



# Bioequivalence of Alendronate and Vitamin D<sub>3</sub> in a Combination Tablet Versus Corresponding-Dose Individual Tablets in Healthy Taiwanese Volunteers, Determined Using a Novel Plasma Alendronate Assay



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## ABSTRACT

**Objective:** This study was designed to demonstrate that alendronate (ALN)/vitamin D<sub>3</sub> combination tablets (ALN/D5600) are bioequivalent to corresponding doses of ALN and vitamin D<sub>3</sub> as individual tablets in healthy Taiwanese volunteers.

**Methods:** In this open-label, randomized, 2-period, crossover study, 68 volunteers were randomized to a single ALN/D5600 combination tablet or corresponding doses of 70 mg ALN + 5600 IU vitamin D<sub>3</sub> (2 × 2800 IU), followed by a 12-day washout period and administration of the alternate formulation. Plasma ALN levels were measured using a newly developed assay. Geometric mean ratios of ALN AUC<sub>0–last</sub>, AUC<sub>0–∞</sub>, and C<sub>max</sub>, and unadjusted vitamin D<sub>3</sub> AUC<sub>0–80h</sub> and C<sub>max</sub> were compared and considered bioequivalent if the 90% CI was within 0.8 to 1.25.

**Results:** The geometric mean ratios were: AUC<sub>0–last</sub>, 1.084 (90% CI, 0.937–1.253); AUC<sub>0–∞</sub>, 1.081 (90% CI, 0.935–1.249); and C<sub>max</sub>, 1.112 (90% CI, 0.959–1.289) for ALN, and AUC<sub>0–80h</sub> 0.953 (90% CI, 0.827–1.098) and C<sub>max</sub>, 0.982 (90% CI, 0.854–1.130) for vitamin D<sub>3</sub> unadjusted for endogenous levels.

**Conclusions:** The combination tablet was considered bioequivalent to coadministration based on ALN AUC<sub>0–∞</sub> and unadjusted vitamin D<sub>3</sub> parameters. Slight differences for ALN AUC<sub>0–last</sub> and C<sub>max</sub> (upper 90% CIs outside the bounds) were not considered clinically significant. The combination tablet was well tolerated. No serious adverse experiences were reported. © 2015. The Authors. Published by Elsevier Inc. All rights reserved.

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## Introduction

Osteoporosis is estimated to affect 200 million women worldwide<sup>1</sup> and causes an estimated 9.0 million fractures annually.<sup>2</sup> In Taiwan, osteoporosis and associated age-related fractures are a major public health problem. The Nutrition and Health Survey in Taiwan, conducted in 2005 to 2008, found prevalence rates of osteoporosis of 23.9% in men and 38.3% in women aged 50 years and older.<sup>3</sup> The prevalence rate of vertebral fractures in urban cities in Taiwan has been reported as 12.5% in men and 20% in women aged 65 years and older.<sup>4</sup> The incidence of hip fractures was reported to increase

between 1996 and 2002, from 496 to 644 per 100,000 persons per year in Taiwan's elderly population (aged 65 years and older).<sup>5</sup>

Providing adequate daily calcium and vitamin D is considered a safe and inexpensive way to help reduce fracture risk for all individuals,<sup>6</sup> and optimal vitamin D status appears necessary to maximize the response to antiresorptive therapy.<sup>7</sup> The antifracture effects of vitamin D supplementation also appear to be over and above those of calcium supplements, which are often given concurrently.<sup>8</sup> Vitamin D deficiency (ie, 25-hydroxyvitamin D [25(OH)D] < 20 ng/mL) is common worldwide, with mean population levels of < 20 ng/mL reported in 37.3% of 195 studies published between 1990 and 2011 involving more than 168,000 patients in 44 countries.<sup>9</sup> In older adults living in a northern Taiwan community, the mean 25(OH)D level was 39.9 ng/mL and 31% of the 215 participants had 25(OH)D levels < 20 ng/mL.<sup>10</sup> In recent years there has been considerable debate regarding what

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constitutes an optimal vitamin D status, with osteoporosis guidelines varying in their recommendations for minimal serum 25(OH)D levels.<sup>11</sup> A minimal serum 25(OH)D level of 20 ng/mL<sup>12</sup> or 30 ng/mL<sup>6,13</sup> is currently recommended for the general population, and a level of 30 ng/mL for fragile elderly patients who are at elevated risk for falls and fracture,<sup>12</sup> with supplementation of 800 to 1000 IU/d for elderly adults with suboptimal levels.

