resistant acid phosphatase isoform 5b (TRAP5b) (METRA TRAP5b Enzyme immunoassay Quidel UK Limited, Hannover, Germany) were assessed. Assays were performed by Charles River Laboratories (Edinburgh, United Kingdom).

PK analysis was performed by Ono Pharmaceutical Co., Ltd. using WinNonlin 4.0.1 software (Pharsight Corp, Mountain View, California). Maximum concentration (C_{max}; ng/mL), time to C_{max} (t_{max}; hours), area under the concentration-time curve to the last measurement (AUC_{last}; ng*h/mL), AUC to infinity (AUC_{inf}; ng*h/mL) and half-life ($t_{1/2}$; hours), were calculated for ONO-5334. Observed accumulation factors (R_{obs}) with 95% confidence interval (CI) and predicted accumulation factors (R) were calculated for C_{max} and AUC_{τ}. AUC_{τ} indicates $AUC_{0\text{-}24h}$ for once daily treatment and $AUC_{0\text{-}12h}$ for twice daily treatment, respectively. R_{obs} was calculated as the ratio of geometric mean dividing AUC_{τ} or C_{max} obtained after the last administration by the value obtained after the first administration. R was calculated as the ratio of the predicted PK parameter determined after the last administration, divided by the value obtained after the first administration. The predicted PK parameters after the first and last administration were calculated from the plasma concentration-time profile predicted from the actual mean plasma concentration-time profile after the first administration using the 2-compartment model.

For bone turnover markers, the percentage change from baseline was summarized using descriptive statistics for each treatment and time point. Urinary markers were corrected for creatinine. Any values below the limit of quantification (BLQ) were assigned

the BLQ value for the assay (BLQs: serum CTX <0.173 μg/L, urinary CTX <99 μg/L, urinary NTX <20 nM bone collagen equivalent [BCE], urinary DPD <15 nmol/L).

An analysis of covariance (ANCOVA) was performed for serum CTX, serum NTX, urinary CTX, and urinary NTX at day 16 (before dosing) for the 15-day multiple-dosing cohort and for PINP, B-ALP, OC, and TRAP5b at day 29 (before dosing) for the 28-day multiple-dosing cohort. This ANCOVA was not a pre-specified analysis but was performed after review of the data to gain an understanding of statistically significant differences versus placebo after multiple dosing.

For each dose of ONO-5334, the log-transformed percentage change from baseline was compared with log-transformed percentage change from baseline for placebo using ANCOVA with treatment as a fixed effect and baseline value as a covariate. P values were calculated, and no adjustments were applied to the type I error to account for multiple treatment comparisons for this exploratory analysis. All tests were 2-tailed and performed at the 5% significance level.

Safety analyses of the study population were summarized by treatment with descriptive statistics and frequency tables. A treatment-emergent adverse event (TEAE) was defined as an adverse event that occurred or worsened after the first investigational product intake.

Results

A total of 120 subjects were randomized, with 96 subjects in the 15-day multiple-dosing cohort and 24 subjects in the 28-day multiple-dosing cohort. Baseline characteristics of the subjects enrolled are summarized in Table 2-1. No subject was withdrawn from the study.

Table 2-1: Baseline Subject Characteristics (Safety Analysis Set)

	Dose, mg	No.[a]	Age, y	Height, cm	Weight, kg
15-day cohort	Placebo (qd)	16	63 ± 5.4	166 ± 6.3	73 ± 10.0
	10 (qd)	9	60 ± 5.6	165 ± 6.6	74 ± 9.4
	30 (qd)	11[b]	59 ± 5.5	166 ± 5.3	67 ± 8.8
	50 (qd)	9	59 ± 6.7	167 ± 4.4	68 ± 7.2
	100 (qd)	9	58 ± 5.0	165 ± 5.7	70 ± 6.7
	300 (qd)	9	62 ± 5.7	166 ± 5.8	74 ± 9.1
	600 (qd)	9	61 ± 3.7	168 ± 5.4	72 ± 10.2
	Placebo (bid)	6	62 ± 7.4	168 ± 8.1	69 ± 4.9
	50 (bid)	9	62 ± 5.6	165 ± 4.0	66 ± 3.0
	300 (bid)	9	61 ± 5.4	168 ± 4.5	75 ± 8.1
28-day cohort	Placebo (qd)	6	63 ± 7.5	164 ± 6.1	71 ± 12.1
	100 (qd)	9	62 ± 6.1	166 ± 5.7	70 ± 7.4
	600 (qd)	9	61 ± 4.3	166 ± 3.9	71 ± 7.2

bid, twice daily; qd, once daily. Data are expressed as mean ± standard deviation.
a. Number of subjects within group. b. The 30-mg group includes all subjects who received 30 mg of ONO-5334 at any time during the study.

The safety analysis set included all subjects who received at least 1 dose of study medication. Four subjects in the 15-day cohort received the incorrect treatment-30 mg of ONO-5334 once daily and placebo were administered incorrectly. During the first administration these subjects were included in the 30 mg ONO-5334 group safety analysis set because all 4 subjects received at least 1 dose of ONO-5334. These subjects were

excluded from the analysis including ANCOVA of bone turnover markers. One additional subject was excluded from the PK analysis before unblinding because the 2-hour post-dose sample was missed (15-day cohort), the point at which the maximum plasma concentration could be expected.

Pharmacokinetic Assessments

At all doses in both the 15- and 28-day treatment periods, the predicted plasma concentration-time profiles during multiple dosing, simulated from PK data of the initial single dose, were similar to the observed plasma concentration-time profile (data not shown). R and R_{obs} for C_{max} and AUC_{τ} are summarized in Table 2-2. The observed ratios of the geometric mean with their 95% CIs were not substantially different than the predicted ratios. Changes in trough plasma concentration of ONO-5334 in the morning are presented in Table 2-3 and Figure 2-1. The trough plasma concentration of ONO-5334 became stable 2 days after the first administration (observed from the 15-day cohort), and the steady-state levels appeared consistent as did all the other PK parameters between the 15- and 28-day dosing cohorts (Table 2-4).

Table 2-2: Observed and Predicted Accumulation Factors for C_{max} and AUC of ONO-5334 After Multiple Oral Administration of ONO-5334

			C_{max}		AUC_t[a]	
	Dose, mg	No.	R	R_{obs}	R	R_{obs}
15-day cohort	10 (qd)	9	1.01	0.85 (0.61-1.19)	1.06	1.01 (0.83-1.23)
	30 (qd)	7	1.01	1.14 (0.80-1.64)	1.04	1.25 (1.07-1.46)
	50 (qd)	9	1.01	1.35 (0.99-1.84)	1.04	1.16 (1.01-1.34)
	100 (qd)	8	1.00	0.69 (0.49-0.98)	1.02	0.89 (0.75-1.06)
	300 (qd)	9	1.00	0.88 (0.55-1.41)	1.02	1.02 (0.77-1.34)
	600 (qd)	9	1.00	0.87 (0.55-1.36)	1.02	0.98 (0.77-1.25)
	50 (bid)	9	1.02	1.63 (1.04-2.54)	1.07	1.55 (1.21-1.99)
	300 (bid)	9	1.02	0.84 (0.50-1.43)	1.02	0.99 (0.75-1.31)
28-day cohort	100 (qd)	9	1.00	0.77 (0.40-1.47)	1.02	1.00 (0.69-1.45)
	600 (qd)	9	1.01	0.93 (0.72-1.20)	1.05	1.15 (0.91-1.46)

AUC, area under the concentration–time curve; C_{max}, maximum concentration; R, simulated ratio; R_{obs}, observed ratio of geometric mean (95% confidence interval).
AUC_t indicates $AUC_{0\text{-}24h}$ for once daily treatment and $AUC_{0\text{-}12h}$ for twice daily treatment, respectively.

Table 2-3: Plasma Concentration of ONO-5334 After 15-Day and 28-Day Multiple Dosing

Dose, mg	15-Day Cohort: Plasma Concentration Before Dosing on the Last Dosing Day (Day 16)	28-Day Cohort: Plasma Concentration Before Dosing on the Last Dosing Day (Day 29)
10 (qd)	0.165 ± 0.043	Not evaluated
30 (qd)	0.285 ± 0.154[a]	Not evaluated
50 (qd)	0.601 ± 0.203	Not evaluated
100 (qd)	0.844 ± 0.285[b]	0.951 ± 0.392
300 (qd)	3.97 ± 1.57	Not evaluated
600 (qd)	15.7 ± 7.5	19.9 ± 9.0
50 (bid)	2.28 ± 0.46	Not evaluated
300 (bid)	15.8 ± 6.8	Not evaluated

bid, twice daily; qd, once daily. Values are expressed as ng/mL, mean ± SD. n = 9. a. n = 7. b. n = 8.

