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Lothar Seefried, … , Uwe Junker, Franz Jakob

Clinical Medicine   Bone biology   Clinical trials

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CONCLUSION. BPS804 treatment was well tolerated and resulted in increases in bone formation biomarkers and bone mineral density, suggesting that sclerostin inhibition could be applied to enhance bone mineral density, stability, and regeneration in non-life-threatening clinical situations in adults with HPP.

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Introduction

Hypophosphatasia (HPP) is a rare genetic metabolic disorder (OMIM 171760) caused by mutations in the alkaline phosphatase (ALP) gene *ALPL (1p36.12)*, which encodes tissue-nonspecific ALP (TNSALP) enzyme and results in reduced activity of the enzyme

(1). HPP can be transmitted in both an autosomal recessive and dominant manner. Clinical manifestation is commonly categorized as perinatal, infantile, childhood, adult, or odonto-HPP, according toe age at onset of the disease or mere dental manifestation. The clinical phenotype is more severe in cases with earlier (perinatal/infantile) manifestation, which is frequently associated with mutations on both alleles, causing a compound heterozygous or, more rarely, a homozygous constellation. Several *ALPL* mutations have been demonstrated to elicit a dominant-negative effect, with the gene product of the mutated allele causing sequestration and/or degradation of the WT monomer (2) or inhibiting activity of the WT monomer in the heterodimeric enzyme complex (3, 4). Such mechanisms may cause autosomal dominant inheritance and are supposed to account especially for milder forms of the disease in patients with one affected allele. Usually, a mutation is assumed to be dominant-negative if ALP activity of cells transfected with the mutated cDNA is less than 50% of the activity of cells transfected with the WT cDNA. Still, since there is no convincing genotype/phenotype correlation (5, 6), mapping the *ALPL* mutation is not sufficient to predict severity of the clinical phenotype, and individual manifestations may vary considerably in patients

with comparable residual ALP activity or even identical genotype (5). Consequently, mutations of one allele may elicit relevant clinical problems, especially in challenge situations (7).

As TNSALP is involved in hydrolysis of extracellular phosphate substrates such as inorganic pyrophosphate (PPi) and pyridoxal-5′-phosphate (PLP; the major circulating isoform of vitamin B6) (8, 9), reduced enzymatic activity of TNSALP leads to accumulation of PPi and PLP (10). PPi is a potent mineralization inhibitor. Specifically, enhanced levels of PPi and the associated increase in the PPi/Pi ratio inhibit extravesicular growth of hydroxyapatite crystals, resulting in impaired skeletal mineralization (11, 12). Although deficient *ALPL* activity in HPP has been shown to affect multiple organ systems, such as the kidney, muscles, and the central nervous system, the predominant clinical phenotype in many patients comprises reduced bone quality and stability, and is usually characterized as deficient mineralization or some form of osteomalacia. Still, current investigations imply that besides PPi, PLP, and likely PEA, nucleotides (e.g., ATP, ADP, AMP), the diphosphoryl form of lipopolysaccharides (LPS), and phosphorylated osteopontin are also TNSALP substrates and thus might contribute to the clinical manifestation of the disease (1). Considering the impact of ALP on the dephosphorylation of nucleotides and associated consequences for purinergic signaling, deficiencies in *ALPL* activity in bone may result in a more complex bone phenotype in HPP. Several receptor families with variable ligand affinities bind ATP, ADP, AMP, and adenosine. Deficient *ALPL* activity may sequentially lead to accumulation of P2X/P2Y purinergic receptor agonists and cause deficient formation of the P1 receptor agonist adenosine (1). This might result in impaired bone formation, as the phenotype of *P2Y2* (ATP-binding) receptor–KO mice showed increased bone mass, whereas *P2Y13* (ADP-binding) receptor– and adenosine receptor–KO mice showed low bone mass with altered osteoblast differentiation and osteoclast activation (13). If these complex phenotypes are considered together, with a focus on ALP activity, it could be hypothesized that substrate accumulation, notably PPi, inhibits bone mineralization and that ADP/adenosine deficiency further contributes to compromised bone formation with a low-turnover bone phenotype (13–17).