Currently available pharmacologic options for osteoporosis approved by the Taiwan Food and Drug Administration include bisphosphonate alendronate (ALN). Treatment with ALN has been shown to increase bone mass and reduce the incidence of fractures, including hip and vertebral fractures, in postmenopausal women with osteoporosis.<sup>14,15</sup> Combination tablets, including a once-weekly formulation of ALN 70 mg plus vitamin D<sub>3</sub> (either 2800 or 5600 IU) in a single tablet, have been developed to ensure that patients with osteoporosis receiving treatment with ALN also receive adequate vitamin D<sub>3</sub> to help reduce the risk of vitamin D insufficiency and deficiency. Previous open-label, 2-period, crossover studies showed that ALN/vitamin D<sub>3</sub> combination tablets (either 2800 or 5600 IU vitamin D<sub>3</sub>) were bioequivalent to an 70-mg ALN tablet with respect to ALN bioavailability, and that the bioavailability of vitamin D<sub>3</sub> was similar in the combination tablets and when vitamin D<sub>3</sub> was administered alone.<sup>16</sup>

The objective of our study (protocol No. 330-00) was to demonstrate bioequivalence between 70 mg ALN/ 5600 IU vitamin D<sub>3</sub> (ALN/D5600) combination tablets and coadministration of corresponding doses of 70 mg ALN and 5600 IU vitamin D<sub>3</sub> (2 × 2800 IU) as individual tablets, in healthy male and female Asian volunteers, to support registration in Taiwan, and to assess safety and tolerability. Of note, the present study used a newly developed, sensitive plasma assay to determine bioequivalence based on the assessment of the pharmacokinetic (PK) characteristics of ALN in plasma, rather than the total urinary excretion method that has been used historically.

## Methods

### Participants

Adults aged 18 to 85 years, in good health (based on medical history, physical examination, vital sign measurements, and laboratory safety tests), and with a body mass index  $\leq 30$  were eligible. Women with reproductive potential required a negative pregnancy test and use of appropriate contraception other than oral or hormone-based methods 14 days before study entry through 14 days poststudy. Volunteers were excluded if they had an estimated creatinine clearance  $\leq 80$  mL/min based on the Cockcroft–Gault equation; were unable to refrain from using any medication, beginning approximately 2 weeks (or 5 half-lives, whichever was longer) before administration of the first dose of study drug; used active vitamin D<sub>3</sub> compounds within 10 days before study entry; received a high dose of vitamin D<sub>3</sub> ( $> 5000$  IU) within 4 weeks before study entry; or had a 25(OH)D level  $< 15$  ng/mL at screening. Subjects were also excluded if they received regular, prolonged sun exposure and were unable to avoid this exposure during the study. Subjects were required to limit direct sunlight exposure at least 10 days before the first dose and throughout the study, to apply sunscreen (at least 45 SPF) if anticipating exposure to direct sunlight for  $> 1$  hour, and to avoid most dairy products, vitamin D<sub>3</sub>-fortified foods, and foods known to be high in vitamin D<sub>3</sub>.

### Study design

This was a single-dose, open-label, randomized, 2-period, crossover study to establish bioequivalence between ALN/D5600

combination tablets and coadministration of corresponding doses of 70 mg ALN and 5600 IU vitamin D<sub>3</sub> (2 × 2800 IU) as individual tablets in healthy male and female Asian volunteers, to support registration in Taiwan. Subjects were assigned an allocation number for open-label treatment using a computer-generated allocation schedule. In the 2 treatment periods, subjects received 1 of 2 single-dose oral treatments in a randomized order: 1 tablet of ALN/D5600, or 1 tablet of 70 mg ALN and 2 tablets of 2800 IU vitamin D<sub>3</sub>. For both treatments, subjects fasted overnight for at least 8 hours, with the exception of water, which was restricted for 1 hour before and 1 hour after study-drug administration. All doses of the study drug were administered with 240 mL water. There was a minimum 12-day washout interval between treatments. Blood samples for determination of plasma ALN concentrations were obtained predose and up to 24 hours postdose in both treatment periods for all enrolled subjects. For the first 40 subjects, blood samples for determination of serum vitamin D<sub>3</sub> (cholecalciferol) were obtained predose and up to 96 hours postdose. Subjects were housed in the study unit and not exposed to direct sunlight from 24 hours predose to 24 hours postdose (96 hours postdose for subjects providing serum vitamin D<sub>3</sub> samples), including the duration of the PK sampling periods; food provided contained minimal, if any, vitamin D<sub>3</sub>.

The protocol was approved by the ethical review committee at the study center and conducted in accordance with the guidelines for Good Clinical Practice. All participants provided written informed consent.