Table 2-4: Pharmacokinetic Parameters for the Last Dosing in the 15- and 28-Day Cohorts

	15-Day Cohort				28-Day Cohort			
Dose, mg	C_{max}, ng/mL	t_{max}, h	$AUC_{0\text{-}24h}$, ng·h/mL	$t_{1/2}$, h	C_{max}, ng/mL	t_{max}, h	$AUC_{0\text{-}24h}$, ng·h/mL	$t_{1/2}$, h
100 qd[a]	198 (115)	2.0 (0.50-4.0)	415 (191)	15 (4)	250 (271)	2.0 (1.0-4.0)	446 (373)	13 (5)
600 qd	2030 (720)	1.5 (1.5-2.0)	4290 (1200)	8.1 (1.4)	1820 (780)	1.5 (0.50-4.0)	4170 (1400)	11 (3)

$AUC_{0\text{-}24h}$, area under the concentration-time curve from 0 to 24 hours; C_{max}, maximum concentration; qd, once daily; t_{max}, time to Cmax; $t_{1/2}$, half-life. ONO-5334 was administered orally once a day for a single day (day 1), followed by 14 or 27 consecutive days. C_{max}, $AUC_{0\text{-}24h}$, and $t_{1/2}$ expressed as mean (standard deviation); t_{max} expressed as median (range). n = 9.
a. n = 8.

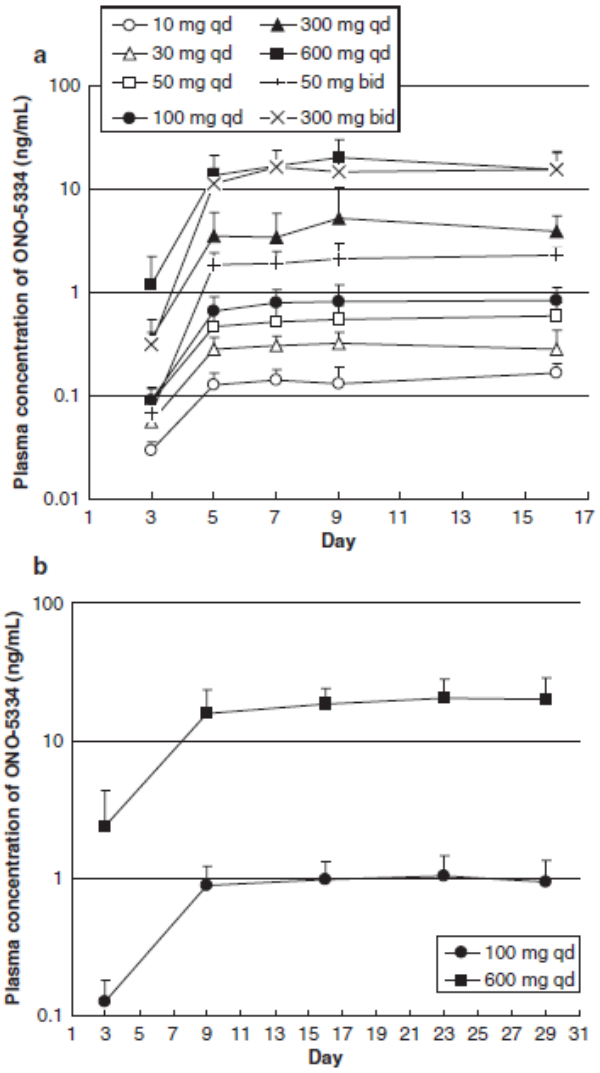

Figure 2-1: Log-transformed mean (± standard error) trough plasma concentrations of ONO-5334 during (a) 15-day multiple-dose cohort and (b) 28-day multiple-dose cohort. Samples were obtained before morning dose. ONO-5334 was administered for a single day (day 1), followed by 14 or 27 consecutive days. n = 7 to 9.

Assessments of Biochemical Markers of Bone Turnover

Once-daily treatment in 15-day multiple-dosing cohort

The acute effects of a single dose of ONO-5334 on bone resorption are presented in Figure 2-2. Serum markers of bone resorption were suppressed quickly (at least within 4 hours), and the maximum effect was observed mainly within 8 hours after dosing (except for 600 mg once daily on serum NTX, where the maximum suppression occurred at 11 hours after dosing). Twenty-four hours after single dose administration, 100, 300 and 600 mg of ONO-5334 reduced urinary CTX by 1.7% ± 23.2% (n = 9), 63.0% ± 6.2% (n = 8) and 86.4% ± 2.5% (n = 9) and urinary NTX by 19.2% ± 5.8% (n = 9), 48.7% ± 5.3% (n = 9) and 72.6% ± 4.2% (n = 8), respectively (mean ± standard error).

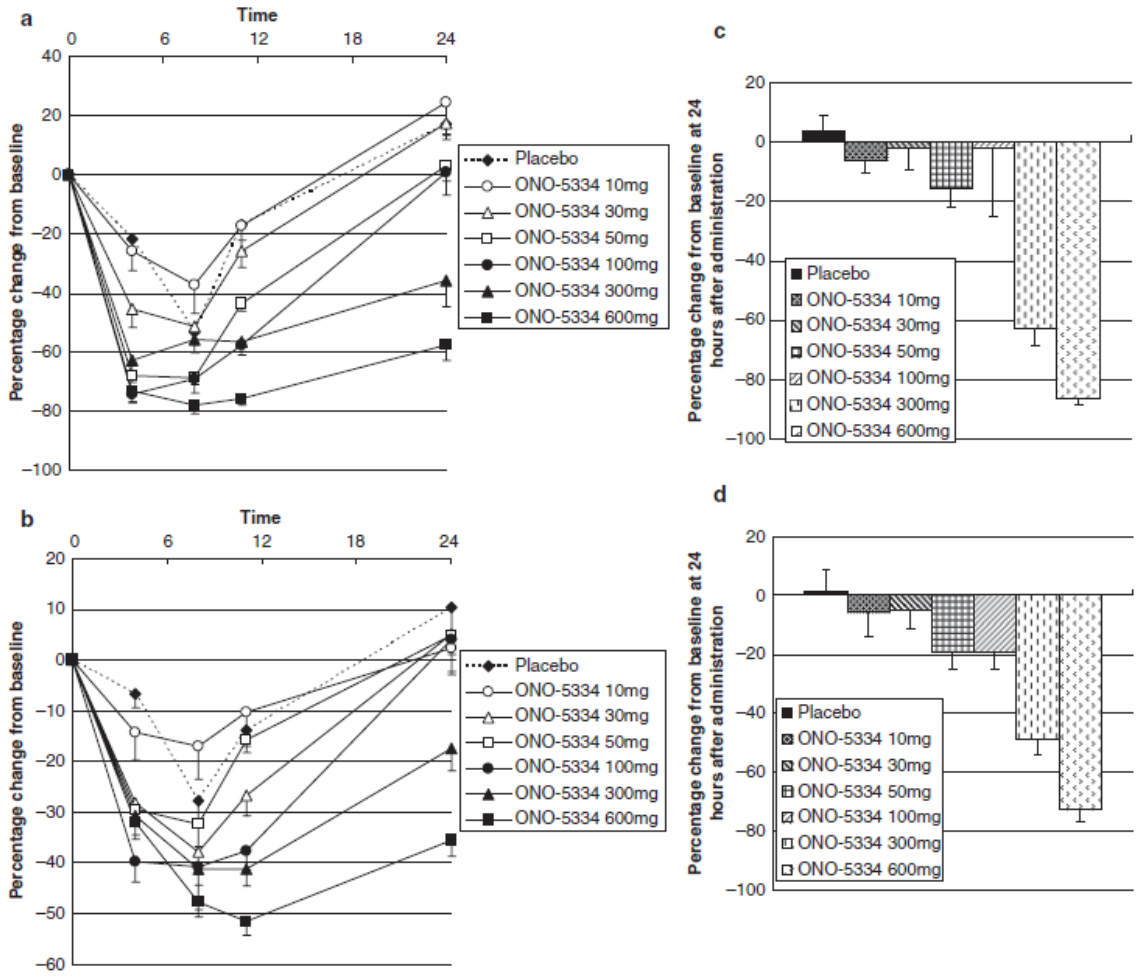

Figure 2-2: Effect of a single dose of ONO-5334 on bone resorption markers. Mean (standard error) percentage change from baseline during 24 hours after administration in (a) serum CTX and (b) serum NTX. Mean (standard error) change at 24 hours after administration (second morning void) in (c) urinary CTX and (d) urinary NTX.

The percentage changes in serum CTX and serum NTX at the time point when the trough PK level is expected (i.e. just before every dosing) and 5 to 7 days post treatment together with percentage changes in urinary CTX and urinary NTX are presented in Table

2-5 and Figure 2-3. After multiple dosing, sustained reduction of both serum CTX and serum NTX was observed at doses of more than 100 mg. With the high doses, 300 and 600 mg, suppression of serum CTX and serum NTX was maintained throughout the study period; even at the follow-up visit (5-7 days after the last dose), levels had not returned completely to baseline. Twenty-four hours after the last dose, 100, 300 and 600 mg of ONO-5334 reduced urinary CTX by 44.9% ± 13.6%, 84.5% ± 4.4% and 92.5% ± 1.3% and urinary NTX by 38.0% ± 4.7%, 67.0% ± 6.2% and 84.4% ± 2.1%, respectively. There was no apparent suppression of serum markers of bone resorption with 50 mg once daily or lower. There seemed to be a more variable and inconsistent dose-response effect on urine markers at 50 mg once daily.