Common disease manifestations of HPP in adults are, in particular, metatarsal and femoral stress fractures or pseudofractures, pathological fractures after minimal trauma, muscle and joint pain, and osteomalacia (18, 19). However, the incidence and clinical manifestation of the latter may also be complicated by an additional vitamin D deficiency, which is frequently seen in HPP patients (20). In addition to the above clinical symptoms, the diagnosis of HPP is based on low serum ALP enzyme activity, high endogenous levels of TNSALP substrates, and ultimately mutations in the *ALPL* gene (9, 18).

Based on the hypotheses discussed above, the combined deficiency of mineralization and regeneration capacity in HPP could be ameliorated by bone anabolic and/or enzyme replacement treatment strategies. Recently asfotase alfa (Strensiq, Alexion), a bone-targeted enzyme replacement therapy, was approved for long-term treatment of pediatric-onset HPP in the United States, Europe, Canada, and Japan. In clinical trials in patients with serious or life-threatening perinatal/infantile onset of the disease, asfotase alfa treatment resulted in superior survival as compared

with a historical cohort, along with improved skeletal mineralization and significant reductions in the TNSALP substrates PPi and PLP (21, 22). However, access to this enzyme replacement therapy is limited by regulatory specifications.

Following the first description of this option in 2007 (23), treatment with recombinant human parathyroid hormone (PTH) analogs (teriparatide, FORTEO, Eli Lilly and Co.; and PTH 1-84, Preotact, Nycomed) has been shown to improve mobility and reduce bone pain, increase bone formation biomarkers, and accelerate fracture healing in individual cases of HPP (24, 25). However, available literature pertaining to that option is limited to a total of 8 individual cases, and thus far, no prospectively structured, comprehensive evaluation is available for such an approach. Moreover, these effects were not sustained over time (26) and could not be reliably reproduced in every instance (27). Still, when focusing on challenging clinical situations in adulthood, such as bone marrow edema and fragility fracture healing, there is an urgent clinical need for therapeutic options, as suitable treatment modalities are limited.

Sclerostin is a novel drug target for the treatment of metabolic bone disorders (28, 29). The protein is encoded by the *SOST* gene, is expressed by osteocytes, and downregulates osteoblastic bone formation by inhibiting the Wnt signaling pathway. Sclerostin prevents the LRP5 receptor from interacting with the frizzled receptor, thus disabling osteoanabolic, canonical Wnt signaling (28). A high bone mass phenotype has been reported in *Sost*-KO mice, and similar symptoms are observed in sclerosteosis, a disease in humans caused by sclerostin deficiency (30). Recently, we and others have demonstrated that sclerostin prevents LRP5 binding to frizzled receptors and that transgenic expression of inhibitors of canonical Wnt signaling (e.g., sclerostin and kremen-2) causes pathology and impairs fracture healing (31–33). We also found that in skeletal precursor cells harvested from osteoporotic patients, sclerostin expression is prematurely enhanced and may be part of the complex pathology of primary osteoporosis (34). In preclinical studies in mice and non-human primates, anti-sclerostin monoclonal antibodies have led to significant increases in bone mineral density (BMD) and bone formation biomarkers and also beneficial effects on fracture healing (28, 35). Moreover, in clinical trials, treatment with humanized sclerostin monoclonal antibodies in healthy volunteers and postmenopausal women with low BMD was shown to increase lumbar spine BMD (LS-BMD) and bone turnover biomarkers such as procollagen type-I N-terminal propeptide (PINP), bone-specific ALP (BSAP), and osteocalcin (OC); and decrease serum C-telopeptide of type I collagen (CTX-1) levels (28, 36).

