

ARTICLE

Phase I studies of the safety, tolerability, pharmacokinetics, and pharmacodynamics of DS-1211, a tissue-nonspecific alkaline phosphatase inhibitor

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Abstract

Tissue-nonspecific alkaline phosphatase (TNAP) hydrolyzes and inactivates inorganic pyrophosphate (PPI), a potent inhibitor of calcification; therefore, TNAP inhibition is a potential target to treat ectopic calcification. These two first-in-human studies evaluated safety, tolerability, pharmacokinetics (PKs), and pharmacodynamics (PDs) of single (SAD) and multiple-ascending doses (MAD) of DS-1211, a TNAP inhibitor. Healthy adults were randomized 6:2 to DS-1211 or placebo, eight subjects per dose cohort. SAD study subjects received one dose of DS-1211 (range, 3–3000 mg) or placebo, whereas MAD study subjects received DS-1211 (range, 10–300 mg) once daily, 150 mg twice daily (b.i.d.), or placebo for 10 days. Primary end points were safety and tolerability. PK and PD assessments included plasma concentrations of DS-1211, alkaline phosphatase (ALP) activity, and TNAP substrates (PPI, pyridoxal 5'-phosphate [PLP], and phosphoethanolamine [PEA]). A total of 56 (DS-1211: $n = 42$; placebo: $n = 14$) and 40 (DS-1211: $n = 30$; placebo: $n = 10$) subjects enrolled in the SAD and MAD studies, respectively. In both studies, adverse events were mild or moderate and did not increase with dose. PKs of DS-1211 were linear up to 100 mg administered as a single dose and 150 mg b.i.d. administered as a multiple-dose regimen. In multiple dosing, there was minimal accumulation of DS-1211. Increased DS-1211 exposure correlated with dose-dependent ALP inhibition and concomitant increases in PPI, PLP, and PEA. In two phase I studies, DS-1211 appeared safe and well-tolerated. Post-treatment PD assessments were consistent with exposure-dependent TNAP inhibition. These data support further evaluation of DS-1211 for ectopic calcification diseases.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

In preclinical studies, successful inhibition of tissue-nonspecific alkaline phosphatase (TNAP) decreased soft-tissue calcification. However, TNAP inhibition is untested in humans, and the safety and pharmacological effects are unknown.

WHAT QUESTION DID THIS STUDY ADDRESS?

What are the safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) effects of TNAP-specific inhibitor DS-1211?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

TNAP inhibition mediated by orally administered DS-1211 appears to be safe and well-tolerated in humans. The PD effects of DS-1211 on alkaline phosphatase, inorganic pyrophosphate, pyridoxal 5'-phosphate, and phosphoethanolamine suggest it can inhibit TNAP in humans. This is the first clinical trial with a TNAP-specific inhibitor; its safety over 10 days and PK-dependent profiles of TNAP inhibition were established.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Ectopic calcification lacks a direct and safe treatment. TNAP inhibition with DS-1211 may provide a novel targeted therapy for excess soft-tissue calcification.

INTRODUCTION

Alkaline phosphatases (ALPs) are ubiquitous membrane-bound glycoproteins that catalyze the hydrolysis of phosphate monoesters.^{1,2} Four ALP isozymes exist in humans, including three with restricted tissue distribution: intestinal ALP, placental ALP, germ cell ALP, and the tissue-nonspecific ALP (TNAP) with wide tissue distribution in bone, liver, and kidneys.² Although mammalian TNAP can hydrolyze or transphosphorylate many phosphorylated compounds in vitro, three have been recognized as endogenous substrates of TNAP: inorganic pyrophosphate (PPi), pyridoxal 5'-phosphate (PLP; vitamin B6), and phosphoethanolamine (PEA).¹⁻³

One well-recognized biological function of TNAP is hydrolysis of extracellular PPi to maintain the balanced inorganic phosphate (Pi) to PPi ratio in skeletal tissues to enable proper skeletal and dental mineralization.² In non-skeletal tissues, TNAP maintains proper PPi levels to prevent ectopic vascular and soft-tissue calcification.^{2,4} A loss of balance through elevated TNAP expression is associated with soft-tissue calcification, whereas severely deficient TNAP enzymatic activity, as in hypophosphatasia (HPP), leads to excessively elevated PPi and may cause conditions such as early loss of primary teeth, stress fractures, and osteomalacia, among others.^{4,5}

The narrow physiological range of the endogenous mineralization inhibitor PPi is regulated by the release of its precursors, which subsequently generates PPi.^{6,7} Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) converts circulating adenosine triphosphate

(ATP) to adenosine monophosphate (AMP) and PPi. ATP binding cassette subfamily C member 6 (ABCC6), an organic anion transporter, facilitates the release of ATP from the liver into circulating plasma.⁸ AMP is further hydrolyzed by an ecto-5'-nucleotidase (*NT5E*, which encodes CD73) into adenosine and Pi.⁴ Adenosine, an endogenous inhibitor of TNAP transcription, further maintains PPi levels.⁹ Dysfunction in this actively regulated PPi metabolic pathway leads to abnormal soft-tissue mineralization in several monogenetic ectopic calcification diseases, including ABCC6 in pseudoxanthoma elasticum (PXE), ENPP1 in generalized arterial calcification of infancy (GACI), and CD73 in arterial calcification due to deficiency of CD73 (ACDC).⁸⁻¹¹ Remediation of these defects is considered an important therapeutic strategy to raise endogenous PPi and prevent ectopic soft-tissue calcification, including use of TNAP inhibitors.^{9,12}

Several inhibitors of ALP isozymes are described in the literature. Earlier compounds, such as levamisole and theophylline, are not specific for TNAP and show weak inhibitory activity.^{13,14} Recently, the pharmacological activities of a TNAP-specific inhibitor, SBI-425, have been reported in animal models of vascular calcification and PXE, further supporting the approach of TNAP inhibition to reduce ectopic calcification.^{6,12,15-17}

DS-1211 is a potent and specific small-molecule inhibitor of TNAP.¹⁸ In vitro and in vivo animal studies have shown that it is effective in inhibiting TNAP activities, increasing PPi levels, and reducing ectopic calcification in animal models (manuscript in preparation). Here, we present the results of the initial clinical characterization

of this TNAP inhibitor from a single-ascending dose (SAD) and a multiple-ascending dose (MAD) study on the safety, tolerability, pharmacokinetics (PKs), and pharmacodynamics (PDs) of DS-1211 in healthy subjects.

METHODS

Ethics statement

Both study protocols were approved by the Institutional Review Board IntegReview. All study subjects provided a written informed consent before any study procedure was performed. The studies were conducted at Worldwide Clinical Trials and were in compliance with the Helsinki Declaration of 1975 (as revised in 1983) and codified in International Council for Harmonization (ICH) E6 (R2), US Food and Drug Administration Good Clinical Practice regulations, and the ICH E6 guideline for Good Clinical Practice.

Subjects

Eligible subjects included healthy adults aged less than or equal to 45 years with a body mass index 18–30 kg/m² with no clinically significant abnormalities in presudy medical history, physical examination, vital signs, electrocardiogram (ECG), and laboratory evaluations. Key exclusion criteria included pregnancy or breastfeeding; presence of proteinuria, hematuria, or abnormal liver function tests—including ALP (outside the normal range by age); consumption of greater than 21 units per week of alcohol for males or greater than 14 units for females; history of alcoholism; tobacco use within 6 months prior to the first dose; or an abnormal ECG or an ECG with a corrected QT interval (QTcF) greater than 450 ms. Subjects enrolled in the SAD study were ineligible for participation in the MAD study.