In general, in both the trough estimations or after 15 days of treatment, CTX and NTX in urine showed greater suppression compared with the same CTX and NTX in serum. A similar but smaller dose suppression response on another bone resorption marker, DPD, was observed (data not shown).

Table 2-5: Levels of Bone Resorption Markers at Baseline and After Multiple Dosing (Prior to the Last Dosing on Day 16)

		Serum CTX, µg/L		Serum NTX, nM BCE		Urinary CTX, µg/L		Urinary NTX, nM BCE	
Dose, mg	No.[a]	Day 1 Pre	Day 16 Pre	Day 1 Pre	Day 16 Pre	Day 1 Pre	Day 16 Pre	Day 1 Pre	Day 16 Pre
Placebo (qd)	16	0.54 ± 0.156	0.66 ± 0.224	15.2 ± 3.75	17.5 ± 4.34	1099 ± 615.8	1072 ± 652.66	273 ± 157.0	234 ± 147.5
10 (qd)	9	0.53 ± 0.133	0.73 ± 0.201	14.2 ± 3.38	15.3 ± 2.67	1222 ± 462.9	1144 ± 613.3	373 ± 218.2	342 ± 146.5
30 (qd)	7	0.74 ± 0.309	0.74 ± 0.270	16.8 ± 4.65	16.3 ± 5.06	2290 ± 1407.3	1084 ± 678.2	426 ± 248.7	234 ± 156.0
50 (qd)	9	0.58 ± 0.170	0.72 ± 0.303	15.9 ± 2.79	16.1 ± 3.84	1523 ± 835.1	955 ± 726.0	330 ± 305.7	209 ± 123.6
100 (qd)	9	0.60 ± 0.191	0.42 ± 0.097	15.7 ± 4.07	12.8 ± 2.22	1225 ± 1026.6	272 ± 239.1	390 ± 278.7	147 ± 79.0
300 (qd)	9	0.48 ± 0.187	0.20 ± 0.061	17.2 ± 2.73	12.0 ± 1.67	1265 ± 551.6	99 ± 0.0	282 ± 119.2	64 ± 22.1
600 (qd)	9	0.72 ± 0.214	0.23 ± 0.092	19.3 ± 2.38	11.2 ± 1.67	1193 ± 351.7	99 ± 0.0	183 ± 57.4	45 ± 23.7
Placebo (bid)	6	0.74 ± 0.331	0.95 ± 0.484	17.3 ± 4.42	19.6 ± 6.37	1321 ± 1031.3	1050 ± 734.4	275 ± 200.5	264 ± 170.5
50 (bid)	9	0.57 ± 0.248	0.31 ± 0.163	14.3 ± 3.27	10.1 ± 2.27	1263 ± 128.8	188 ± 182.3	306 ± 254.2	59.6 ± 37.9
300 (bid)	9	0.59 ± 0.252	0.21 ± 0.058	16.4 ± 2.17	10.1 ± 2.28	1298 ± 751.3	110 ± 31.7	242 ± 156.7	78 ± 51.7

BCE, bone collagen equivalent; bid, twice daily; CTX, C-terminal telopeptide of type I collagen; NTX, N-terminal telopeptide of type I collagen; qd, once daily. ONO-5334 was administered orally once a day for a single day (day 1), followed by 14 consecutive days. Data expressed as mean ± standard deviation.
a. Number of subjects included in the summary analysis set.

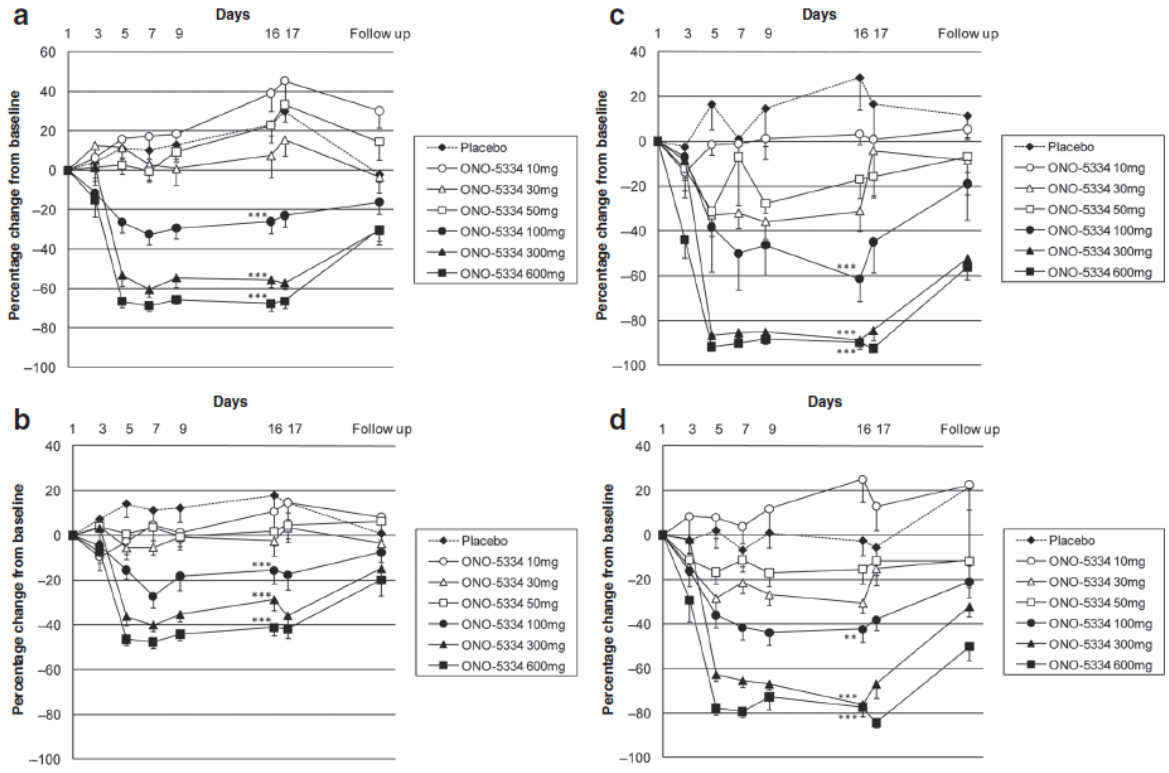

Figure 2-3: Percentage change from baseline in (a) serum CTX, (b) serum NTX, (c) urinary CTX, and (d) urinary NTX during and after 15 days multiple dosing with ONO-5334 once daily. Mean ± standard error. Samples were obtained just prior to morning dosing. ONO-5334 or placebo was administered on day 1 and day 3 onward. Follow-up was 5 to 7 days after last dosing. Statistical comparison was performed between placebo and ONO-5334 groups before dosing on the last dose (day 16) (*P < .05, **P < .01, ***P < .001 vs placebo).

Twice-daily treatment in 15-day multiple-dosing cohort

The pharmacodynamic effects of 50 and 300 mg of ONO-5334 twice daily on biochemical markers of bone turnover at steady state are presented in Table 2-5 and Figure 2-4 along with comparison of 100 and 600 mg of ONO-5334 once daily as equivalent total daily doses to 50 mg twice daily and 300 mg twice daily. A dose of 300 mg twice daily showed a similar magnitude of effect on any bone resorption markers compared with 600 mg once daily, whereas 50 mg twice daily appeared to show more consistent and greater suppression of all bone resorption markers compared with 100 mg once daily.