### Assessments

Blood samples for determination of plasma ALN concentrations were collected predose and 10, 20, 30, 40, and 50 minutes, and 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 12, and 24 hours postdose. Plasma samples were assayed by PharmaNet Canada, Inc. (Québec, Canada), using a validated HPLC method with a lower limit of quantification of 100 pg/mL. The analyte and its internal standard alendronic acid-d<sub>6</sub> were extracted from a 0.2 mL aliquot of human plasma prepared with K2 EDTA, using a protein precipitation procedure, followed by a solid phase extraction and a derivatization step. The extracted samples were injected into a liquid chromatograph equipped with a Zorbax 300-SCX (Agilent, Santa Clara, CA, USA), 50 × 3 mm, 5  $\mu$ m column. The mobile phase A and B were mixtures of Milli-Q<sup>®</sup> type water and acetonitrile with ammonium acetate and glacial acetic acid at different proportions. The detection method used was tandem mass spectrometry.

Within-run accuracy and precision evaluations of the assay were performed by analyzing replicate concentrations of alendronic acid in human EDTA K2 plasma. The run consisted of a calibration curve plus a total of 30 spiked samples; 6 replicates of each of the lower and higher limits of quantitation; and low, medium, and high quality control samples. The within-run coefficients of variation ranged between 1.27% and 4.47%. The within-run percentages of bias ranged between -4.19% and 6.34%. The data meet the pre-established acceptance criteria. The assay was demonstrated to be selective for alendronic acid; common over-the-counter medications as well as vitamin D<sub>3</sub> were confirmed not to interfere with the analysis.

Blood samples for determination of serum vitamin D<sub>3</sub> concentrations were collected at -18, -12, and -6 hours predose, and 1, 2, 4, 6, 8, 9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 14, 15, 16, 18, 20, 24, 48, 72, and 96 hours postdose. Serum samples for vitamin D<sub>3</sub> concentrations were assayed using a validated HPLC/tandem mass spectrometry method with an lower limit of quantification of 0.50 ng/mL.<sup>16</sup>

Plasma ALN and serum vitamin D<sub>3</sub> concentrations and actual sampling times relative to the time of dose were used to determine

noncompartmental PK parameters using WinNonlin Professional (version 5.2.1) (Certara, Princeton, NJ, USA). Individual baseline vitamin D<sub>3</sub> concentration adjustments were made using arithmetic mean vitamin D<sub>3</sub> concentrations at –18, –12, and –6 hours before dosing. Baseline adjusted vitamin D<sub>3</sub> concentrations were obtained by subtracting the arithmetic mean predose vitamin D<sub>3</sub> concentration from each individual postdose sample.

Safety and tolerability were assessed by the daily recording of adverse experiences (AEs) throughout the study. A complete physical examination was performed at pre- and poststudy visits, and complete vital signs were obtained at prestudy, before each study-drug administration, and poststudy. Laboratory safety tests, including serum chemistry, hematology, and urinalysis, were performed on samples obtained at the prestudy visit (approximately 2–4 weeks before the first study drug administration) after at least an 8-hour fast.

### Statistical Analysis

#### Sample size and power

If the true geometric mean ratio (GMR) (ALN/D5600:ALN + vitamin D<sub>3</sub>) for the AUC<sub>0–last</sub> and C<sub>max</sub> of ALN was 0.99 and 0.95, respectively, then a sample size of 60 subjects would provide this study with > 90% probability of observing the 90% CIs of 2 ALN end points to be contained within a range of 0.80 to 1.25,<sup>17</sup> assuming a nonnegative correlation between the 2 test statistics for AUC<sub>0–∞</sub> and C<sub>max</sub> of ALN. If the true GMR for the AUC<sub>0–80h</sub> and C<sub>max</sub> of vitamin D<sub>3</sub> was 0.94, then a sample size of 36 subjects would provide this study with > 90% probability of observing the 90% CIs of the 2 end points to be contained within the range of 0.80 to 1.25, assuming a nonnegative correlation between the 2 test statistics for AUC<sub>0–80h</sub> and C<sub>max</sub>. For these calculations, the within-subject SD of ~0.20 (log scale) for the AUC<sub>0–80h</sub> and C<sub>max</sub> of vitamin D<sub>3</sub> was obtained from internal data (MK-0217A P253). The within-subject SD of 0.30 (log scale) for the AUC<sub>0–last</sub> and C<sub>max</sub> of ALN was obtained from Rhim et al.<sup>18</sup> A nonnegative correlation among the 4 primary end points was assumed for the calculations.