Study design

The SAD study was a phase I, randomized, double-blind (sponsor-unblinded), placebo-controlled, first-in-human, sequential dose-escalation study. The study consisted of seven cohorts of eight subjects each: six randomized to DS-1211 and two randomized to placebo (Figure S1A). Screening occurred less than or equal to 21 days prior to check-in. Subjects checked in to the clinical pharmacology unit (CPU) and fasted for greater than or equal to 10 h before receiving either a single oral dose of DS-1211 in powder dosage form reconstituted as oral solution or corresponding placebo on day 1 of the study, and they remained fasted

until 4 h postdose. Following study drug administration, a series of blood and urine samples were collected to assess the PKs and PDs of DS-1211. Subjects were discharged from the CPU 72 h postdose and returned once between 7 and 10 days postdose for follow-up assessments.

The MAD study was a phase I, randomized, double-blind, placebo-controlled dose-escalating study consisting of five cohorts randomized 6:2 to DS-1211 or placebo, with eight subjects per dose cohort. Subjects were screened for eligibility less than or equal to 21 days prior to check-in. Four cohorts of subjects received 10, 30, 100, or 300 mg of oral DS-1211 in powder dosage form reconstituted as oral solution or received matching placebo once daily (q.d.) for 10 days. Cohort five received 150 mg oral DS-1211 or matching placebo for 10 days, administered twice daily (b.i.d.) ~12 h apart (Figure S1B). All subjects fasted for greater than or equal to 8 h before the morning dosing and continued to fast for 2–4 h post-morning dose. Subjects on the 150 mg b.i.d. regimen also fasted from 2 h predose to 2 h postdose for the evening dose. All subjects checked out of the CPU 72 h after their last dose and returned for follow-up assessments within 4–7 days.

Subjects were asked to refrain from consuming grapefruit/grapefruit juice, Seville oranges, and food or beverages containing caffeine/xanthine or alcohol until the end of the study. Additionally, dose escalation in both studies proceeded following the review of safety, tolerability, and PKs (systemic exposure assessed in the first 24 h postdose) data of the previous cohort. Data were evaluated against predetermined stopping criteria prior to dose escalation. If any stopping criteria were met, dose escalation stopped until a full safety review was complete with a risk estimate of proceeding to the higher dose level.

Study end points

In both studies, the primary end points were safety and tolerability of DS-1211. Secondary end points included DS-1211 PK and PD parameters.

Study assessments

Safety assessments

Safety assessments included clinical examinations, changes in vital signs, clinical assessments for treatment-emergent adverse events (TEAEs), ECG readings, and clinical laboratory assessments. All TEAEs were coded by system organ class and according to the Medical Dictionary for Regulatory Activities and were evaluated for intensity. Vital signs included blood pressure, temperature, and

respiratory rate. ECGs were performed in triplicate and assessed ECG parameters and heart rate, comparing results to potentially clinically important values and to baseline. Clinical laboratory assessments included hematology, coagulation, serum chemistry, and urinalysis. During physical examinations, investigators noted abnormal findings.

Pharmacokinetic assessments

In both studies, blood samples were collected for the determination of plasma DS-1211 concentrations. In the SAD study, samples were collected at predose and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48, and 72 h postdose. In the MAD study, samples were collected at predose and at 0.5, 1, 1.5, 2, 4, 6, 12, and 24 h on days 1 and 10; additional samples were collected for the 150 mg b.i.d. cohort at 12.5, 13, and 14 h (relative to the first dose). Plasma DS-1211 concentrations were quantified by a validated liquid chromatography-tandem mass spectrometry method (LC-MS/MS). Plasma concentration-time data were analyzed through noncompartmental methods using Phoenix WinNonlin using actual sample times relative to time of dose. For the SAD study, PK parameters included maximum observed plasma concentration (C_{max}), time to C_{max} (T_{max}), area under the concentration-time curve to last measurable concentration (AUC_{last}), terminal half-life ($t_{1/2}$), and area under the curve extrapolated to infinity (AUC_{inf}). For the MAD study, the following PK parameters were estimated for the morning doses of days 1 and 10: C_{max} , T_{max} , AUC_{inf} , and AUC_{last} (or AUC over a dosing interval [AUC_{tau}]). Additionally, the accumulation ratio (AR) was calculated based on the exposure (C_{max} and AUC_{last} or AUC_{tau}) ratios of day 10 and day 1.

Pharmacodynamic assessments

Blood samples for assessment of ALP activity and PPI and PLP levels were collected at predose and at 0.5, 1, 2, 6, 12, 24, and 48 h postdose on day 1 in both studies and also on day 10 in the MAD study. Additional samples were collected for the MAD 150 mg b.i.d. cohort at 12.5, 13, and 14 h (relative to the first dose). Blood samples were also collected in the MAD study on day 7 (predose) and day 13 (72 h after the last dose) relative to dosing on day 10 for PPI and PLP assessment; samples for ALP were collected during follow-up.

In the SAD study only, blood samples to assess PEA were collected at predose and at 1, 2, 6, 12, and 24 h postdose. Urine samples were also collected at various intervals from predose to 72 h postdose for PEA assessment. PEA was not assessed in the 3-mg and 10-mg dose cohorts or in the MAD study.

Blood specimens were centrifuged immediately to yield plasma for PPI, PLP, and PEA analysis and serum for the ALP activity assay. ALP activity was analyzed following the conventional International Federation of Clinical Chemistry method with 4-nitrophenyl phosphate as a substrate within the clinical unit (Roche Cobas, Roche Diagnostics).¹⁹ Frozen plasma and urine specimens were shipped and analyzed at analytical laboratories. Plasma PLP (vitamin B6) levels were determined using a commercially available LC-MS/MS assay (LabCorp).²⁰ PEA levels were evaluated in plasma and urine using a commercially available assay at Genova Diagnostics. PPI levels were determined using ATP sulfurylase at QPS, as previously described.⁸

Statistical analysis

In both the SAD and MAD studies, the safety analysis set included subjects who received greater than or equal to 1 dose of study drug. The PK analysis set included subjects who received greater than or equal to 1 dose of DS-1211 and had greater than or equal to 1 corresponding measurable plasma concentration; subjects receiving placebo were not included for PK analysis. The PD analysis set encompassed subjects who had received greater than or equal to 1 dose of study treatment and had greater than or equal to 1 corresponding PD assessment. Data from placebo-treated subjects were pooled for PD analysis.

Demographics, safety data, and PK and PD assessments were summarized using descriptive statistics including sample size (n), mean, SD, median, minimum, maximum, frequency, and percentages, as appropriate. Noncompartmental analysis was used for PK analysis. The relationship between dose and exposure parameters (C_{max} , AUC_{last} , and AUC_{inf}) was examined graphically. Dose-proportionality based on C_{max} , AUC_{last} , and AUC_{tau} (150 mg b.i.d. cohort only) was assessed using a power model and dose-normalization approach.

Percent change from baseline for PD data was described numerically and graphically by subject for each applicable PD parameter. The last assessment prior to the first dose of DS-1211 was defined as baseline. Data were summarized by dose cohort and study day, if applicable. All analyses were performed using SAS version 9.3 (SAS Institute).

RESULTS

Demographics and baseline characteristics

Fifty-six subjects were randomized across seven cohorts in the SAD study (DS-1211: $n = 42$; placebo: $n = 14$), and 40 subjects were randomized across five cohorts

in the MAD study (DS-1211: $n = 30$; placebo: $n = 10$). All randomized subjects completed the SAD study; one subject withdrew from the MAD study due to TEAEs. Subject demographics and baseline characteristics were comparable between the studies. The mean (SD) ages of subjects were 31.9 (6.7) years and 31.7 (7.2) years for the SAD and MAD studies, respectively (Table 1). The SAD and MAD studies included 37 (66%) and 20 (50%) female subjects, respectively.