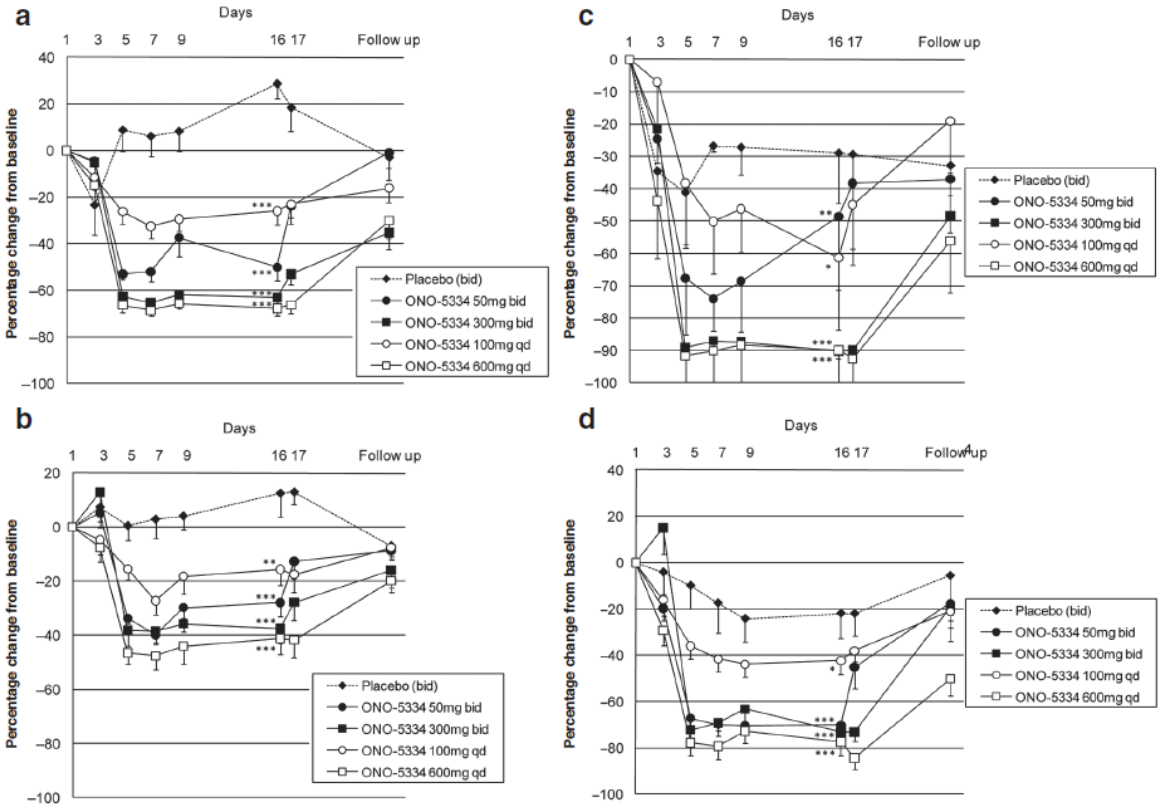

Figure 2-4: Effects of dose regimen on bone resorption markers at trough ONO-5334 PK levels during 15 days of multiple dosing. Samples were obtained prior to administration. ONO-5334 or placebo was administered on day 1 and day 3 onward. Mean percentage changes from baseline in (a) serum CTX, (b) serum NTX, (c) urinary CTX, and (d) urinary NTX. Mean ± standard error. Statistical comparison was performed between placebo and ONO-5334 groups before dosing on the last dose (day 16) (*P < .05, **P < .01, ***P < .001 vs placebo).

Twenty-eight-day multiple-dosing cohort

In the 28-day multiple-dosing cohort, similar PD effects on serum CTX, serum NTX, urinary CTX, urinary NTX, and DPD compared with the 15-day multiple-dosing cohort were observed. These data are not shown, both to ensure more concise reporting and because a clearer and more detailed dose-response profile could be obtained and shown with the 15-day cohort. Changes in other biochemical markers of bone turnover, PINP, B-ALP, OC and TRAP5b, are summarized in Table 2-6 and Figure 2-5. Changes from baseline in PINP, B-ALP and TRAP5b 24 hours after the last multiple dosing of 600 mg once daily of ONO-5334 were -19.4% ± 5.6%, -8.6% ± 4.3% and 4.5% ± 9.3%, respectively (mean ± standard error).

Table 2-6: Levels of Bone Formation Markers at Baseline and After Multiple Dosing (Before the Last Dosing on Day 29)

		PINP, µg/L		B-ALP, IU/L		OC, µg/L		TRAP5b, U/L	
Dose, mg	No.[a]	Day 1 Pre	Day 16 Pre	Day 1 Pre	Day 16 Pre	Day 1 Pre	Day 16 Pre	Day 1 Pre	Day 16 Pre
Placebo (qd)	6	43.2 ± 9.14	46.1 ± 10.84	20.3 ± 3.02	23.8 ± 4.56	10.3 ± 2.39	10.0 ± 2.95	2.62 ± 0.147	2.78 ± 0.412
100 (qd)	9	47.9 ± 17.08	42.8 ± 15.73	19.4 ± 6.50	21.7 ± 6.76	9.9 ± 2.10	11.7 ± 3.43	2.60 ± 0.000	2.60 ± 0.000
600 (qd)	9	50.2 ± 11.89	44.9 ± 12.07	23.2 ± 6.13	24.7 ± 5.65	5.4 ± 3.37	5.6 ± 3.32	2.62 ± 0.254	2.59 ± 0.203

B-ALP, bone-specific alkaline phosphatase; OC, osteocalcine; PINP, procollagen type I intact N-terminal propeptide; qd, once daily; TRAP5b, tartrateresistant acid phosphatase isoform 5b. ONO-5334 was administered orally once a day for a single day (day 1), followed by 27 consecutive days. Data expressed as mean ± standard deviation. a. Number of subjects included in the summary analysis set.

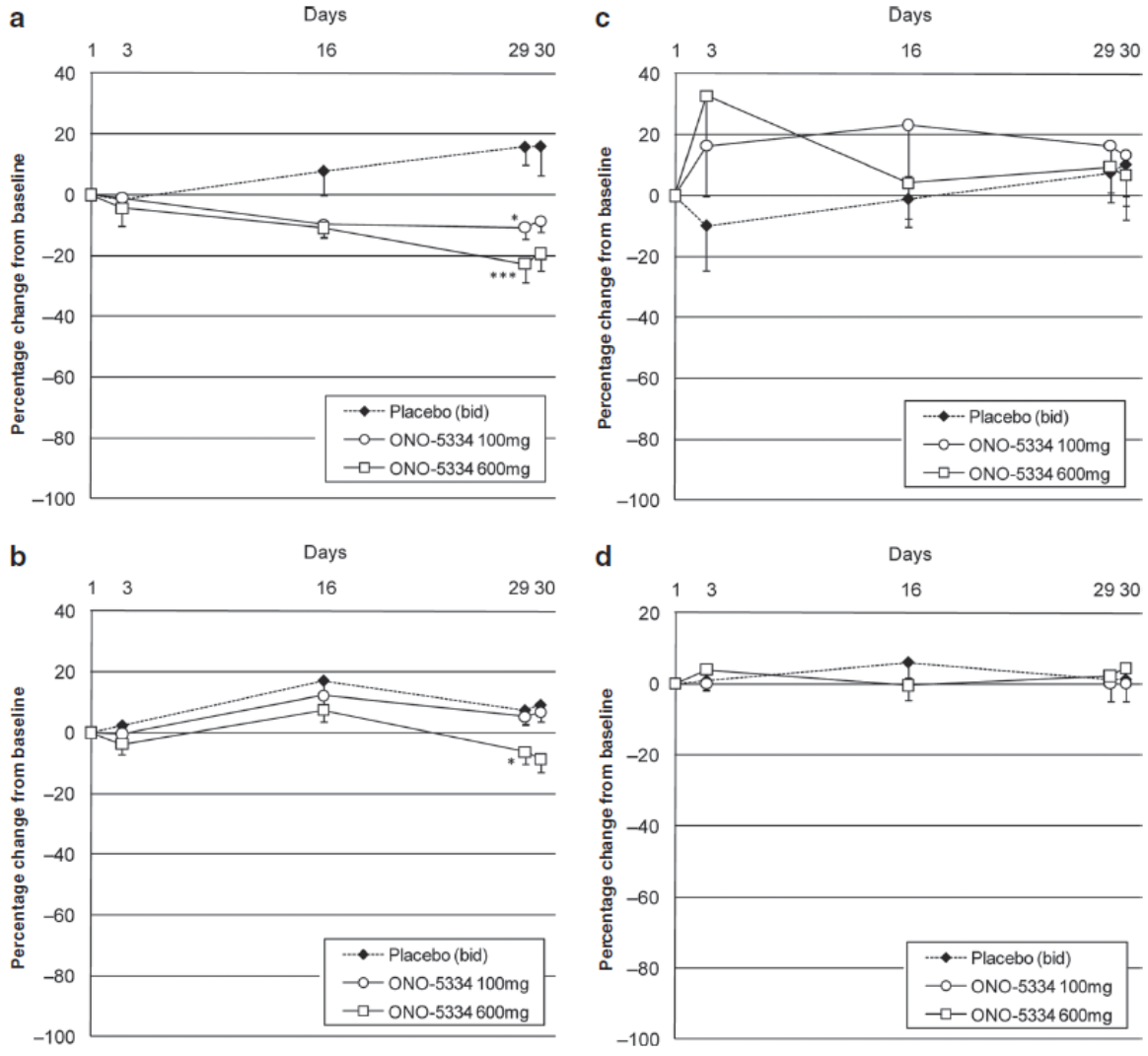

Figure 2-5: Effects of ONO-5334 on (a) PINP, (b) B-ALP, (c) OC, and (d) TRAP5b during 28 days of treatment. Samples were obtained prior to administration. ONO-5334 or placebo was administered on day 1 and day 3 onward. Mean ± standard error. Statistical comparison was performed between placebo and ONO-5334 groups before dosing on the last dose (day 29) (*P < .05, **P < .01, ***P < .001 vs placebo).

Safety Assessments

In the 15-day multiple-dosing cohorts, the incidence of subjects reporting at least 1 TEAE was between 56% (300 mg once daily) and 100% (placebo twice daily, 50 mg twice daily, and 100 mg once daily) across the treatment groups. The most commonly reported TEAEs were within the nervous system disorders, general disorders and administration site conditions, musculoskeletal and connective tissue disorders, and gastrointestinal disorders system organ classes (SOCs) and occurred evenly throughout the whole dose range including placebo. Thirty-four subjects across all treatment groups reported 61 headaches with no evidence of any dose relationship. Twenty-two hot flushes were reported by 15 subjects, the majority of which occurred in the 10 mg dose group. Twenty subjects across all treatment groups except the 100 mg once-daily group reported 1 incidence each of catheter site-related reaction, and 15 subjects from a number of treatment groups who received up to 100 mg of ONO-5334 in a day reported 19 episodes of back pain. Nausea was the most frequently reported adverse event in the gastrointestinal disorders SOC and accounted for 9 reports from 7 subjects, again from the lower dose groups. Only 2 subjects experienced vomiting, both in the placebo group.