#### Statistical methods

Statistical and PK analysis was conducted by Cytel Inc. (Cambridge, MA, USA). There were 4 primary end points in this study: ALN AUC<sub>0–last</sub>, ALN C<sub>max</sub>, vitamin D<sub>3</sub> AUC<sub>0–80h</sub>, and vitamin D<sub>3</sub> C<sub>max</sub>. The PK parameters for ALN (AUC<sub>0–last</sub>, AUC<sub>0–∞</sub>, and C<sub>max</sub>) and vitamin D<sub>3</sub> (unadjusted AUC<sub>0–80h</sub> and C<sub>max</sub>) following a single oral dose of ALN/vitamin D<sub>3</sub> combination tablet or coadministration of ALN + vitamin D<sub>3</sub> were compared using a linear mixed-effects model appropriate for a 2-period, crossover design. The model contained period and treatment as fixed effects, and subject as a random effect. A log transformation was applied to the AUC and C<sub>max</sub> before analysis.

The GMR of ALN AUC<sub>0–80h</sub>, AUC<sub>0–last</sub>, AUC<sub>0–∞</sub>, and C<sub>max</sub>, and vitamin D<sub>3</sub> AUC<sub>0–80h</sub> and C<sub>max</sub>, unadjusted for endogenous vitamin D<sub>3</sub>, were compared and bioequivalence defined by the 90% CI (based on the *t*-distribution) of the GMR for each primary end point being contained within prespecified bounds (ie, 0.80–1.25).

Data were also examined for departures from the assumptions of the statistical model and the use of a distribution-free method considered if a serious departure from the assumptions of the model was observed. Normality assumptions for the linear mixed model were not met due to several extreme values. Therefore, a nonparametric analysis that requires no normality assumption about the distribution of the data was also conducted for the ALN AUC<sub>0–last</sub>, AUC<sub>0–∞</sub>, and C<sub>max</sub> data. Specifically, the Hodges-Lehmann point estimate and the associated 90% CI were calculated for the median of all pairwise differences of log-transformed

AUC<sub>0–last</sub>, AUC<sub>0–∞</sub>, and C<sub>max</sub>. The point estimate and 90% CI were then exponentiated to the linear scale to obtain the estimate of median ratio and the associated 90% CI.

Descriptive statistics were provided for other plasma PK parameters (T<sub>max</sub> and t<sub>1/2</sub>). Minimum, median, and maximum were provided for all PK parameters. In addition, the %CV for AUC and C<sub>max</sub> were calculated according to the following formula:  $100 \times \sqrt{\exp(s^2) - 1}$ , where  $s^2$  is the observed variance on the natural log scale. Harmonic mean and jackknife SD were provided for apparent t<sub>1/2</sub>.

## Results

### Subjects

From March 2012 through May 2012, 69 healthy male (n = 53) and female (n = 16) volunteers (mean [range] age 29.0 [20–54] years; height, 169.8 [150.0–192.0] cm; weight 56.0 [45.0–95.0] kg; and body mass index, 22.9 [17.4–29.3]) were enrolled and were included in the safety evaluation; 68 subjects completed the study per protocol and were included in the PK analysis. One male subject was discontinued from the study after dosing with concomitant ALN + vitamin D<sub>3</sub> in the first treatment period due to noncompliance with the protocol.

### PK of ALN

Mean plasma concentration-time profiles of ALN following administration of an ALN/D5600 combination tablet and coadministration of corresponding doses of ALN and vitamin D<sub>3</sub> as individual tablets are shown in **Figure 1A**. One subject (a woman aged 29 years) had no measurable plasma concentrations for ALN on administration of the reference formulation (ALN + vitamin D<sub>3</sub>), so was excluded from the PK analysis for ALN.

Summary statistics for PK parameters of plasma ALN for subjects with measurable PK in both treatment periods are shown in **Table I**. The 90% CIs of the GMR for ALN AUC<sub>0–last</sub> and C<sub>max</sub> were not contained within the prespecified bioequivalence bounds (ie, 0.80–1.25); the upper 90% CIs slightly exceeded 1.25 with actual values of 1.253 and 1.289, respectively. However, the 90% CI of the GMR for ALN AUC<sub>0–∞</sub> was contained within the bounds. The median T<sub>max</sub> (1.2 hours and 1.0 hour for ALN/D5600 and ALN + vitamin D<sub>3</sub>, respectively) and harmonic mean apparent t<sub>1/2</sub> (7.8 hours and 7.7 hours, respectively) for plasma ALN were comparable between treatments.

ALN exhibited higher within-subject variability for both AUC and C<sub>max</sub> (%CV, 50%–51%) than was anticipated from the literature (%CV, ~30% for both AUC and C<sub>max</sub>)<sup>18</sup> when the study was designed.