Safety

Single ascending dose

Overall, 16 TEAEs were reported from 14 (25%) subjects (Table 2). Seven (12.5%) subjects experienced a total of eight (50%) drug-related TEAEs; of these, two occurred in two subjects receiving placebo. Most TEAEs were mild and none were severe. No subject discontinued the study due to TEAEs and there were no deaths or serious adverse events (SAEs). The most common TEAE was headache (5.4%), followed by abdominal pain (3.6%; Table S2). Types of TEAE frequency did not increase in a dose-ordered manner. Subjects receiving DS-1211 experienced TEAEs at a rate comparable to that of subjects receiving placebo (Table 2).

Multiple ascending dose

In the MAD study, 27 TEAEs were reported from 16 (40%) of 40 subjects (Table 2). Seven (17.5%) subjects experienced 14 (51.8%) drug-related TEAEs; of these, three occurred in two subjects receiving placebo. There were no deaths or severe TEAEs, and a majority (69%) of subjects experienced mild TEAEs. One subject discontinued the study due to experiencing two TEAEs: mild diarrhea and moderate hemorrhoids (Table S2). Across all treatment groups, the most common TEAEs were anemia (7.5%), headache (7.5%), and constipation (5.0%). There was no dose-ordered relationship of TEAEs, and the rate of TEAEs observed in the placebo group was comparable to that in the 10-, 100-, and 300-mg DS-1211 cohorts (Table 2).

Pharmacokinetics

Single ascending dose

After oral administration, DS-1211 was quickly absorbed with a median T_{max} of 0.5–1.0 h (across a 3–3000-mg

dose range). DS-1211 exposure increased with dose (Figure 1a,b), with an approximate dose-proportional increase from 3–100 mg; increases were less than dose proportional greater than 100 mg (Figure 2a,b). The mean half-life (SD) ranged from 1.4 (0.2) to 2.5 (1.0) h over a dose range of 3–30 mg. At dose levels greater than 30 mg, longer mean $t_{1/2}$ estimates were observed, ranging from 11.5 (9.4) to 38.4 (24.2) h (Table 3).

Multiple ascending dose

During multiple dosing, DS-1211 was rapidly absorbed and plasma concentration increased rapidly in all dose groups, with a median T_{max} of 0.5–1.0 h postdose on day 1 and day 10 (Figure 1c,d). The highest mean C_{max} (SD) were 3590 (1237) ng/ml and 4555 (889) ng/ml on days 1 and 10, respectively, in the 300 mg q.d. cohort (Table 3). The mean half-life (SD) for all doses was ~2 h and was similar on day 1 and day 10. The C_{max} , AUC_{last} , and AUC_{tau} demonstrated an approximate dose-proportional increase for dose levels of 10–100 mg q.d. and 150 mg b.i.d. (based on the exposure after the morning dose) on day 1 and day 10; increases were less than dose proportional for the 300 mg q.d. dose (Figure 2c,d). There was no apparent accumulation following multiple administrations of DS-1211 at 10–100 mg q.d., and minimal accumulation occurred with 300 mg q.d. and 150 mg b.i.d.

Pharmacodynamics

Single ascending dose

Overall, the degree of ALP inhibition correlated with increasing exposures of DS-1211. Inhibition of ALP activity was observed as early as 0.5 h postdose and reached a maximum mean (SD) change from baseline of –58.5% (8.4) at the highest 3000-mg dose at 1 h postdose, which approximately corresponds to the T_{max} for DS-1211 plasma concentration (Figure 3a). There was a concomitant and dose-dependent increase in PPI, reaching a maximal mean (SD) change from baseline of 147% (30) at 6 h postdose for the highest 3000-mg dose (Figure 3b). Similarly, dose-ordered PLP increases were observed in all treatment groups, with a peak at 6 h for dose cohorts of 100 mg or higher, and they reached approximately >10-fold increases from baseline at the highest 3000-mg dose (Figure 3c). In subjects receiving 1000 and 3000 mg, PLP remained elevated from baseline 48 h postdose. Plasma PEA increased from baseline at 1 h in an apparent dose-dependent manner, with a maximal mean change from baseline (SD) of 1271% (486)

TABLE 1 Subject demographics and baseline characteristics

	SAD study								Overall N = 56
	3 mg q.d. n = 6	10 mg q.d. n = 6	30 mg q.d. n = 6	100 mg q.d. n = 6	300 mg q.d. n = 6	1000 mg q.d. n = 6	3000 mg q.d. n = 6	Placebo q.d. n = 14	
Age, years, mean (SD)	31.2 (8.0)	27.7 (5.1)	30.3 (7.2)	30.0 (5.3)	31.8 (6.7)	35.0 (6.3)	31.7 (2.4)	34.5 (8.2)	31.9 (6.7)
Sex, female	4 (66.7)	4 (66.7)	3 (50.0)	6 (100)	4 (66.7)	3 (50.0)	4 (66.7)	9 (64.3)	37 (66.1)
Race									
White	4 (66.7)	5 (83.3)	2 (33.3)	3 (50.0)	4 (66.7)	5 (83.3)	4 (66.7)	7 (50.0)	34 (60.7)
Black or African American	2 (33.3)	1 (16.7)	4 (66.7)	3 (50.0)	2 (33.3)	0	2 (33.3)	7 (50.0)	21 (37.5)
Asian	0	0	0	0	0	1 (16.7)	0	0	1 (1.8)
BMI, kg/m ² , mean (SD)	26.5 (1.9)	24.8 (2.3)	24.5 (3.1)	24.5 (2.2)	25.7 (3.1)	24.6 (1.0)	24.0 (2.7)	27.1 (2.8)	25.5 (2.6)
	MAD study								Overall N = 40
	10 mg q.d. n = 6	30 mg q.d. n = 6	100 mg q.d. n = 6	300 mg q.d. n = 6	150 mg b.i.d. n = 6	300 mg q.d. n = 6	150 mg b.i.d. n = 6	Placebo n = 10	
Age, years, mean (SD)	33.8 (7.7)	25.5 (6.9)	34.2 (4.4)	34.3 (7.9)	35.3 (7.0)	34.3 (7.9)	35.3 (7.0)	28.8 (6.1)	31.7 (7.2)
Sex, female	2 (33.3)	3 (50.0)	5 (83.3)	3 (50.0)	1 (16.7)	3 (50.0)	1 (16.7)	6 (60.0)	20 (50.0)
Race									
White	3 (50.0)	3 (50.0)	1 (16.7)	4 (66.7)	4 (66.7)	4 (66.7)	4 (66.7)	2 (20.0)	17 (42.5)
Black or African American	2 (33.3)	3 (50.0)	5 (83.3)	2 (33.3)	2 (33.3)	2 (33.3)	2 (33.3)	7 (70.0)	21 (52.5)
Asian	1 (16.7)	0	0	0	0	0	0	0	1 (2.5)
American Indian or Alaska Native	0	0	0	0	0	0	0	1 (10.0)	1 (2.5)
BMI, kg/m ² , mean (SD)	25.7 (2.2)	26.2 (2.4)	25.4 (2.5)	25.4 (2.9)	25.7 (2.1)	25.4 (2.9)	25.7 (2.1)	26.2 (2.1)	25.8 (2.2)

Note: Data shown as n (%) unless otherwise specified.

Abbreviations: b.i.d., twice daily; BMI, body mass index; MAD, multiple ascending dose; q.d., once daily; SAD, single ascending dose; SD, standard deviation.