In the 28-day multiple-dosing cohorts, the most commonly reported adverse events were within the gastrointestinal disorders, general and administration site conditions, nervous system disorders, skin and subcutaneous tissue disorders, and musculoskeletal disorders SOCs and occurred in all treatment groups including placebo. A total of 15 headaches were reported by 7 subjects, of which 11 were reported by 4 subjects who

received 600 mg of ONO-5334 once daily, suggesting an increased incidence on the highest dose level. Flatulence was reported on 7 occasions by 7 subjects, but these subjects were all receiving either placebo or the 100 mg dose of ONO-5334 once daily, suggesting no dose relationship. There were 5 reports of skin reaction to ECG/telemetry patches by 5 subjects, and again none of these subjects received the 600 mg dose of ONO-5334. No other adverse event was reported by more than 3 subjects (12.5%) across the total study population. More subjects reported musculoskeletal events in the 600 mg group, but the nature of these events varied.

Discussion

During multiple dosing of ONO-5334, the predicted plasma concentration-time profiles during multiple dosing, simulated from PK data of single doses, were visually similar to the plasma concentrations observed at all doses. The observed accumulation factors for C_{max} and AUC were not substantially different from the predicted accumulation factors for all doses and length of treatment. Plasma concentrations of ONO-5334 rapidly reached steady state, and the trough concentration remained almost unchanged from the second day of dosing. From these findings, I concluded that there was no accumulation of ONO-5334 or any evidence to show that ONO-5334 plasma concentrations can be reduced with repeated dosing.

A single dose of at least 100 mg of ONO-5334 showed consistent suppression of the bone resorption markers serum CTX and serum NTX. The effect appeared maximal between 8 and 11 hours after dosing regardless of dose (in general). ONO-5334 doses of 300 and 600 mg continued to suppress serum CTX and serum NTX for at least 24 hours after treatment.

At the end of the follow-up period (5-7 days after 15 days of multiple dosing), the suppressive effect of 300 mg and 600 mg of ONO-5334 remained (e.g. 56.1% on urinary CTX and 50.2% on urinary NTX, respectively). This may suggest that higher doses of ONO-5334 could be used as once-weekly medication.

A similar but smaller dose suppression response on DPD was observed (compared with urinary CTX and NTX), and there was no reliable suppression of DPD at the follow-

up visit. Whether these differences in effect on bone resorption markers are specific to the mechanism of action or ONO-5334 metabolism is unknown.

Fracture risk reduction is the ultimate goal of osteoporosis prevention and treatment. This may be achievable through an increase in bone mineral density and suppression of bone resorption markers. CTX and NTX are recognized markers of bone resorption and are used to evaluate osteoporosis treatment. Bisphosphonates show a characteristic bone resorption profile with these markers. In this study, ONO-5334 suppressed serum CTX and urinary NTX by approximately 60% to 80% compared with baseline. Recently, the cathepsin K inhibitor odanacatib showed a similar suppression on these markers (approximately 60%) and clear increases in bone mineral density [45-46]. Marketed antiresorptive agents, such as bisphosphonates and denosumab, suppress these markers with similar magnitude by approximately 60% to 70% [31, 47-48], and although it is too early to confirm an effect on fracture risk reduction for ONO-5334, the suppression of CTX and NTX shows potential antiresorptive efficacy.

There may be a difference in dosing regimen, highlighted by the observation that 50 mg twice daily consistently showed greater suppression of bone resorption markers compared with 100 mg once daily. Although there was no difference between 300 mg twice daily and 600 mg once daily, it may be that maximal suppression was already achieved and thus differences attributable to dose regimen may not be apparent at higher doses. However, this suggests that the use of a sustained-release formulation that can keep higher trough concentration for 24 hours may be of interest (at certain doses) for further development of ONO-5334.

There were no apparent effects on TRAP5b levels during 28 days of multiple treatment, indicating that osteoclast number is not affected by ONO-5334.

In contrast to the clear suppressive effects of ONO-5334 on bone resorption, there were far smaller changes during 28 days of multiple dosing in the bone formation markers PINP, B-ALP and OC. However, changes in PINP with 600 mg of ONO-5334 once daily may suggest a slight suppression in bone formation with high doses. It is difficult to conclude what effect ONO-5334 has on bone formation markers since this short-term study may not be sufficiently long to show a change in bone formation, especially considering the complex biochemical interaction between osteoclasts and osteoblasts. No dynamic histomorphometry data are available for ONO-5334 on measures such as osteoclast number or activation frequency to confirm any effect on bone formation. However, the cathepsin K inhibitor odanacatib has shown an increase in BMD without reducing bone formation [49]. In a monkey ovariectomized model, Yamada et al [50] showed that ONO-5334 significantly prevented the increase in bone formation and bone resorption markers due to ovariectomy with a corresponding increase in BMD [50]. The magnitude of effect on bone resorption markers was far greater compared with bone formation markers. Tanaka et al [51] reported that ONO-5334 increases cortical thickness and bone mass and considered that these effects may be attributable to the fact that ONO-5334 exerted no significant effect on bone formation while suppressing bone resorption at cortical surfaces. Given that currently available antiresorptive agents clearly suppress both bone formation and bone resorption [31, 47-48] and considering the lack of effect on these formation markers in our

study, assessment with longer term treatment is of interest to explore ONO-5334's mode of action on bone remodeling further in humans.

In conclusion, ONO-5334 was generally well tolerated up to a dose of 600 mg/day for 28 days. Plasma ONO-5334 concentration reached steady state after 2 days of administration and provided fast onset of pharmacodynamic effects on bone resorption markers and a sustained suppression 5 to 7 days after dosing with high doses. The magnitude of effect of ONO-5334 on bone resorption markers suggests that ONO-5334 may show potential as an antiresorptive agent in the treatment of osteoporosis, but unlike traditional antiresorptive agents like bisphosphonates, ONO-5334 (depending on dose) may show little or no effect on bone formation. Although effective in treating osteoporosis, bisphosphonates are known to suppress bone formation, and hence bone turnover is reduced. Whether reducing bone turnover for many years gives rise to complications such as atypical fractures of the femur is a cause for concern [52-53]. New treatments for osteoporosis are therefore still of interest.

General Discussion

In the pre-clinical studies with ovariectomized cynomolgus monkeys, ONO-5334 reduced bone resorption without affecting bone formation with the results of increase in bone mineral density (BMD) and bone strength [54-55]. These studies also suggested more potent increase in cortical BMD compared to those on trabecular region. In addition, effect of higher doses of ONO-5334 on cortical bone looked more potent compared to active control group, one of popular bisphosphonates - alendronate. The authors concluded that, although ONO-5334 may have different effects on bone formation at different anatomical sites in bone, the effect on bone formation was none or minimal compared with antiresorptive effect with ONO-5334. Ochi et al performed another pre-clinical study to evaluate efficacy of ONO-5334 with ovariectomized rat model and reached similar conclusion that ONO-5334 has potential of better efficacy on cortical bone which is more potent compared with alendronate [56].

Fragility fractures occurred under osteoporosis is usually discussed by being classified as vertebral and non-vertebral fractures since context of bone significantly differ between these 2 types of bones: spinal vertebrae are richer with trabecular bone, while non-vertebral bone has more cortical bone compartment. Although all such fractures are clinically important because of deterioration of quality-of-life however one of typical non-vertebral fractures, those on hip including femoral neck, are recognized as the most serious fragility fractures [57]. Fractures on hip require surgeric approaches to treat and patients have to be hospitalized for certain period. This may be related higher mortality in such

patient population which has been reported approximately 10.1% at 1 year after the incidence, in Japan [58]. Considering profile of ONO-5334 of having better efficacy on cortical bone, which may be more significant than those of current standard of care, bisphosphonates, ONO-5334 cathepsin K inhibitor may be very attractive as a new type of agents for osteoporosis.