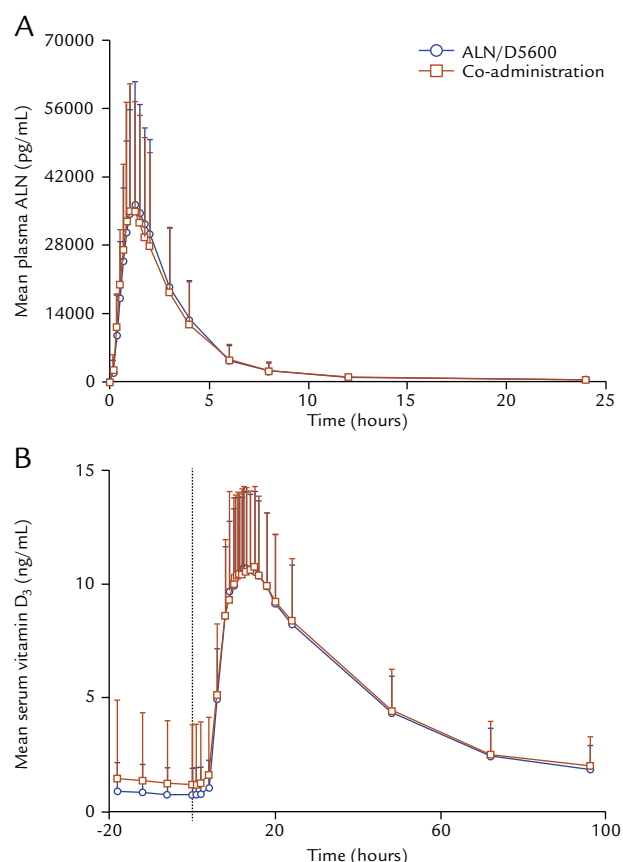
With the nonparametric analysis that requires no normality assumption about the distribution of the data and is less sensitive to extreme values, the 90% CI for the ratio of the medians for AUC<sub>0–last</sub> and AUC<sub>0–∞</sub> was within the bounds of bioequivalence (ie, 0.80–1.25) (**Table II**). The upper bound of the 90% CI for ALN C<sub>max</sub> median ratio marginally exceeded 1.25.

The individual ratios, GMRs, and corresponding 90% CIs of the plasma AUC<sub>0–last</sub>, AUC<sub>0–∞</sub>, and C<sub>max</sub> for ALN are shown in **Figure 2A**.

### PK of Vitamin D<sub>3</sub>

#### Baseline unadjusted vitamin D<sub>3</sub>

Mean plasma concentration-time profiles of serum unadjusted vitamin D<sub>3</sub> following administration of an ALN/D5600 combination tablet and coadministration of corresponding doses of ALN and vitamin D<sub>3</sub> as individual tablets are shown in **Figure 1B**.



**Figure 1.** (A) Arithmetic mean (upper SD) plasma alendronate (ALN) and (B) serum vitamin D<sub>3</sub> concentration-time profiles following single-dose administration of either a 70 mg ALN/5600 IU vitamin D<sub>3</sub> combination tablet or coadministration of corresponding doses of ALN and vitamin D<sub>3</sub> as individual tablets.

Summary statistics for PK parameters of serum unadjusted vitamin D<sub>3</sub> for subjects with measurable PK data in both treatment periods are shown in **Table I**. The 90% CIs of the GMR for vitamin D<sub>3</sub> AUC<sub>0–80h</sub> and C<sub>max</sub>, unadjusted for endogenous vitamin D<sub>3</sub>, were contained within the prespecified bioequivalence bounds (ie, 0.80–1.25). The median T<sub>max</sub> (12.5 hours and 13.5 hours, respectively) and harmonic mean apparent t<sub>1/2</sub> (29.9 hours and

31.2 hours, respectively) for baseline unadjusted serum vitamin D<sub>3</sub> were comparable between ALN/D5600 and ALN + vitamin D<sub>3</sub> treatments.

The individual ratios, GMRs, and corresponding 90% CIs of the serum unadjusted AUC<sub>0–80h</sub> and C<sub>max</sub> for vitamin D<sub>3</sub> are shown in **Figure 2B**.

#### Baseline adjusted vitamin D<sub>3</sub>

For most subjects, postdose vitamin D<sub>3</sub> levels were above predose levels. However, in some subjects, vitamin D<sub>3</sub> levels declined to such an extent that at 80 hours postdose, they had substantially lower vitamin D<sub>3</sub> concentrations than their predose values. In some instances, this decline resulted in negative concentration values when adjusted for predose vitamin D<sub>3</sub>. The GMRs (90% CI) of serum vitamin D<sub>3</sub> after baseline correction were: 1.101 (90% CI, 0.895–1.356) and 1.049 (90% CI, 0.893–1.231) for AUC<sub>0–80h</sub> and C<sub>max</sub>, respectively. Whereas both point estimates and the entire 90% CI for C<sub>max</sub> were within the prespecified bioequivalence bounds of 0.80 to 1.25, the upper limit of the 90% CI for AUC<sub>0–80h</sub> fell outside these bounds.