TABLE 2 Overall summary of TEAEs by dose

	SAD study										Overall N = 56
	3 mg q.d. n = 6	10 mg q.d. n = 6	30 mg q.d. n = 6	100 mg q.d. n = 6	300 mg q.d. n = 6	1000 mg q.d. n = 6	3000 mg q.d. n = 6	Placebo n = 14			
Subjects reporting ≥1 TEAE	0	1 (16.7)	2 (33.3)	4 (66.7)	1 (16.7)	1 (16.7)	2 (33.3)	3 (21.4)	14 (25.0)		
Total number of TEAEs	0	1	2	6	1	1	2	3	16		
Subjects reporting a drug-related TEAE	0	0	2 (33.3)	2 (33.3)	0	0	1 (16.7)	2 (14.3)	7 (12.5)		
Total number of drug-related TEAEs	0	0	2	3	0	0	1	2	8		
MAD study											
	10 mg q.d. n = 6	30 mg q.d. n = 6	100 mg q.d. n = 6	300 mg q.d. n = 6	150 mg b.i.d. n = 6	Placebo n = 10	Overall N = 40				
Subjects reporting ≥1 TEAE	2 (33.3)	4 (66.7)	2 (33.3)	2 (33.3)	3 (50.0)	3 (30.0)	16 (40.0)				
Total number of TEAEs	2	10	4	2	4	5	27				
Subjects reporting a drug-related TEAE	0	3 (50.0)	1 (16.7)	0	1 (16.7)	2 (20.0)	7 (17.5)				
Total number of drug-related TEAEs	0	8	1	0	2	3	14				
Subjects reporting a TEAE leading to discontinuation	0	0	0	0	1 (16.7)	0	1 (2.5)				
Total number of TEAEs leading to discontinuation	0	0	0	0	2	0	2				

Note: All data shown as n (%), unless otherwise noted. Abbreviations: b.i.d., twice daily; MAD, multiple ascending dose; q.d., once daily; SAD, single ascending dose; TEAE, treatment-emergent adverse event.

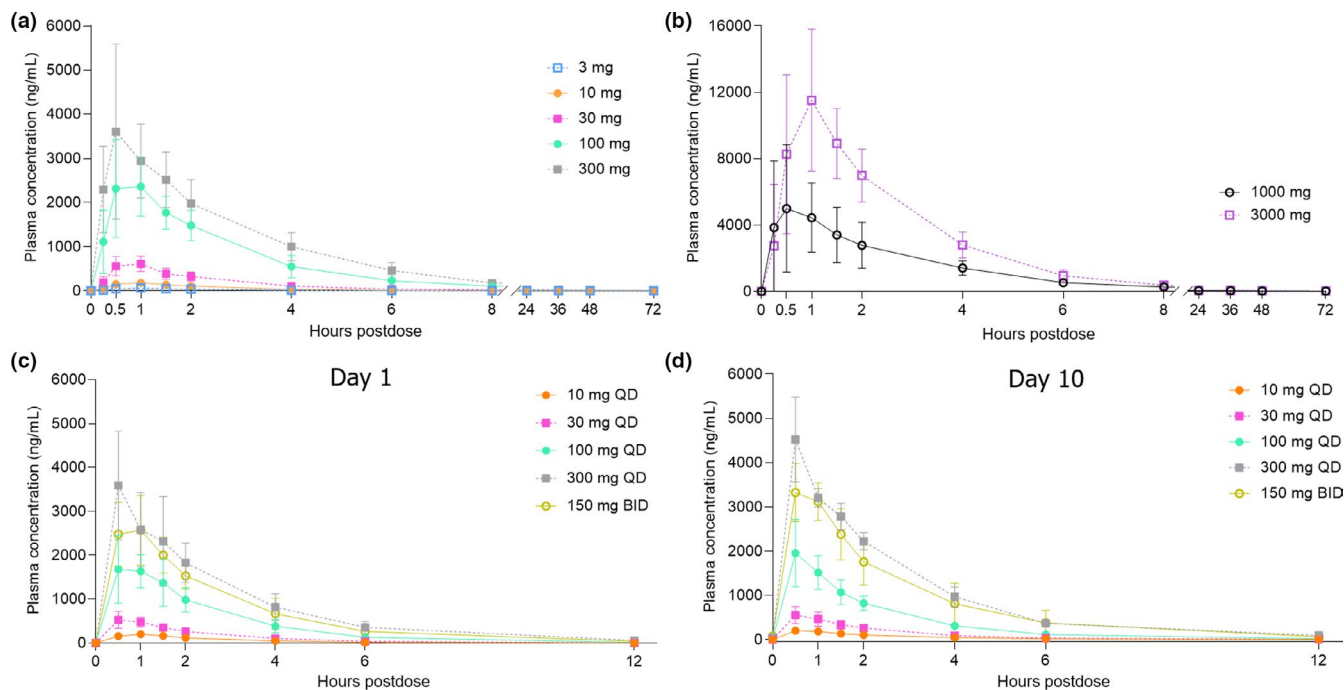


FIGURE 1 Mean (SD) plasma concentration after administration of DS-1211 for (a) and (b) single ascending dose study, (c) multiple ascending dose study day 1, and (d) multiple ascending dose study day 10. Plasma concentration for the multiple ascending dose 150 mg b.i.d. is reported for the first morning dose. BID, twice daily; QD, once daily; SD, standard deviation

(Figure S2). Urinary PEA was highest at the 0- to 4-h time point for all assessed doses; PEA levels remained above baseline through 48–72 h for the 100–3000-mg doses (Table S1). All biomarkers returned close to pre-dose levels within 24 h postdose, except the 1000- and 3000-mg doses, which still had substantially elevated PLP and PEA at the 24-h time point.

Multiple ascending dose

Exposure-dependent ALP inhibition was observed between 0.5 and 6 h postdose on both day 1 and day 10. The maximum decrease in ALP activity occurred in the 300-mg q.d. dose with a maximal mean (SD) change from baseline of -53.5% (8.1) at 1 h postdose on day 1, and -47.4% (10.5) at 0.5 h postdose on day 10 (Figure 4a). There was a dose-dependent increase in PPI across all doses, peaking between 1 and 2 h postdose (Figure 4b); subjects receiving 150 mg b.i.d. had a maximal mean (SD) increase from baseline of 155% (53) and 258% (138) at 2 h postdose on day 1 and day 10, respectively (Figure S3). Similarly, there was a dose-dependent increase in PLP concentration with increasing doses of DS-1211 administered on day 1 and on day 10 (Figure 4c). Concentration of PLP increased after a single dose of DS-1211, with the greatest increase from baseline observed at 6 h postdose in subjects receiving the highest daily dose of 300 mg on day 1. In subjects

receiving 150 mg b.i.d., PLP peaked ~ 14 h after the first daily dose. PLP was still elevated at 12 h after the first 150-mg dose (end of the dosing interval) and at 2 h after the second daily dose; an additional increase was observed, resulting in the daily maximum PLP. The increases in PLP concentration on day 10 were similar to those on day 1 in subjects that received q.d. dosing. In contrast, in subjects that received 150 mg b.i.d., on day 10, there were markedly elevated PLP levels at predose (12 h postdose on day 9) and a reduced response postdose on day 10.