Through 2 Phase I studies described in Part 1 and 2, I demonstrated potent antiresorptive efficacy of ONO-5334 in human, along with relatively smaller effect on bone formation. It was similar to those findings in pre-clinical studies and thus findings in ovariectomized monkeys and rats could be extrapolated onto human, and this means it can be expected significant increase in BMD and bone strength in human osteoporosis patients. Also, it could be the case that cathepsin K inhibitor shows greater efficacy on non-vertebral fractures compared with standard antiresorptive agents, bisphosphonates. Based on this assumption, I conducted Phase II study named the OCEAN (ONO-5334 cathepsin K inhibitor European study) in collaboration with Eastell [59-60]. In the OCEAN study, 100 and 300 mg once daily and 30 mg twice daily regimen of ONO-5334 were compared with placebo and 70 mg once weekly alendronate. Effect on bone turnover markers were mirrored to Phase I studies, i.e. significant suppression on bone resorption markers along with minimal effect on bone formation markers which was far smaller than those of alendronate. Furthermore, ONO-5334 demonstrated significant increase in BMD. Effect on BMD may suggested better efficacy on cortical-rich bones such as femoral neck since the highest dose of ONO-5334, 300 mg once daily, showed greater increase in femoral neck BMD than alendronate, while those are comparable at lumbar spine which is typical

trabecular-rich bone. In order to explore more detailed changes in bone structure, in collaboration with Engelke, I evaluated changes in bone geometry with quantitative computed tomography (QCT) which enable to assess increase in bone geometry separately between cortical and trabecular region [61]. The observation was not clear but the assessment with QCT in the OCEAN study suggested potential for ONO-5334 to add more new bone to cortical as to the trabecular compartment. The reason of seeing inconclusive results with QCT may because of patient variability in clinical trial setting, or limitations on assessment of QCT, but unknown. It may be beneficial to implement such assessment in future larger trails in order to further clarify difference with bisphosphonates, since only 24-34 patients/group are included in QCT assessments in the OCEAN study (57 patients/group randomized for BMD assessments).

Among some of cathepsin K inhibitors under clinical development, odanacatib (Merck) is on the most advanced stage. In the reported clinical trial, odanacatib suppressed bone resorption with minimal effect on bone formation, similarly to ONO-5334, during treatment however significant rebound was observed after termination of treatment [62-63]. Immediately after stopping treatment, both bone resorption and formation markers elevates to far higher than baseline levels which tends to return to baseline within 1 year after discontinuation. Within 1 year post-treatment, BMD at all region evaluated including lumber spine, total hip, femoral neck and trochanter returned to baseline level while continuous treatment kept increasing BMD for the entire study period of 5 years. Interestingly, treatment with odanacatib increases TRAP5b over 3-year treatment, compared with placebo, which may explain rebound effect as the result of increased

number of osteoclasts. Neither Phase I nor Phase II (OCEAN) studies for ONO-5334 evaluated long term post-treatment effect therefore it is unknown if ONO-5334 shows rebound effect on bone turnover markers and BMD. However, it makes great sense to expect similar findings for ONO-5334 since histological assessments in pre-clinical study with ovariectomized monky suggested increase in number of osteoclasts [54-55]. In addition, the OCEAN study showed increase in TRAP5b over 2 years [59-60]. It is important to further evaluate post-treatment changes with ONO-5334 in order to better understand profile of cathepsin K inhibitors.

Needless to say, one of most popular agents for long-term treatment of osteoporosis is bisphosphonates. Bisphosphonates show an efficacy by being once deposited in bone and taken into osteoclast to lead apoptosis of cells which is one of key mode of action. Because of extremely long half-line in bone, bisphosphonates provides highly sustained effect even after discontinuation. This specific profile of bisphosphonates has been utilized to deliver intermittent dose formulation to patients, such as weekly alendronate or once-yearly zoledronate. In contrast to this feature, I have demonstrated in Phase I studies, described in Part 1 and 2, that ONO-5334 shows an efficacy depending on pharmacokinetics which is most likely blood concentration-dependent. In order to explore how PK-dependent profile is reflected on effect on bone turnover markers, I conducted another Phase I study to compare conventional formulation of ONO-5334 (tested in Phase I studies described in Part 1 and 2) and newly developed sustained-release formulation [64]. In this study, compared with conventional formulation, the sustained-release formulation shows reduced C_{max}, greater C_{24h}, but roughly comparable overall AUC_{inf} dose for dose. The sustained-release

formulation showed clear dose-response suppression on bone resorption markers and a greater pharmacodynamic effect dose for dose versus the conventional formulation after a single dose of ONO-5334. Based on this finding, it is obvious that ONO-5334, cathepsin K inhibitor, has different pharmacologic effect compared with bisphosphonates, which may result in different pattern in anti-fracture efficacy; ultimate goal of osteoporosis treatment. It is of significant interest to explore difference in anti-fracture efficacy and perhaps safety profile between bisphosphonates and cathepsin K inhibitors.

Considering all the above discussions, further clinical studies are warranted to investigate the long-term efficacy and safety of cathepsin K inhibitor, ONO-5334, as a potential treatment for osteoporosis. Through such clinical studies, it should be beneficial to explore differences with bisphosphonates such as more potent effect on cortical bone.

In terms of safety of cathepsin K inhibitor, ONO-5334, no clinically relevant concerns were found in Phase I studies described in Part 1 and 2, for at least 28 days multiple treatment, however safety profile during long-term use is of interest if consider nature of target disease, osteoporosis. Among some cathepsin K inhibitors in clinical stage, balicatib demonstrated increase in BMD along with antiresorptive effect on bone turnover markers [65]. However development of balicatib has been terminated due to skin adverse events found in 7 of 709 patients enrolled [66]. Balicatib has lysosomotropic character which resulted in its accumulation in lysosomes and in nonselective off-target effects which may explain the dramatically decreased selectivity [67]. Cathepsins other than cathepsin K such as cathepsins B and L are expressed generally ubiquitously [68-69]. It is considered that balicatib showed non-selective off-target effect on those ubiquitous cathepsins to cause

skin adverse events. On the other hand, ONO-5334 and odanacatib are known as non-lysosomotropic compound and thus no relevant skin adverse events have been reported in clinical setting so far.

Cathepsin K is predominantly expressed in osteoclasts however its expression has been demonstrated in various other cell types including synovial fibroblasts, skin fibroblasts, macrophages, dendritic cells, chondrocytes, epithelial cells and melanocytes [70-76]. Treatment on osteoporosis is expected to continue for long duration and therefore there is a potential that cathepsin K inhibitors may show on-target but off-osteoclast effect. Although exact background has not been revealed or published, Merck challenged US FDA for odanacatib to be launched in the country however the application has been withdrawn by themselves because of higher incidence of cardiovascular events compared with placebo. The reason of higher cardiovascular events is unknown however off-target effect may be part of mechanisms. Odanacatib has longer half-live reported as 66-93 hours [77] compared with ONO-5334 (9-22 hours – See Part 1) thus it is dosed as once-weekly treatment in its clinical trials. Although it is totally unknown either continuous or intermittent suppression on bone resorption is better in anti-fracture efficacy or safety profile, pharmacokinetic difference between odanacatib and ONO-5334 may worth being evaluated in future large clinical trials.

Acknowledgments

I sincerely thank Associate Professor Kazuichi Sakamoto, Professor Tomoki Chiba, Associate Professors Kentaro Nakano and Hidekazu Kuwayama for guiding my work and valuable discussions through my doctoral program.

I thank Dr. Steve Deacon, Tomohiro Kuwayama, Maria Small, Yoshitaka Hashimoto and Michiyo Ohyama, Ono Pharma UK Ltd and Ono Pharmaceutical Co., Ltd. for their collaboration to design and conduct studies.

Finally, I would like to appreciate all the people who have given considerable supports on me to conduct researches and complete doctoral program.

References

1. Frost HM. Treatment of osteoporoses by manipulation of coherent bone cell populations. Clin Orthop Relat Res. 1979; 143: 227–244.
2. Frost HMA. A 2003 update of bone physiology and Wolff's Law for clinicians. Angle Orthod. 2004; 74: 3–15.
3. Parfitt AM. Morphological basis of bone mineral measurements: transient and steady state effects of treatment in osteoporosis. Miner Electrolyte Metab. 1980; 4: 273–287.
4. Teitelbaum SL. Bone resorption by osteoclasts. Science. 2000; 289: 1504-1508.
5. Ducy P, Schinke T, Karsenty G. The osteoblast: a sophisticated fibroblast under central surveillance. Science. 2000; 289: 501-504.
6. Gong JK, Arnold JS, Cohn SH. Composition of Trabecular and Cortical Bone. Anat Rec. 1964; 149: 325-331.
7. Boskey AL, Posner AS. Bone structure, composition, and mineralization. Orthop Clin North Am. 1984; 15: 597-612.
8. Blair HC, Teitelbaum SL, Ghiselli R, Gluck S. Osteoclastic bone resorption by a polarized vacuolar proton pump. Science. 1989; 245: 855-857.
9. Chatterjee D, Chakraborty M, Leit M, Neff L, Jamsa-Kellokumpu S, Fuchs R, Baron R. Sensitivity to vanadate and isoforms of subunits A and B distinguish the osteoclast proton pump from other vacuolar H+ ATPases. Proc Natl Acad Sci U S A. 1992; 89: 6257-6261.