#### Safety and Tolerability

Of the 69 subjects included in the safety analysis (1 of whom did not receive the combination tablet), 51 reported a total of 128 clinical AEs, of which 125 were considered by the investigator to be possibly related to the study drug. All AEs were transient and considered mild in intensity by the investigator. There were no serious AEs reported and no subjects discontinued because of an AE. Two laboratory AEs were reported at prestudy: alanine aminotransferase increased for 1 subject and aspartate aminotransferase increased for a second subject. There were no consistent treatment-related changes in routine clinical safety parameters, including vital signs and physical examination.

The most common drug-related AEs were arthralgia (reported by 25 and 27 subjects after receiving ALN/D5600 and ALN + vitamin D<sub>3</sub>, respectively), pyrexia (12 vs 10 subjects), and headache (9 vs 10 subjects). Gastrointestinal disorders were reported in 4 and 6 patients, respectively, and included abdominal distension, diarrhea, vomiting (all 1 subject each) and toothache (2 subjects) in the ALN/D5600 group, and diarrhea (5 subjects), toothache (2 subjects), and nausea (1 subject) in the ALN + vitamin D<sub>3</sub> group. There were no clinically meaningful differences in

**Table I**

Summary results for pharmacokinetic parameters of plasma alendronate (ALN) and serum vitamin D<sub>3</sub> following administration of a 70 mg ALN/5600 IU vitamin D<sub>3</sub> (D5600) combination tablet or corresponding doses of ALN and vitamin D<sub>3</sub> as individual tablets (linear mixed-model approach).

Pharmacokinetic parameter	ALN/D5600 combination tablet			ALN + vitamin D <sub>3</sub> coadministration			ALN/D5600:ALN + vitamin D <sub>3</sub>		
	n	GM*	95% CI	n	GM*	95% CI	GMR	90% CI	%CV†
<b>Plasma ALN</b>									
AUC <sub>0–last</sub> , ng·h/mL	67	111.71	96.72–129.03	67	103.08	88.20–120.48	1.084	0.937–1.253	50.45
AUC <sub>0–∞</sub> , ng·h/mL	67	116.73	101.18–134.66	67	108.02	92.63–125.98	1.081	0.935–1.249	50.21
C <sub>max</sub> , ng/mL	67	36.48	31.46–42.30	67	32.82	28.25–38.13	1.112	0.959–1.289	51.29
T <sub>max</sub> ‡, h	67	1.2	0.5–2.0	67	1.0	0.3–3.0	–	–	–
Apparent t <sub>1/2</sub> §, h	67	7.8	1.6	67	7.7	1.8	–	–	–
<b>Serum vitamin D<sub>3</sub></b>									
AUC <sub>0–80h</sub> , ng·h/mL	40	382.81	318.83–459.62	40	401.86	351.01–460.08	0.953	0.827–1.098	37.68
C <sub>max</sub> , ng/mL	40	10.45	8.86–12.32	40	10.64	9.29–12.19	0.982	0.854–1.130	37.14
T <sub>max</sub> ‡, h	40	12.5	9.0–24.1	40	13.5	10.0–16.0	–	–	–
Apparent t <sub>1/2</sub> §, h	40	29.9	8.8	40	31.2	7.6	–	–	–

GM = geometric mean.

\* GM computed from least squares estimate from a linear mixed-effects model performed on the natural log-transformed values.

† %CV, derived from variance components, provides an estimate of the pooled within-subject coefficient of variation.

‡ Median, minimum, and maximum reported for T<sub>max</sub>.

§ Harmonic mean and jackknife SD provided for apparent t<sub>1/2</sub>.

**Table II**  
Summary results for pharmacokinetic parameters of plasma alendronate (ALN) and serum vitamin D<sub>3</sub> after administration of 70 mg ALN and 5600 IU vitamin D<sub>3</sub> as a combination tablet or corresponding doses of ALN and vitamin D<sub>3</sub> as individual tablets (nonparametric approach).