DISCUSSION

These studies evaluated the safety, tolerability, PKs, and PDs of the DS-1211 molecule in healthy adults. The results described here represent the first clinical characterization of a TNAP-specific inhibitor. DS-1211 demonstrated a favorable safety profile and was well-tolerated with no dose-limiting toxicities up to 3000 mg in single oral doses and up to 300 mg q.d. and 150 mg b.i.d. in repeated oral doses for 10 days. In the SAD and MAD studies, DS-1211 systemic exposure increased over 200-fold (dose range of 3–3000 mg) and more than 20-fold (dose range of 10–300 mg/day), respectively. Overall, the increase in exposure was dose-proportional up to ~ 150 mg, with a subsequent increase that was less than dose-proportional. This indicates absorption-limited kinetics of DS-1211

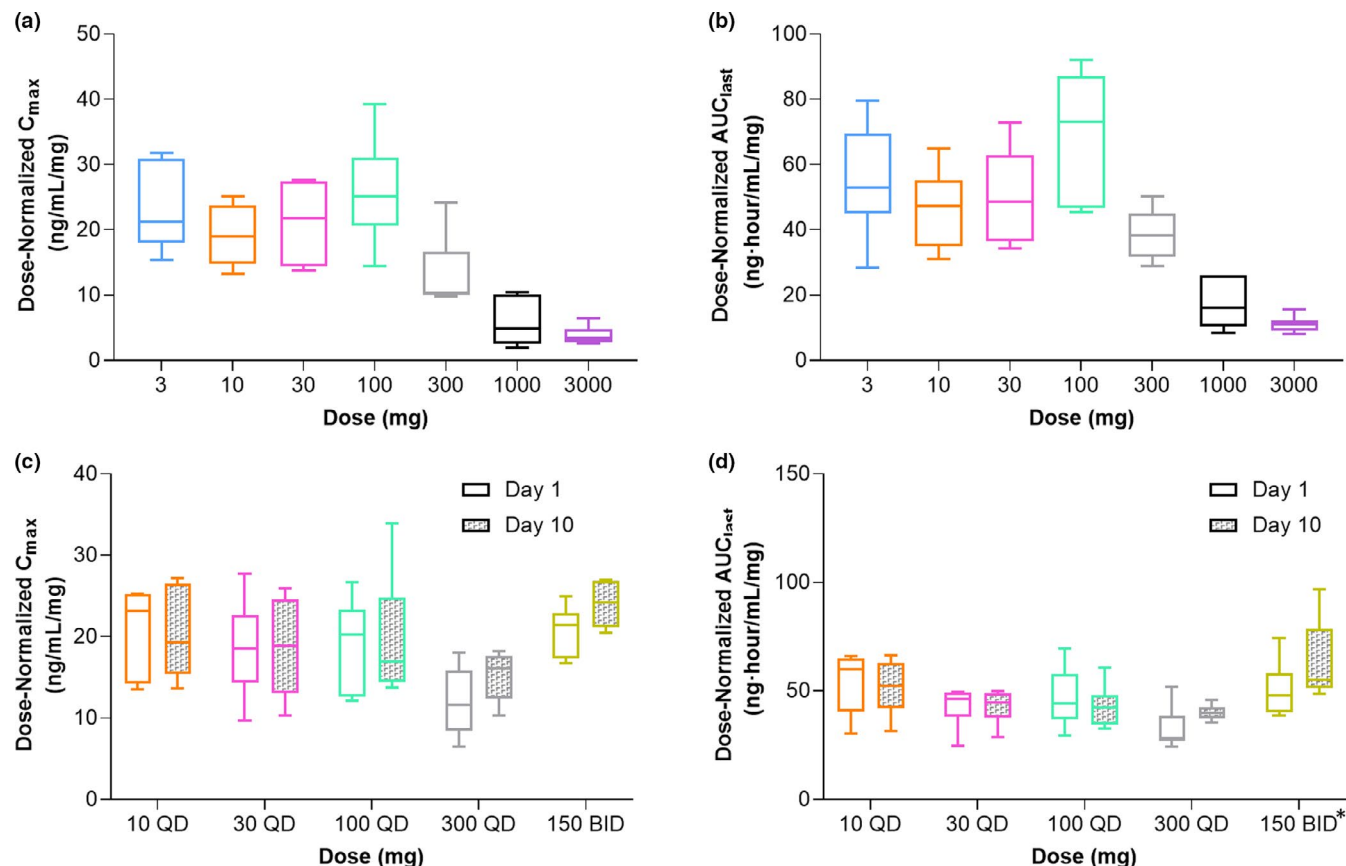


FIGURE 2 Dose-normalized plasma pharmacokinetic parameters of DS-1211 (a) C_{max} of single ascending dose, (b) AUC_{last} of single ascending dose, (c) C_{max} day 1 and day 10 of multiple ascending dose, and (d) AUC_{last} day 1 and day 10 of multiple ascending dose. All doses of DS-1211 shown were received once daily. *Data shown for 150 mg b.i.d. cohort are $2 \times AUC_{tau}$. The last sampling for the q.d. cohorts was 24 h; therefore, AUC_{last} captured the postdose 24-h period and AUC_{last} is reflective of AUC_{tau} in these cohorts. AUC_{last} , area under the curve to last measurable concentration; AUC_{tau} , area under the curve during dosing interval; BID, twice daily; C_{max} , maximum concentration; QD, once daily

following oral administration. PD changes were consistent with the mechanism of action of this TNAP inhibitor, including the dose-ordered inhibition of ALP activities and dose-ordered elevation of the TNAP substrates, PPi, PLP, and PEA.

The wide dose range in the SAD study covered exposures 1/10th to greater than or equal to 100-fold of the exposure at the pharmacological active dose in nonclinical studies (manuscript in preparation). The starting dose of 3 mg was selected for this initial clinical study in humans based on the human equivalent doses calculated by allometric scaling with body surface area from the dose level at which no adverse effects were observed in the most sensitive species, the monkey, from the toxicology assessment. Overall, DS-1211 half-life is short, ~2 h at the dose range of clinical interest (up to 30 mg). After a single dose of DS-1211, ALP activity was inhibited, and the concentration of ALP substrates, plasma PPi, and PLP increased transiently and dose-dependently, consistent with ALP inhibition. Increases in plasma and urinary PEA were also observed. Pharmacological activities of DS-1211, by

inhibition of ALP and increases in PPi and PLP, became observable starting at 10 mg. The time profile of ALP inhibition follows the plasma concentration time profile of DS-1211 with no rebound effect. The activity of ALP and its substrates generally returned toward baseline levels within 24 h postdose, except at the highest 1000- and 3000-mg doses. Consistent with the ALP inhibition at the highest doses, PPi and PLP levels were still elevated after 24 h.

In the MAD study, after repeated daily dosing of DS-1211 in healthy subjects for 10 days, the safety and PK profiles of DS-1211 were as predicted from the SAD study. There was no accumulation of plasma exposure following q.d. administration of DS-1211 10 mg–100 mg and a small degree of accumulation observed with 300 mg q.d. and 150 mg b.i.d. doses. In the MAD study, AUC_{tau} following the morning dose of the 150 mg b.i.d. cohort was used to compare daily plasma exposure and assess dose-proportionality. For this cohort, AUC_{tau} was more appropriate than AUC_{last} , as the limited PK sampling did not accurately capture the PK profile following the evening