10. Silver IA, Murrills RJ, Etherington DJ. Microelectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. Exp Cell Res. 1988; 175: 266-276.
11. Fonovic M, Turk B. Cysteine cathepsins and extracellular matrix degradation. Biochim Biophys Acta. 2014; 1840: 2560–2570.
12. Littlewood-Evans A, Kokubo T, Ishibashi O, Inaoka T, Wlodarski B, Gallagher JA, Bilbe G. Localization of cathepsin K in human osteoclasts by in situ hybridization and immunohistochemistry. Bone. 1997; 20: 81–86.
13. Yamaza T, Goto T, Kamiya T, Kobayashi Y, Sakai H, Tanaka T. Study of immunoelectron microscopic localization of cathepsin K in osteoclasts and other bone cells in the mouse femur. Bone. 1998; 23: 499–509.
14. Garnero P, Borel O, Byrjalsen I, Ferreras M, Drake FH, McQueney MS, Foged NT, Delmas PD, Delaissé JM. The collagenolytic activity of cathepsin K is unique among mammalian proteinases. J Biol Chem. 1998; 273: 32347-32352.
15. Kafienah W, Brömme D, Buttle DJ, Croucher LJ, Hollander AP. Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. Biochem J. 1998; 331: 727-732.
16. Gelb BD, Shi GP, Chapman HA, Desnick RJ. Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. Science. 1996; 273: 1236–1238.
17. Saftig P, Hunziker E,Wehmeyer O, Jones S, Boyde A, Rommerskirch W, Moritz JD, Schu P, von Figura K. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. Proc Natl Acad Sci U S A. 1998; 95: 13453–13458.

18. Gowen M, Lazner F, Dodds R, Kapadia R, Feild J, Tavaria M, Bertoncello I, Drake F, Zavarselk S, Tellis I, Hertzog P, Debouck C, Kola I. Cathepsin K knockout mice develop osteopetrosis due to a deficit in matrix degradation but not demineralization. J Bone Miner Res. 1999; 14: 1654–1663.
19. Weivoda MM, Ruan M, Pederson L, Hachfeld C, Davey RA, Zajac JD, Westendorf JJ, Khosla S, Oursler MJ. Osteoclast TGF-β Receptor Signaling Induces Wnt1 Secretion and Couples Bone Resorption to Bone Formation. J Bone Miner Res. 2016; 31: 76-85.
20. Pederson L, Ruan M, Westendorf JJ, Khosla S, Oursler MJ. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. Proc Natl Acad Sci U S A. 2008; 105: 20764-20769.
21. Lotinun S, Kiviranta R, Matsubara T, Alzate JA, Neff L, Lüth A, Koskivirta I, Kleuser B, Vacher J, Vuorio E, Horne WC, Baron R. Osteoclast-specific cathepsin K deletion stimulates S1P-dependent bone formation. J Clin Invest. 2013; 123: 666-681.
22. Nishi Y, Atley L, Eyre DE, Edelson JG, Superti-Furga A, Yasuda T, Desnick RJ, Gelb BD. Determination of bone markers in pycnodysostosis: effects of cathepsin K deficiency on bone matrix degradation. J Bone Miner Res. 1999; 14: 1902–1908.
23. Christgau S. Circadian variation in serum crosslaps concentration is reduced in fasting individuals. Clin Chem. 2000; 46: 431.
24. Ju HS, Leung S, Brown B, Stringer MA, Leigh S, Scherrer C, Shepard K, Jenkins D, Knudsen J, Cannon R. Comparison of analytical performance and biological variability of three bone resorption assays. Clin Chem. 1997; 43: 1570–1576.

25. Nenonen A, Cheng S, Ivaska KK, Alatalo SL, Lehtimäki T, Schmidt-Gayk H, Uusi-Rasi K, Heinonen A, Kannus P, Sievänen H, Vuori I, Väänänen HK, Halleen JM. Serum TRACP 5b is a useful marker for monitoring alendronate treatment: comparison with other markers of bone turnover. J Bone Miner Res. 2005; 20: 1804–1812.
26. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res. 1987; 2: 595–610.
27. Burtis CA, Ashwood EA and Burns DE. Teitz textbook of clinical chemistry and molecular diagnostics. Vol 4. St Louis: Elsevier, 2006.
28. Simon LS, Krane SM, Wortman PD, Krane IM, Kovitz KL. Serum levels of type I and III procollagen fragments in Paget's disease of bone. J Clin Endocrinol Metab. 1984; 58: 110–120.
29. Watts NB, Diab DL. Long-term use of bisphosphonates in osteoporosis. J Clin Endocrinol Metab. 2010; 95: 1555–1565.
30. Boonen S, Laan RF, Barton IP, Watts NB. Effect of osteoporosis treatments on risk of non-vertebral fractures: review and metaanalysis of intention-to-treat studies. Osteoporos Int. 2005; 16: 1291–1298.
31. Black DM, Delmas PD, Eastell R, Reid IR, Boonen S, Cauley JA, Cosman F, Lakatos P, Leung PC, Man Z, Mautalen C, Mesenbrink P, Hu H, Caminis J, Tong K, Rosario-Jansen T, Krasnow J, Hue TF, Sellmeyer D, Eriksen EF, Cummings SR; HORIZON

Pivotal Fracture Trial. Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. N Engl J Med. 2007; 3: 1809–1822.

32. Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, Delmas P, Zoog HB, Austin M, Wang A, Kutilek S, Adami S, Zanchetta J, Libanati C, Siddhanti S, Christiansen C; FREEDOM Trial. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med. 2009; 20: 756–765.
33. Karsdal MA, Martin TJ, Bollerslev J, Christiansen C, Henriksen K. Are nonresorbing osteoclasts sources of bone anabolic activity? J Bone Miner Res. 2007; 22: 487–494.
34. Deal C. Potential new drug targets for osteoporosis. Nat Clin Pract Rheumatol. 2009; 5: 20–27.
35. Hasegawa C, Ohno T, Umemura T, Honda N, Ohyama M, Nagase S, Small M, Deacon S, Ogawa M, Ieiri I. Population pharmacokinetic and pharmacodynamic modeling of different formulations of ONO-5334, cathepsin K inhibitor, in Caucasian and Japanese postmenopausal females. J Clin Pharmacol. 2014; 54: 23-34.
36. Data on File. Inhibitory effect of ONO-5334 on human recombinant cathepsin K (E05QA012). Osaka: Ono Pharmaceutical Co., Ltd, 2005.
37. Data on File. Inhibitory effect of ONO-5334 on 6 cysteine proteases (E05QA025). Osaka: Ono Pharmaceutical Co., Ltd, 2005.
38. Data on File. An investigation of Ono's compound (ONO-5334) on 9 enzyme and 3 receptor binding assays (1056356). Osaka: Ono Pharmaceutical Co., Ltd, 2005.
39. Yamada H, Mori H, Nakanishi Y, Kunishige A, Nishikawa S, Tanaka M, Shiroya T. Orally active cathepsin K inhibitor, ONO-5334, potently improved bone mineral

density not only in trabecular bone but also in cortical bone in ovariectomized Cynomolgus monkeys. A09002146, ASBMR 2009.

40. Data on File. Inhibitory effect of ONO-5334 on bone resorption after single administration in normal rats (E05QA022). Osaka: Ono Pharmaceutical Co., Ltd, 2005.
41. Cloos PAC, Fledelius C, Christgau S, Christiansen C, Engsig M, Delmas P, Body J-J, Garnero P. Investigation of bone disease using isomerized and racemized fragments of type I collagen. Calcif Tissue Int. 2003; 72: 8–17.
42. Hoshino H, Takahashi M, Kushida K, Ohishi T, Inoue T. The relationship between the degree of b-isomerisation of type I collagen degradation products in the urine and ageing, menopause and osteoporosis with fractures. Osteoporos Int. 1999; 9: 405–409.
43. Reginster JY, Henrotin Y, Christainsen C, Gamwell-Henriksen E, Bruyere O, Collette J, Christgau S. Bone resorption in post-menopausal women with normal and low BMD assessed with biochemical markers specific for telopeptide derived degradation products of collagen type 1. Calcif Tissue Int. 2001; 69: 130–137.
44. Data on file. A phase I, three-part, study to investigate the safety, tolerability, pharmacokinetic profile and pharmacodynamics effect of single and multiple doses of ONO-5334 in post-menopausal females, ONO-5334POE001 study. Ono Pharmaceutical Co., Ltd.
45. Bone HG, McClung MR, Roux C, Recker RR, Eisman JA, Verbruggen N, Hustad CM, DaSilva C, Santora AC, Ince BA.. Odanacatib, a cathepsin-K inhibitor for osteoporosis: a two-year study in postmenopausal women with low bone density. J Bone Miner Res. 2010; 25: 937-947.

46. Stoch SA, Zajic S, Stone J, Miller DL, Van Dyck K, Gutierrez MJ, De Decker M, Liu L, Liu Q, Scott BB, Panebianco D, Jin B, Duong LT, Gottesdiener K, Wagner JA. Effect of the cathepsin K inhibitor odanacatib on bone resorption biomarkers in healthy postmenopausal women: two double-blind, randomized, placebocontrolled phase I studies. Clin Pharmacol Ther. 2009; 86: 175-182.
47. Reid DM, Hosking D, Kendler D, Brandi ML, Wark JD, Weryha G, Marques-Neto JF, Gaines KA, Verbruggen N, Melton ME. Alendronic acid produces greater effects than risedronic acid on bone density and turnover in postmenopausal women with osteoporosis: results of FACTS international. Clin Drug Investig. 2006; 26: 63-74.
48. Brown JP, Prince RL, Deal C, Recker RR, Kiel DP, de Gregorio LH, Hadji P, Hofbauer LC, Alvaro-Gracia JM, Wang H, Austin M, Wagman RB, Newmark R, Libanati C, San Martin J, Bone HG. Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: a randomized, blinded, phase 3 trial. J Bone Miner Res. 2009; 24: 153-161.
49. Pennypacker BL, Duong LT, Cusick TE, Masarachia PJ, Gentile MA, Gauthier JY, Black WC, Scott BB, Samadfam R, Smith SY, Kimmel DB. Cathepsin K inhibitors prevent bone loss in estrogen-deficient rabbits. J Bone Miner Res. 2011; 26: 252-262.
50. Yamada H, Mori H, Nakanishi Y, et al. Orally active cathepsin K inhibitor, ONO-5334, potently improved bone mineral density not only in trabecular bone but also in cortical bone in ovariectomized cynomolgus monkeys. Paper presented at: American Society

for Bone and Mineral Research 2009 Annual Meeting Denver, CO. Abstract A09002146.