Treatment*	n	Minimum	Median	Maximum	Interquartile range	ALN/D5600: ALN + vitamin D <sub>3</sub>	P value
<b>AUC<sub>0–last</sub>, ng · h/mL</b>							
ALN/D5600 combination tablet	67	20.79	119.43	326.98	96.81	1.081 (0.944–1.243)	0.330
ALN + vitamin D <sub>3</sub> coadministration	67	19.16	100.17	496.04	107.25		–
<b>AUC<sub>0–∞</sub>, ng · h/mL</b>							
ALN/D5600 combination tablet	67	21.78	124.42	344.96	100.41	1.081 (0.941–1.244)	0.339
ALN + vitamin D <sub>3</sub> coadministration	67	19.91	104.49	510.21	108.82		–
<b>C<sub>max</sub>, ng/mL</b>							
ALN/D5600 combination tablet	67	7.11	40.08	102.73	33.41	1.120 (0.964–1.287)	0.190
ALN + vitamin D <sub>3</sub> coadministration	67	8.32	33.17	172.74	31.56		–

\* Comparison: Hodges-Lehmann estimate of median ratio with 90% CI; P value from Wilcoxon signed-rank test. P value > 0.1 indicates no statistically significant difference between the 2 treatments.

tolerability between the ALN/D5600 combination tablet and the coadministered dose of ALN and vitamin D<sub>3</sub>.

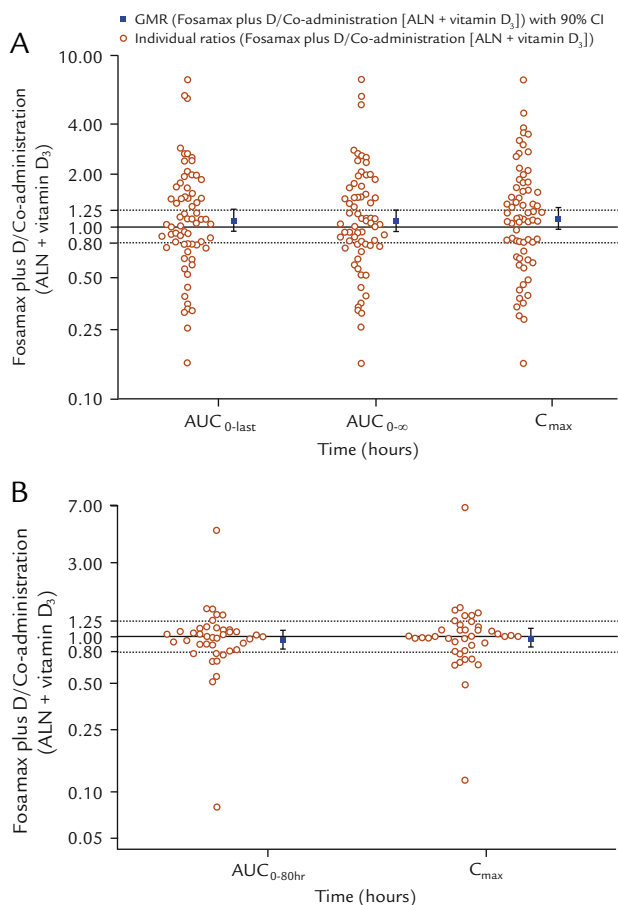
## Discussion

Although the 90% CIs of the observed GMRs for the plasma AUC<sub>0–last</sub> and C<sub>max</sub> of ALN for subjects with measurable PK in both treatment periods were not all contained within the prespecified bounds for bioequivalence (ie, 0.80–1.25), the 90% CI of the observed GMR for AUC<sub>0–∞</sub> of ALN was within the bounds. In addition, the 90% CI of the ratio of medians for AUC<sub>0–last</sub> obtained

from the nonparametric analysis was within the prespecified bounds. The ALN/D5600 combination tablet and coadministration of ALN + vitamin D<sub>3</sub> resulted in similar plasma concentration-time profiles (Figure 1). This is supported by the fact that for both GMRs the AUC<sub>0–last</sub> and C<sub>max</sub> were within the lower bound of the 90% CI of ALN AUC<sub>0–last</sub> and C<sub>max</sub> (there was only a minor excursion of the upper bound of the 90% CI of ALN AUC<sub>0–last</sub> and C<sub>max</sub>), and the entire 90% CI for AUC<sub>0–∞</sub> was within the bioequivalence bounds (ie, 0.80–1.25).

The observed differences between ALN AUC<sub>0–last</sub> and C<sub>max</sub> are not believed to be clinically significant, in that the slightly higher AUC<sub>0–last</sub> and C<sub>max</sub> of the ALN/D5600 combination tablet would not be expected to result in an additional safety risk or to have an influence on efficacy compared with concomitant ALN + vitamin D<sub>3</sub>. This is because the differences were small and thus would likely fall within the range experienced among the broad population of patients using ALN, and because of the mechanism of action of ALN, which relies on its deposition in bone. Incorporation of ALN in bone over time is correlated clinically with increased bone mass and reduced fracture risk,<sup>19</sup> such that small differences in AUC or C<sub>max</sub> are unlikely to have a major effect. In addition, based on the highly variable drug characteristics of ALN and using the proposed bioequivalence approach of the European Medicines Agency for high variability values (intrasubject variability > 30%),<sup>20</sup> the C<sub>max</sub> of ALN falls within the expanded CI of 0.698 to 1.432. The highly variable drug status of ALN was not prespecified in the hypothesis because this was the first study conducted by the sponsor to employ a newly developed assay for plasma ALN. For this reason, internal data on which to base variability estimates were lacking, and data from the literature on ALN were limited in this regard.