BPS804 is a high-affinity, fully human, neutralizing, anti-sclerostin monoclonal antibody that has demonstrated potent in vitro activity and efficacy in animal models of osteoporosis. In healthy postmenopausal osteopenic women, single i.v. administrations of BPS804 significantly increased biomarkers of bone formation and BMD (unpublished observations; ClinicalTrials.gov NCT01406548). We hypothesized that treatment with BPS804 in adult patients with HPP would improve bone formation and increase BMD along with upregulation of serum TNSALP and BSAP levels through the stimulation of osteoblast activity by sclerostin antagonism. The present study was
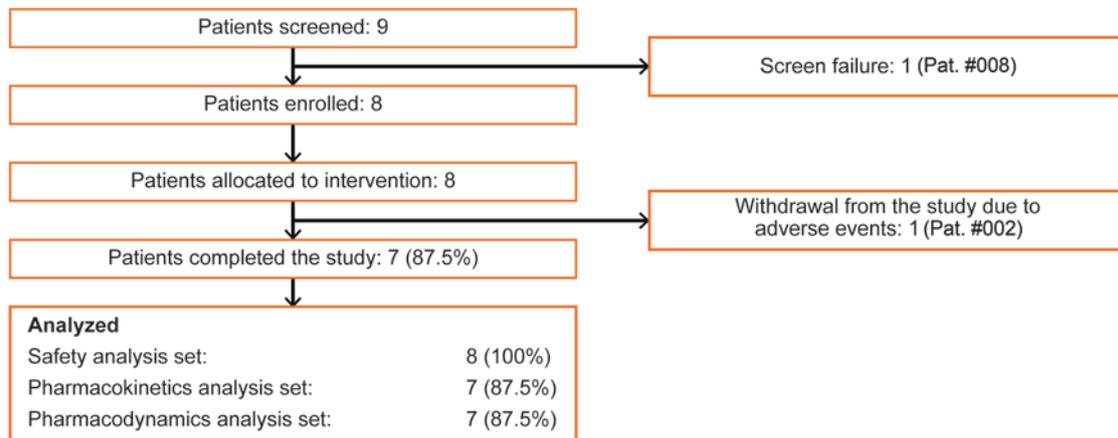
Figure 1. Consolidated Standards of Reporting Trials (CONSORT) diagram. A total of 9 patients were screened for the study. Only 8 patients were enrolled in the study, as 1 patient did not meet the study entry criteria. Of the 8 patients, 7 completed the study as per the protocol. One patient discontinued the study due to SAEs of angina pectoris and dyspnea. All patients who received the study drug were included in the safety analysis set; patients with evaluable pharmacokinetic (PK) parameter data were included in the PK analysis set; and patients with evaluable pharmacokinetic (PD) parameter data were included in the PD analysis set.

designed to assess the pharmacodynamics (PD), pharmacokinetics (PK), preliminary efficacy, and safety of BPS804 in adult patients with HPP.

Results

Of 9 patients screened, 8 with a mean age of 47.8 years (range 21–69) and a mean body mass index of 25.5 kg/m² were eligible, and 7 patients completed the study (Figure 1). Baseline demographics, disease characteristics, and *ALPL* genotype of patients enrolled are summarized in Table 1. All patients had baseline ALP and BSAP levels below normal age- and sex-matched levels.

Patient characteristics. The study cohort comprised patients harboring one as well as those with two mutations. Five participants (nos. 1, 4, 5, 8, and 9) were compound heterozygous with two distinct mutations, while 4 patients (nos. 2, 3, 6, and 7) had only one mutation. In patient 8, screening procedures revealed an additional illness requiring near-term surgical intervention and precluding her further participation. Patient 2 was discontinued

early during first dosing due to serious adverse events (SAEs; angina pectoris and dyspnea) detailed under *Safety* below.

In patients 3 and 6, the documented mutation was previously shown to elicit a dominant-negative effect (6). Patient 6 additionally carried a polymorphism in exon 12, c.1565T>G; p.V522A. The p.K99N mutation in patient 7 was not reported previously, but the nearby mutation, p.T100M, was already described to elicit a dominant-negative effect with 28.2% residual ALP activity (see details in Table 1).