TABLE 3 Plasma pharmacokinetic parameters for DS-1211 by dose

	SAD study						
	3 mg q.d. n = 6	10 mg q.d. n = 6	30 mg q.d. n = 6	100 mg q.d. n = 6	300 mg q.d. n = 6	1000 mg q.d. n = 6	3000 mg q.d. n = 6
C _{max} , ng/ml	70 (20)	191 (45)	635 (180)	2580 (809)	3940 (1690)	5790 (3690)	11,500 (4280)
T _{max} , h ^{d,f}	1.0 (0.5–1.5)	1.0 (0.5–1.0)	0.5 (0.5–1.0)	0.8 (0.5–1.5)	0.5 (0.3–1.5)	0.8 (0.3–1.1)	1.0 (1.0–1.0)
t _{1/2} , h	1.4 (0.2)	1.4 (0.2)	2.5 (1.0)	11.5 (9.4) ^a	19.8 (11.5) ^a	38.4 (24.2)	30.8 (13.0) ^d
AUC _{last} , ng • h/ml	165 (52)	465 (122)	1510 (430)	6930 (1980)	11,600 (2420)	17,400 (8010)	33,100 (7670)
	MAD study						
	10 mg q.d. n = 6	30 mg q.d. n = 6	100 mg q.d. n = 6	300 mg q.d. n = 6	150 mg b.i.d. n = 6		
C _{max} , day 1, ng/ml	208 (54)	557 (179)	1903 (565)	3590 (1237)	3102 (462)		
T _{max} , day 1, h ^f	1.0 (0.5–1.0)	0.8 (0.5–1.0)	0.8 (0.5–1.5)	0.5 (0.5–0.5)	0.5 (0.5–1.0)		
t _{1/2} , day 1, h	1.6 (0.2)	1.6 (0.2)	2.2 (0.7) ^c	3.0 (NC) ^e	1.9 (0.2)		
AUC _{last} , day 1, ng • h/ml	540 (141)	1293 (286)	4681 (1375)	9747 (2996)	15,820 (4432)		
C _{max} , day 10, ng/ml	203 (55)	560 (199)	1965 (756)	4555 (889)	3608 (434) ^a		
T _{max} , day 10, h ^f	0.5 (0.5–1.0)	0.5 (0.5–1.0)	0.5 (0.5–1.0)	0.5 (0.5–1.0)	0.5 (0.5–1.0) ^a		
t _{1/2} , day 10, h	1.7 (0.3)	2.8 (1.6) ^b	2.4 (0.5) ^c	NC	2.0 (0.2) ^a		
AUC _{last} , day 10, ng • h/ml	517 (124)	1284 (231)	4274 (986)	11,999 (1100)	18,862 (5392) ^a		

Note: All data shown as mean (SD), unless otherwise indicated.

Abbreviations: AUC_{last}, area under the curve to last measurable concentration; b.i.d., twice daily; C_{max}, maximum concentration; MAD, multiple ascending dose; NC, not calculated; q.d., once daily; SAD, single ascending dose; SD, standard deviation; t_{1/2}, terminal half-life; T_{max}, time to C_{max}.

^aCalculated on the basis of *n* = 5 subjects.

^bCalculated on the basis of *n* = 2 subjects.

^cCalculated on the basis of *n* = 3 subjects.

^dCalculated on the basis of *n* = 4 subjects.

^eCalculated on the basis of *n* = 1 subject.

^fData shown as median (range).

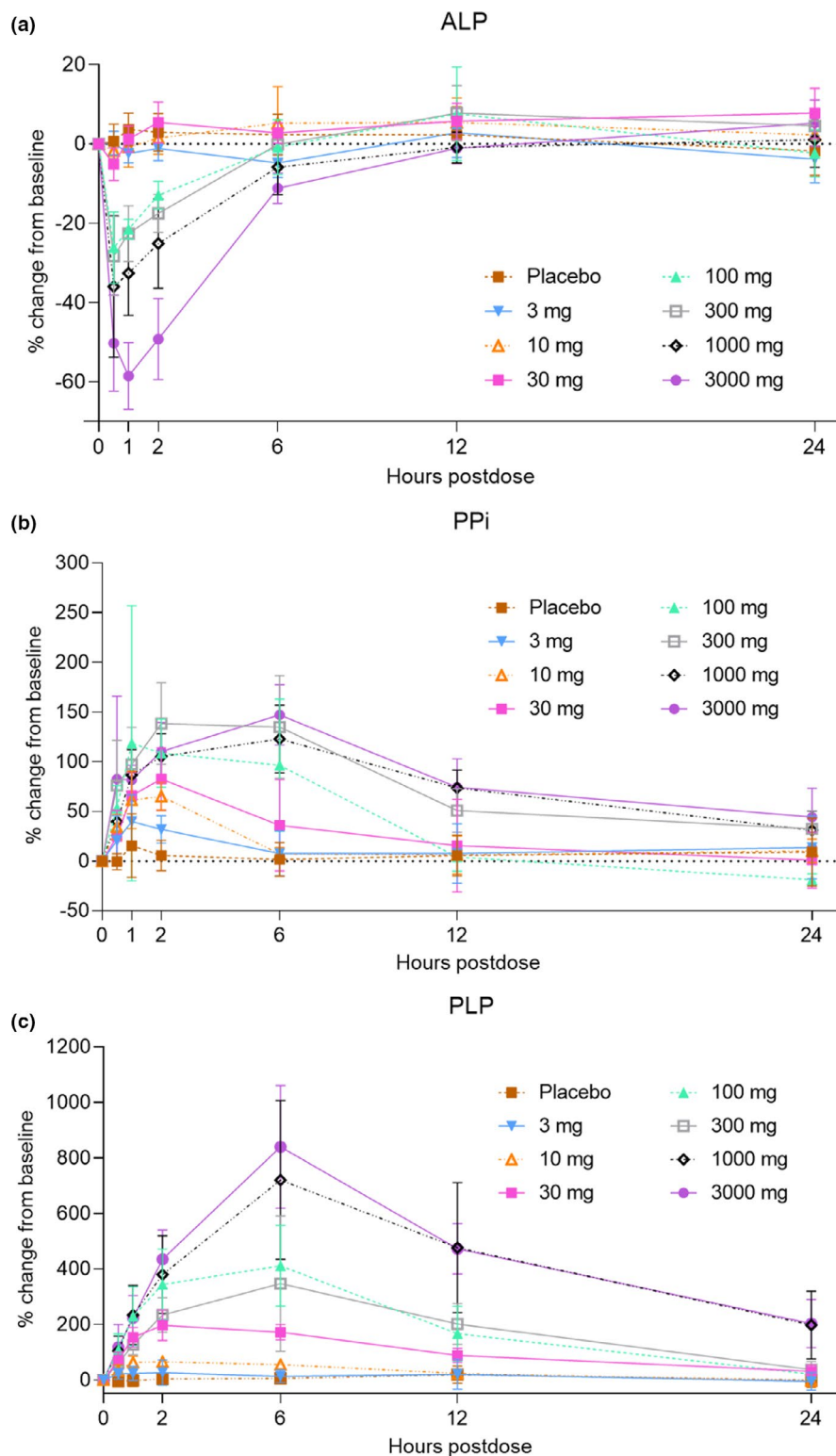
dose during the b.i.d. regimen. AUC_{last} was selected instead of AUC_{tau} for the q.d. cohorts due to some subjects having concentrations measured slightly outside the 24-h window for AUC_{tau}. For the q.d. cohorts, the last blood sample was collected at 24 h; therefore, AUC_{last} captured the postdose 24-h period, and AUC_{last} is reflective of AUC_{tau} in these cohorts. Dose-dependent decreases in ALP activity were observed, with dose-dependent increases in plasma PPI and PLP levels after DS-1211 administration on day 1 and day 10, further confirming the mechanism of action of DS-1211 as a TNAP inhibitor. The activity of ALP and its substrates returned to baseline levels within 24 h post-dose at most doses on day 1 and day 10.

Although there was a wide range of systemic exposure to DS-1211 in both SAD and MAD studies, there was no trend or dose-ordered relationship in the frequency, type, or drug-relatedness of TEAEs across the placebo or DS-1211 treatment groups. Common TEAEs reported in this study were headache, anemia, and constipation, occurring in the placebo group as well and not uncommon in daily living.^{21,22} Further studies should determine potential AEs associated with long-term use in patients.