The GMR and 90% CIs for unadjusted serum vitamin D<sub>3</sub> for AUC<sub>0–80h</sub> and C<sub>max</sub> were within the prespecified bioequivalence bounds. After baseline correction of serum vitamin D<sub>3</sub>, both point estimates and the entire 90% CI for C<sub>max</sub> were within the prespecified bounds; however, the upper limit of the 90% CI for AUC<sub>0–80h</sub> fell outside these bounds. This was because some subjects had lower serum vitamin D<sub>3</sub> concentrations at 80 hours postdose than at predose. The likely explanation for this is the restrictions that were placed on subjects to prevent exposure to vitamin D<sub>3</sub> except for study treatment, including the avoidance of sun exposure and a diet containing minimal, if any, vitamin D<sub>3</sub>. The baseline adjusted approach attempts to separate the contribution of the administered vitamin D<sub>3</sub> from endogenous vitamin D<sub>3</sub> levels on an individual basis, and in doing so, also adjusts for potential differences in baseline vitamin D<sub>3</sub> levels across periods. However, this approach assumes that the endogenous level will remain constant, which appeared to be incorrect for some subjects. The PK parameters AUC<sub>0–80h</sub> and C<sub>max</sub> obtained from the data unadjusted for baseline vitamin D<sub>3</sub> concentrations are therefore favored



**Figure 2.** Individual ratios, geometric mean ratios, and 90% CIs for (A) plasma alendronate (ALN) AUC<sub>0–last</sub>, AUC<sub>0–∞</sub>, and C<sub>max</sub>, and (B) serum unadjusted vitamin D<sub>3</sub> AUC<sub>0–80h</sub>, and C<sub>max</sub>. Dotted lines show the prespecified bioequivalence bounds (0.80–1.25).

because this approach requires no assumptions; these data also agree with those presented previously.<sup>16</sup>

Historically, the primary PK parameter of ALN was urinary excretion of ALN. This was because plasma concentrations were too low for analysis and elimination of bioavailable ALN is essentially entirely via urinary excretion.<sup>19</sup> However, urinary analysis of ALN requires complex processes, is labor intensive, and typically requires more than 200 subjects to demonstrate bioequivalence.<sup>16</sup> Recently, HPLC-MS/MS detection has been used to measure plasma ALN levels. Use of a plasma-based assessment required fewer subjects to demonstrate bioequivalence compared with urine-based assessments.<sup>18</sup> Once the extreme values in the ALN data were accommodated, the data generated from this study are in good agreement with those published previously using a urine-based approach to demonstrate bioequivalence,<sup>16</sup> which supports the applicability of the plasma ALN assay. Because the plasma  $t_{1/2}$  of ALN is long (up to 10 years), calculation of the apparent terminal  $t_{1/2}$  and therefore the  $AUC_{0-\infty}$  may be underestimated. Hence, the  $AUC_{0-last}$  was proposed as the primary PK end point upon which to assess bioequivalence for ALN.

There were no clinically meaningful differences in tolerability between the ALN/D5600 tablet and the coadministration of ALN + vitamin D<sub>3</sub>. Although the combination tablet showed slightly higher  $C_{max}$  and  $AUC_{0-last}$  for ALN, the safety profile remained consistent with that of the marketed drug.

### Conclusions

In this study of healthy male and female volunteers in Taiwan, the ALN/D5600 combination tablet was considered bioequivalent to coadministration based on ALN  $AUC_{0-\infty}$  and nonparametric  $AUC_{0-last}$  and unadjusted vitamin D<sub>3</sub>  $AUC_{0-80h}$  and  $C_{max}$ . The magnitude of the difference between ALN/D5600 and ALN + vitamin D<sub>3</sub> for ALN  $AUC_{0-last}$  and  $C_{max}$  was not considered to be clinically significant, and both parameters fall within the expanded CIs of the European Medicines Agency's proposed bioequivalence approach for high variability values. The combination tablet was well tolerated, with no serious AEs reported, and is comparable to the coadministration of corresponding doses of ALN and vitamin D<sub>3</sub>.

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### Conflicts of Interest

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