51. Tanaka M, Yamada H, Mori H, et al. Efficacy of ONO-5334, a cathepsin K inhibitor, on bone geometry and histomorphometry in cortical bone in ovariectomized cynomolgus monkeys. Paper presented at: American Society for Bone and Mineral Research 2010 Annual Meeting. Toronto, OT, Canada. Abstract SU0436.
52. Goh SK, Yang KY, Koh JS, Wong MK, Chua SY, Chua DT, Howe TS. Subtrochanteric insufficiency fractures in patients on alendronate therapy: a caution. J Bone Joint Surg Br. 2007; 89: 349-353.
53. Odvina CV, Zerwekh JE, Rao DS, Maalouf N, Gottschalk FA, Pak CY. Severely suppressed bone turnover: a potential complication of alendronate therapy [published online ahead of print December 14, 2004]. J Clin Endocrinol Metab. 2005; 90: 1294-1301.
54. Ochi Y, Yamada H, Mori H, Nakanishi Y, Nishikawa S, Kayasuga R, Kawada N, Kunishige A, Hashimoto Y, Tanaka M, Sugitani M, Kawabata K. Effects of eight-month treatment with ONO-5334, a cathepsin K inhibitor, on bone metabolism, strength and microstructure in ovariectomized cynomolgus monkeys. Bone. 2014; 65: 1-8.
55. Yamada H, Ochi Y, Mori H, Nishikawa S, Hashimoto Y, Nakanishi Y, Tanaka M, Bruce M, Deacon S, Kawabata K. Effects of 16-month treatment with the cathepsin K inhibitor ONO-5334 on bone markers, mineral density, strength and histomorphometry in ovariectomized cynomolgus monkeys. Bone. 2016; 86: 43-52.

56. Ochi Y, Yamada H, Mori H, Kawada N, Kayasuga R, Nakanishi Y, Tanaka M, Imagawa A, Ohmoto K, Kawabata K. ONO-5334, a cathepsin K inhibitor, improves bone strength by preferentially increasing cortical bone mass in ovariectomized rats. J Bone Miner Metab. 2014; 32: 645-652.
57. Japan osteoporosis society. Japanese 2015 guideline for prevention and treatment of osteoporosis.
58. Sakamoto K, Nakamura T, Hagino H, Endo N, Mori S, Muto Y, Harada A, Nakano T, Yamamoto S, Kushida K, Tomita K, Yoshimura M, Yamamoto H. Report on the Japanese Orthopaedic Association's 3-year project observing hip fractures at fixed-point hospitals. J Orthop Sci. 2006; 11: 127-134.
59. Eastell R, Nagase S, Ohyama M, Small M, Sawyer J, Boonen S, Spector T, Kuwayama T, Deacon S. Safety and efficacy of the cathepsin K inhibitor ONO-5334 in postmenopausal osteoporosis: the OCEAN study. J Bone Miner Res. 2011; 26: 1303-1312.
60. Eastell R, Nagase S, Small M, Boonen S, Spector T, Ohyama M, Kuwayama T, Deacon S. Effect of ONO-5334 on bone mineral density and biochemical markers of bone turnover in postmenopausal osteoporosis: 2-year results from the OCEAN study. J Bone Miner Res. 2014; 29: 458-466.
61. Engelke K, Nagase S, Fuerst T, Small M, Kuwayama T, Deacon S, Eastell R, Genant HK. The effect of the cathepsin K inhibitor ONO-5334 on trabecular and cortical bone in postmenopausal osteoporosis: the OCEAN study. J Bone Miner Res. 2014; 29: 629-638.

62. Eisman JA, Bone HG, Hosking DJ, McClung MR, Reid IR, Rizzoli R, Resch H, Verbruggen N, Hustad CM, DaSilva C, Petrovic R, Santora AC, Ince BA, Lombardi A. Odanacatib in the treatment of postmenopausal women with low bone mineral density: three-year continued therapy and resolution of effect. J Bone Miner Res. 2011; 26: 242-251.
63. Langdahl B, Binkley N, Bone H, Gilchrist N, Resch H, Rodriguez Portales J, Denker A, Lombardi A, Le Bailly De Tilleghem C, Dasilva C, Rosenberg E, Leung A. Odanacatib in the treatment of postmenopausal women with low bone mineral density: five years of continued therapy in a phase 2 study. J Bone Miner Res. 2012; 27: 2251-2258.
64. Nagase S, Ohyama M, Hashimoto Y, Small M, Sharpe J, Manako J, Kuwayama T, Deacon S. Bone turnover markers and pharmacokinetics of a new sustained-release formulation of the cathepsin K inhibitor, ONO-5334, in healthy post-menopausal women. J Bone Miner Metab. 2015; 33: 93-100.
65. Adami S, Supronik J, Haha T, Brown JP, Garnero P, Haemmerle S.Ortmann CE, Bousset F, TrechelU. Effect of one year treatment with the cathepsin-K inhibitor, balicatib, on bone mineral density (BMD) in postmenopausal women with osteopenia/osteoporosis. J Bone Miner Res. 2006; 21(suppl 1)
66. Runger TM, Adami S, Benhamou CL, Czerwinski E, Farrerons J, Kendler DL, Mindeholm L, Realdi G, Roux C, Smith V. Morphea-like skin reactions in patients treated with the cathepsin K inhibitor balicatib. J Am Acad Dermatol. 2012; 66: e89–e96

67. Falgueyret JP1, Desmarais S, Oballa R, Black WC, Cromlish W, Khougaz K, Lamontagne S, Massé F, Riendeau D, Toulmond S, Percival MD. Lysosomotropism of basic cathepsin K inhibitors contributes to increased cellular potencies against off-target cathepsins and reduced functional selectivity. J Med Chem. 2005; 48: 7535-7543.
68. Brix K, Dunkhorst A, Mayer K, Jordans S. Cysteine cathepsins: cellular roadmap to different functions. Biochimie. 2008; 90: 194-207.
69. Fonović M, Turk B. Cysteine cathepsins and extracellular matrix degradation. Biochim Biophys Acta. 2014; 1840: 2560-2570.
70. Bühling F, Gerber A, Häckel C, Krüger S, Köhnlein T, Brömme D, Reinhold D, Ansorge S, Welte T. Expression of cathepsin K in lung epithelial cells. Am J Respir Cell Mol Biol. 1999; 20: 612-619.
71. Tepel C, Brömme D, Herzog V, Brix K. Cathepsin K in thyroid epithelial cells: sequence, localization and possible function in extracellular proteolysis of thyroglobulin. J Cell Sci. 2000; 113: 4487-4498.
72. Hou WS, Li Z, Gordon RE, Chan K, Klein MJ, Levy R, Keysser M, Keyszer G, Brömme D. Cathepsin k is a critical protease in synovial fibroblast-mediated collagen degradation. Am J Pathol. 2001; 159: 2167-2177.
73. Bühling F, Waldburg N, Krüger S, Röcken C, Wiesner O, Weber E, Welte T. Expression of cathepsins B, H, K, L, and S during human fetal lung development. Dev Dyn. 2002; 225: 14-21.

74. Dejica VM , Mort JS, Laverty S, Percival MD, Antoniou J, Zukor DJ, Poole AR. Cleavage of type II collagen by cathepsin K in human osteoarthritic cartilage. Am J Pathol. 2008; 173: 161-169.

75. Vinardell T, Dejica V, Poole AR, Mort JS, Richard H, Laverty S. Evidence to suggest that cathepsin K degrades articular cartilage in naturally occurring equine osteoarthritis. Osteoarthritis Cartilage. 2009; 17: 375-383.

76. Bone HG, Mcclung M, Verbruggen N, Rybak-Feiglin A, CasilVA C, Santora AC, Ince A. A randomized double-blind, placibo-controlled study of cathepsin K inhibitor in the treatment of postmenopausal women with low BMD: one year results. J Bone Miner Res. 2008; 22: S37.

77. Stoch SA, Zajic S, Stone J, Miller DL, Van Dyck K, Gutierrez MJ, De Decker M, Liu L, Liu Q, Scott BB, Panebianco D, Jin B, Duong LT, Gottesdiener K, Wagner JA. Effect of the cathepsin K inhibitor odanacatib on bone resorption biomarkers in healthy postmenopausal women: two double-blind, randomized, placebo-controlled phase I studies. Clin Pharmacol Ther. 2009; 86: 175-182.