From a clinical perspective, 3 of the patients screened (nos. 4, 5, and 8)—all with a compound heterozygous genotype—had been previously diagnosed with childhood HPP. In all other participants, symptoms were not interpreted in the context of HPP until adult age, although retrospectively all of them reported symptoms in childhood (e.g., fractures, muscular weakness, early loss of deciduous teeth), suggesting, but not proving, earlier onset of the disease.

There was no observation of a more severe clinical phenotype in patients with two mutations as compared with those with a sin-

Table 1. Patient baseline demographics and disease characteristics

Patient no.	Sex	Age (yr)	ALP[A] (U/l)	BSAP[B] (mU/ml)	*ALPL* genotype (% WT)
1	Female	57	12	11.60	Exon 6: c.535G>A; p.A179T (NA), exon 6: c.571G>A; p.E191K (79.5)
2	Female	43	20	11.76	Exon 10: c.1171C>T; p.R391C (50.5)
3	Female	57	26	11.90	Exon 11: c.1250A>G; p.N417S (26.5)
4	Male	21	7	6.68	Exon 4: c.203C>T; p.T68M (NA), exon 6: c.526G>A; p.A176T (58)
5	Male	26	8	5.25	Exon 6: c.542C>T; p.S181L (NA), exon 9: c.920C>T; p.P307L (NA)
6	Female	61	25	11.85	Exon 11: c.1250A>G; p.N417S (26.5)
7	Female	48	16	8.66	Exon 4: c.297G>C; p.K99N (NA) (p.T100M = 28.2%)
8	Female	43	NA	NA	Exon 5: c.379A>G; p.T127A (NA), exon 6: c.526G>A; p.A176T (58.0)
9	Female	69	19	16.64	Exon 6: c.571G>A; p.E191K (79.5), exon 10: c.1001G>A; p.G334D (40.0)

The table presents baseline demographics and underlying genotype of HPP in each patient. [A]ALP lower limit of normal: female >35; male >40. [B]BSAP lower limit. %WT, ALP activity of cells transfected with mutated cDNAs, expressed as a percentage of the activity found in cells transfected with WT cDNA, according to Fauvert et al. (6) and the Tissue Nonspecific Alkaline Phosphatase Gene Mutations Database (http://www.sesep.uvsq.fr/03_hypo_mutations.php). NA, not available.
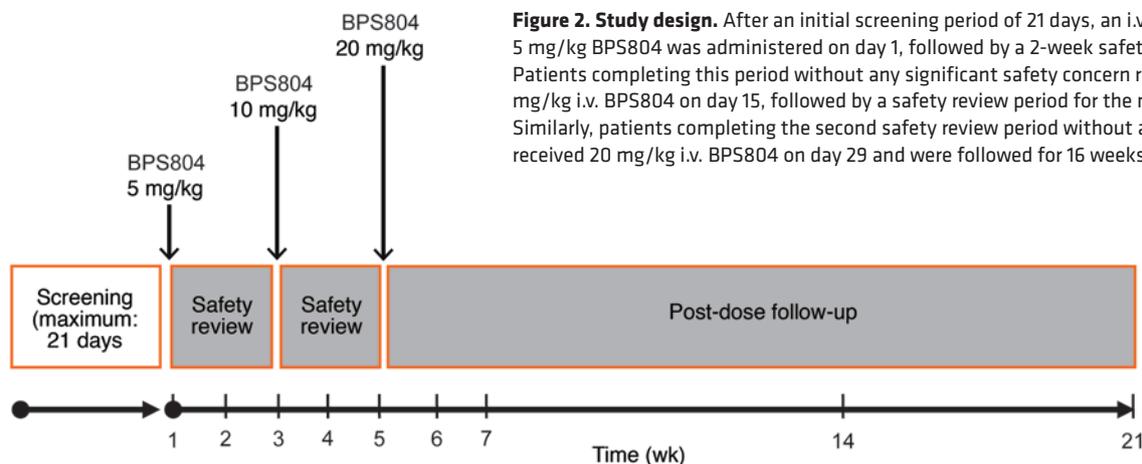
Figure 2. Study design. After an initial screening period of 21 days, an i.v. dose of 5 mg/kg BPS804 was administered on day 1, followed by a 2-week safety review. Patients completing this period without any significant safety concern received 10 mg/kg i.v. BPS804 on day 15, followed by a safety review period for the next 2 weeks. Similarly, patients completing the second safety review period without any concern received 20 mg/kg i.v. BPS804 on day 29 and were followed for 16 weeks.

gle mutation. However, there was a tendency toward lower baseline ALP values in those patients carrying two mutations.