Markedly reduced PPI is associated with ectopic calcification diseases such as GACI, ACDC, and PXE.^{23,24} Because PPI is hydrolyzed and inactivated by TNAP, inhibition of TNAP is potentially a viable therapeutic approach for treatment of these diseases by increasing endogenous PPI levels. In both the SAD and MAD studies, plasma PPI increased with DS-1211 within 2 h of treatment, reaching a maximal concentration a few hours after the maximal DS-1211 concentration. This treatment-related increase in PPI was not attenuated after repeated dosing over 10 days. Taken together, the observed decrease in ALP activity and increases in PPI, PLP, and PEA are consistent with the mechanism of TNAP inhibition and confirms target engagement in humans. This also indicates potential therapeutic application of DS-1211 in ectopic calcification.

As TNAP accounts for the majority of the circulating ALP activity, plasma ALP measurements can accurately approximate the TNAP activity and its inhibition in lieu of a TNAP-specific assay.^{14,25} However, it should be noted that, in this study, the standardized autoanalyzer ALP assay system required a 51-fold dilution of the plasma sample. Therefore, the reported inhibition is likely an

FIGURE 3 Mean percent change from baseline (SD) in (a) alkaline phosphatase, (b) pyrophosphate, and (c) pyridoxal 5'-phosphate after administration of a single oral dose of DS-1211. ALP, alkaline phosphatase; PLP, pyridoxal 5'-phosphate; PPI, pyrophosphate; SD, standard deviation



underestimation of true inhibition due to dilution of DS-1211 in the assay. Nonetheless, inhibition of TNAP enzymatic activity was consistently demonstrated after the administration of DS-1211 by increases in the endogenous substrates PPI, PLP, and PEA.

Prolonged or excessive inhibition by TNAP inhibitors risks recapitulating the condition of HPP, a rare

mineralization disorder caused by accumulation of PPI in the extracellular matrix.^{5,24} In the SAD and MAD studies, transient decreases in ALP and dose-dependent increases in plasma PPI and PLP levels were consistently observed after DS-1211 administration; PPI returned to baseline level before the next dosing in 30- and 100-mg dose groups, whereas PLP elevations were more pronounced

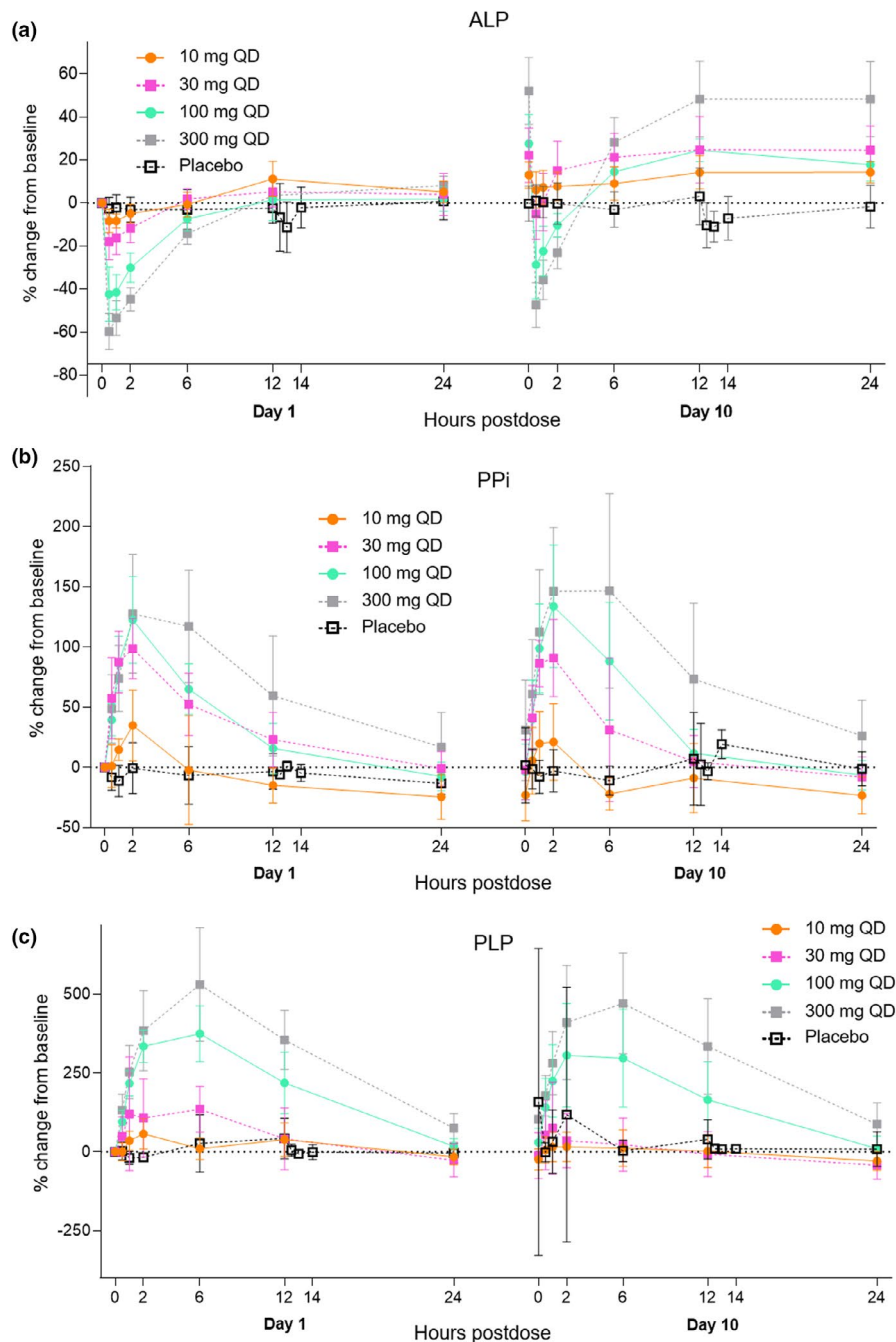


FIGURE 4 Mean percent change from baseline (SD) in (a) alkaline phosphatase, (b) pyrophosphate, and (c) pyridoxal 5'-phosphate after administration of a repeated dose on day 1 and day 10. ALP, alkaline phosphatase; PLP, pyridoxal 5'-phosphate; PPi, pyrophosphate; QD, once daily; SD, standard deviation

and prolonged at 100- and 300-mg dose levels. The PPi levels in PXE patients are reported to be about 50% of that in healthy individuals.^{8,26} Therefore, a two-fold increase from baseline observed in these phase I studies in healthy subjects may produce PPi elevations in PXE patients that are within the normal range. The PPi and PLP data suggest that a dose less than 100 mg may provide a better balance between achieving a sufficient two-fold PPi increase without excessive PLP elevation.

Interpretations of these results are limited by the scope of these clinical studies in healthy subjects for a short duration of up to 10 days, with evaluation of short-term biomarker responses relevant to the TNAP mechanism

of action and TNAP inhibition methodology. The SAD study had a wide range of doses with a small sample size and was not powered as a formal dose-proportionality trial. Plasma half-life in the SAD study varied with dose, which is likely an artifact of sampling time and quantifiable plasma concentrations over the wide dose range using a validated LC-MS/MS method. At low dose levels (≤ 30 mg), plasma concentrations of DS-1211 were detected through only 8–12 h postdose for most subjects, and the elimination phase may not have been well-characterized. At higher doses, plasma concentrations were measurable over a longer sampling period, generating a half-life that may not be clinically meaningful. Half-life is challenging to

accurately assess in first-in-human studies, especially with low sample sizes and wide dose ranges. In this study, $t_{1/2}$ values in the target clinical dose range (10–100 mg) were well-characterized. The estimated $t_{1/2}$ values beyond the dose-proportional range may not reflect the effective half-life, as minimal accumulation was observed during multiple dosing in the subsequent MAD study. Some limitations of the SAD study were addressed by the MAD study, but it still had a small sample size, limiting the strength of comparisons between cohorts. Last, these safety, PK, and PD results were reported from healthy subjects and will need to be further evaluated in patients with ectopic calcification.