Efficacy outcomes. Treatment with BPS804 with 3 ascending dose levels of 5, 10, and 20 mg/kg given i.v. on days 1, 15, and 29, respectively (Figure 2), resulted in a variable increase in ALP and BSAP enzymatic activity during the study (Figure 3). The mean ALP and BSAP enzymatic activity substantially increased from day 2 to 29 after the third infusion as compared with baseline values. The average ALP enzymatic activity increase from baseline was between 27% and 48% during days 2–29 after the third infusion of BPS804. A maximum increase in BSAP enzymatic activity levels ranging from approximately 8 to 20 mU/ml was observed during the course of the study. In 2 participants (patients 4 and 5), BSAP values were particularly low and intermittently below the limit of quantification (LOQ). The average increase in the area under the curve from baseline through days 2–29 was 37% for ALP enzymatic activity and 30% for BSAP enzymatic activity (Figure 3, A and B).

The HPP-associated disease biomarkers PLP and phosphorylethanolamine (PEA) showed large inter-individual variability. Following BPS804 administration, PLP levels temporarily and reproducibly dropped in those 2 participants with excessively elevated baseline values (patients 4 and 5). PLP values in those patients with only slightly increased values at baseline did not show a reproducible

decline. Likewise, BPS804 administration did not elicit a conclusive response in PEA values over time. The individual plots for PEA and PLP values over time, with ratio from baseline for all patients, are presented in Figure 3, C and D.

Treatment with BPS804 transiently increased the bone formation markers PINP and OC, with maximum mean increases of 101% (on day 43) and 92% (on day 43), respectively. Concomitantly, PTH levels increased by 60% (on day 57), and a decrease in the bone resorption marker CTX-1 was observed after BPS804 administration, with a maximum decrease of 35% on day 36 (Figure 4). Lumbar spine BMD (LS-BMD; dual-energy X-ray absorptiometry [DXA]) obtained at baseline, day 85, and end of study (EoS) showed a consistent increase. As compared with baseline, mean values on day 85 and EoS increased by 3.0% and 3.9%, respectively (Table 2). The BMD increase at EoS was most pronounced in those 3 patients (nos. 1, 4, and 5) with the lowest ALP and highest PLP baseline levels. In these patients, there was a reproducible decrease in PLP levels upon administration of BPS804.

PK. The mean plasma concentration–time profile following infusion of each dose of BPS804 is presented in Figure 5A. Serum BPS804 concentrations were quantifiable in all of the patients throughout the treatment phase. A dose-proportional increase in maximum serum concentration (C_{max}) was observed across 5 mg/kg (158 ± 36 μg/ml), 10 mg/kg (314 ± 71 μg/ml), and 20 mg/kg (679 ± 140 μg/ml). Peak plasma concentration of BPS804 was reached approximately 2 hours after each infusion.

A minimal carryover of PK exposure from the first and second doses was observed on the last dose of 20 mg/kg. Exposure following the last dose of 20 mg/kg (area under the plasma concentration–time curve from 0 to the last quantifiable concentration [AUC_{last}] was ~8,080 day μg/ml), or about 30% higher compared with the 20 mg/kg (AUC_{last} ~6,148 day μg/ml) dose level tested in the single ascending dose study in healthy subjects with low BMD. The terminal elimination half-life ($t_{1/2}$) following the last dose (20 mg/kg) averaged 11.8 days, consistent with the observed data from other clinical trials with BPS804. The

Table 2. Lumbar spine BMD

Patient no.	LS-BMD (mg/cm²)/z-score			Change from baseline (%)	
	Baseline	Day 85	EoS	Day 85	EoS
1	980/−1.0	1,033/−0.5	1,053/−0.3	5.4	7.4
2	991/−1.1	NP[A]	NP[A]	–	–
3	903/−1.6	924/−1.4	927/−1.4	2.3	2.7
4	991/−1.6	1,075/−0.8	1,055/−0.9	8.5	6.5
5	917/−2.4	955/−1.9	975/−1.8	4.1	6.3
6	1,087/0.1	1,080/0.1	1,119/0.4	−0.6	2.9
7	1,019/−0.8	1,011/−0.8	1,025/−0.7	−0.8	0.6
9	1,512/4.5	1,548/4.8	1,527/4.6	2.4	1.0
Mean				3.0	3.9

BMD z-scores indicate the relationship of individual BMD values to sex- and age-adjusted normal BMD values and are expressed as above or below the mean by number of SDs. [A]LS-BMD was not assessed, as patient 2 was discontinued early from the study. NP, not performed.
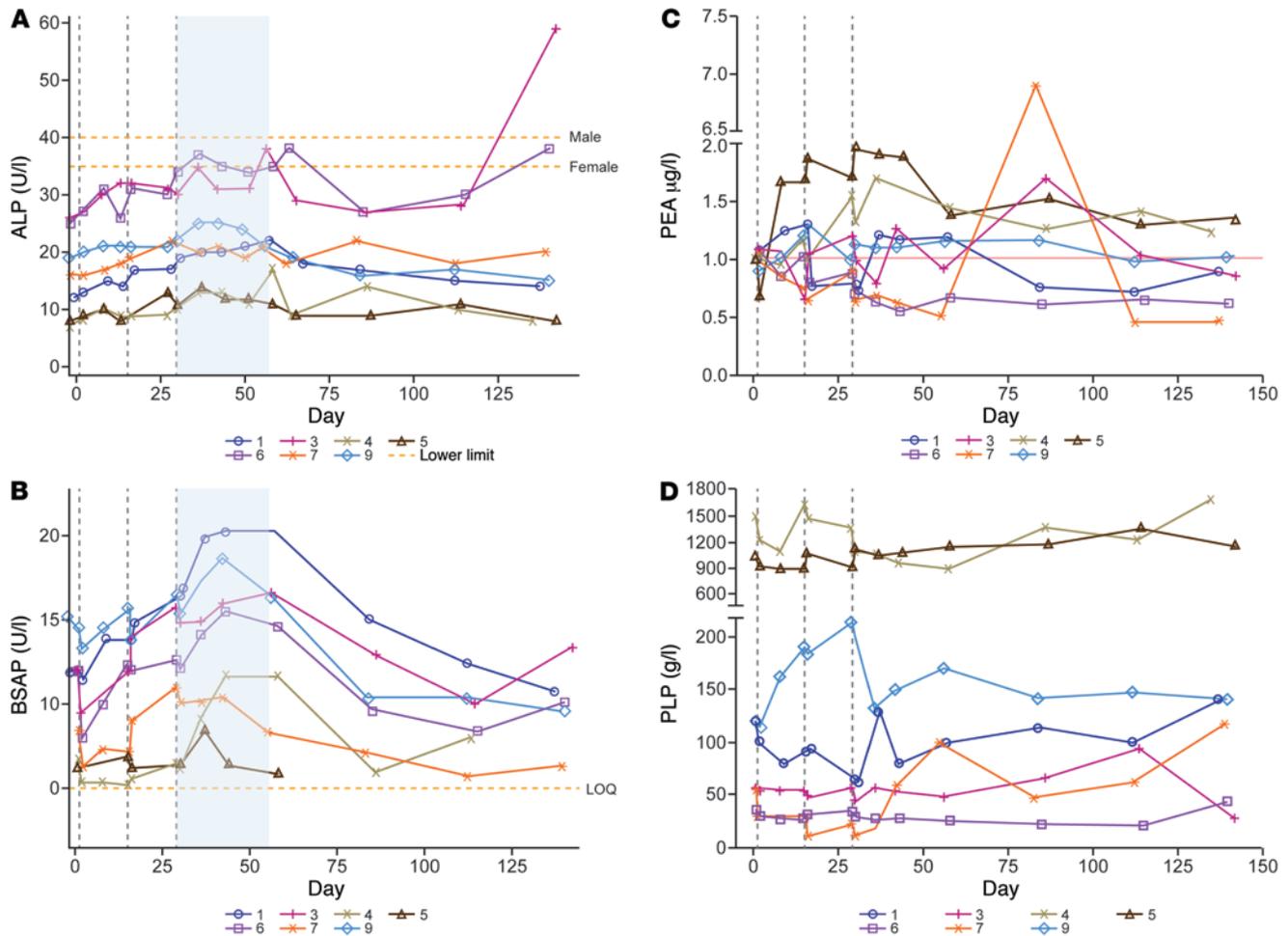
Figure 3. Plots of individual patient PD data over time. After administration of BPS804, mean ALP enzymatic activity (**A**) increased between days 2 and 29 after the third infusion compared with baseline. (**B**) Similarly, for BSAP, the enzymatic activity increased between days 2 and 29 after the third infusion compared with baseline. Individual BSAP values for patient 4 (screening, EoS) and patient 5 (days 1, 2, 8, 85, and 133 and EoS) were below the LOQ and are not represented in the graph. (**C**) PEA levels showed inter-individual variability; thus, changes during treatment are given as ratio versus individual baseline. (**D**) PLP values showed large intra- and inter-patient variability. A consistent decline upon treatment was seen in patients 4 and 5, who had the highest baseline values. Vertical lines indicate time of infusion (days 1, 15, and 29). Shaded area indicates the time period of days 2–29 after the third infusion. Horizontal lines indicate lower normal limit for ALP by sex in **A**, LOQ in **B**, and normalized baseline in **C**. The y axis in **C** and **D** is broken to include outliers and still clearly visualize lower individual PEA/PLP levels.