CONCLUSIONS

Healthy subjects appeared to tolerate a wide range of doses of DS-1211, and PD data suggests DS-1211 inhibits TNAP activity. The findings of these studies support further investigation of the efficacy and safety of DS-1211 in populations vulnerable to ectopic calcification.

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CONFLICT OF INTEREST

H.S.C. and B.T. are employees of Daiichi Sankyo, Inc. S.M., H.V., H.Z., D.F., and T.L. were employees of Daiichi Sankyo, Inc., at the time the clinical studies were conducted. T.I. is an employee of Daiichi Sankyo Co., Ltd. C.A.Z. and J.G.S. are employees of Worldwide Clinical Trials.

AUTHOR CONTRIBUTIONS

T.I., T.L., H.Z., J.G.S., and H.S.C. wrote the manuscript. S.M., H.V., T.I., H.Z., B.T., and H.S.C. designed the research. S.M., H.V., H.Z., D.F., C.A.Z., J.G.S., and H.S.C. performed the research. S.M., H.V., T.I., T.L., H.Z., B.T., J.G.S., and H.S.C. analyzed the data. All authors reviewed and approved submission of the manuscript.

REFERENCES

- Whyte MP, Landt M, Ryan LM, et al. Alkaline phosphatase: placental and tissue-nonspecific isoenzymes hydrolyze phosphoethanolamine, inorganic pyrophosphate, and pyridoxal 5'-phosphate. Substrate accumulation in carriers of hypophosphatasia corrects during pregnancy. *J Clin Invest.* 1995;95:1440-1445.
- Millán JL. *Mammalian Alkaline Phosphatases: From Biology to Applications in Medicine and Biotechnology.* Wiley-VCH Verlag GmbH & Co.; 2006.
- Whyte MP, Mahuren JD, Vrabel LA, Coburn SP. Markedly increased circulating pyridoxal-5'-phosphate levels in hypophosphatasia. Alkaline phosphatase acts in vitamin B6 metabolism. *J Clin Invest.* 1985;76:752-756.
- St Hilaire C, Ziegler SG, Markello TC, et al. NT5E mutations and arterial calcifications. *N Engl J Med.* 2011;364:432-442.
- Millán JL, Whyte MP. Alkaline phosphatase and hypophosphatasia. *Calcif Tissue Int.* 2016;98:398-416.
- Li Q, Huang J, Pinkerton AB, et al. Inhibition of tissue-nonspecific alkaline phosphatase attenuates ectopic mineralization in the Abcc6(-/-) mouse model of PXE but not in the Enpp1 mutant mouse models of GACI. *J Invest Dermatol.* 2019;139:360-368.
- Harmey D, Hesse L, Narisawa S, Johnson KA, Terkeltaub R, Millán JL. Concerted regulation of inorganic pyrophosphate and osteopontin by akp2, enpp1, and ank: an integrated model of the pathogenesis of mineralization disorders. *Am J Pathol.* 2004;164:1199-1209.
- Jansen RS, Duijst S, Mahakena S, et al. ABCC6-mediated ATP secretion by the liver is the main source of the mineralization inhibitor inorganic pyrophosphate in the systemic circulation—brief report. *Arterioscler Thromb Vasc Biol.* 2014;34:1985-1989.
- Rashdan NA, Rutsch F, Kempf H, Varadi A, Leftheriotis G, MacRae VE. New perspectives on rare connective tissue calcifying diseases. *Curr Opin Pharmacol.* 2016;28:14-23.
- Jin H, St. Hilaire C, Huang Y, et al. Increased activity of TNAP compensates for reduced adenosine production and promotes ectopic calcification in the genetic disease ACDC. *Sci Signal.* 2016;9:ra121.
- Uitto J, Li Q, van de Wetering K, Váradi A, Terry SF. Insights into pathomechanisms and treatment development in heritable ectopic mineralization disorders: summary of the PXE international biennial research symposium-2016. *J Invest Dermatol.* 2017;137:790-795.
- Sheen CR, Kuss P, Narisawa S, et al. Pathophysiological role of vascular smooth muscle alkaline phosphatase in medial artery calcification. *J Bone Miner Res.* 2015;30:824-836.
- Kozlenkov A, Le Du MH, Cuniase P, Ny T, Hoylaerts MF, Millán JL. Residues determining the binding specificity of uncompetitive inhibitors to tissue-nonspecific alkaline phosphatase. *J Bone Miner Res.* 2004;19:1862-1872.
- Narisawa S, Harmey D, Yadav MC, O'Neill WC, Hoylaerts MF, Millán JL. Novel inhibitors of alkaline phosphatase suppress vascular smooth muscle cell calcification. *J Bone Miner Res.* 2007;22:1700-1710.
- Pinkerton AB, Sergienko E, Bravo Y, et al. Discovery of 5-((5-chloro-2-methoxyphenyl)sulfonamido)nicotinamide (SBI-425), a potent and orally bioavailable tissue-nonspecific alkaline phosphatase (TNAP) inhibitor. *Bioorg Med Chem Lett.* 2018;28:31-34.
- Tani T, Fujiwara M, Orimo H, et al. Inhibition of tissue-nonspecific alkaline phosphatase protects against medial arterial calcification and improves survival probability in the CKD-MBD mouse model. *J Pathol.* 2020;250:30-41.
- Ziegler SG, Ferreira CR, MacFarlane EG, et al. Ectopic calcification in pseudoxanthoma elasticum responds to inhibition of tissue-nonspecific alkaline phosphatase. *Sci Transl Med.* 2017;9:eaal1669.
- Miyazaki S, Yamamoto Y, Suzuki K, et al. Pyridoxazinone derivatives as TNAP inhibitors. WIPO Patent No. WO2017007943A1. 2017.

19. Schumann G, Klauke R, Canalias F, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C. Part 9: reference procedure for the measurement of catalytic concentration of alkaline phosphatase International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Scientific Division, Committee on Reference Systems of Enzymes (C-RSE) (1). *Clin Chem Lab Med*. 2011;49:1439-1446.
20. Cabo R, Kozik K, Milanowski M, et al. A simple high-performance liquid chromatography (HPLC) method for the measurement of pyridoxal-5-phosphate and 4-pyridoxic acid in human plasma. *Clin Chim Acta*. 2014;433:150-156.
21. Peery AF, Crockett SD, Barritt AS, et al. Burden of gastrointestinal, liver, and pancreatic diseases in the United States. *Gastroenterology*. 2015;149:1731-41.e3.
22. Global Collaborators Group 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2018;392:1789-1858.
23. Murshed M, Harmey D, Millán JL, McKee MD, Karsenty G. Unique coexpression in osteoblasts of broadly expressed genes accounts for the spatial restriction of ECM mineralization to bone. *Genes Dev*. 2005;19:1093-1104.
24. Russell RG, Bisaz S, Donath A, Morgan DB, Fleisch H. Inorganic pyrophosphate in plasma in normal persons and in patients with hypophosphatasia, osteogenesis imperfecta, and other disorders of bone. *J Clin Invest*. 1971;50:961-969.
25. Azpiazu D, González-Parra E, Egido J, Villa-Bellosta R. Hydrolysis of extracellular pyrophosphate increases in post-hemodialysis plasma. *Sci Rep*. 2018;8:11089.
26. Sánchez-Tévar AM, García-Fernández M, Murcia-Casas B, et al. Plasma inorganic pyrophosphate and alkaline phosphatase in patients with pseudoxanthoma elasticum. *Ann Transl Med*. 2019;7:798.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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