intra-patient variability in PK in this study was ~20% and is similar to that observed between patients in earlier studies.

PK/PD correlation. Levels of total sclerostin measured by the chosen assay include free sclerostin as well as the sclerostin-antibody complex formed after dosing of BPS804, i.e., levels indicate a sclerostin capture effect. Accordingly, total sclerostin in serum showed a dose-dependent increase, with maximum levels observed at each dose of 5, 10, and 20 mg/kg (Figure 5A). Only a slight difference in the peak sclerostin level was observed between 10 and 20 mg/kg. The peak PD effect on serum sclerostin lagged by approximately 2–4 weeks behind the BPS804 PK time course (Figure 5B).

Safety. In total, 50 adverse events (AEs) were reported by 6 patients during the course of the study. The most frequently reported AEs were related to musculoskeletal and connective tissue disorders (Table 3), including pain in extremity (*n* = 3; 37.5%), arthralgia (*n* = 2; 25.0%), and bone pain (*n* = 2; 25.0%). One patient (no. 2) reported dyspnea and symptoms of angina pectoris during the first administration of BPS804. The infusion was immediately discon-

tinued. The patient was hospitalized for further surveillance and clinical work-up, which fulfilled the criteria of an SAE. Thorough and extensive cardiorespiratory work-up did not reveal any abnormalities that could be attributed to the treatment. Considering the patient's emerging anxiety during the infusion and the fact that only a minimal amount of BPS804 was administered, the investigators concluded that the SAE was unrelated to the study drug. The SAE resolved during the course of hospitalization, and the patient was discharged without sequelae. Only one patient experienced AEs that were considered drug-related, comprising eye swelling, myalgia, pain in extremity, and unspecific paresthesia. These as well as all other AEs were mild in intensity, transient in nature, and resolved without further treatment. No deaths were reported during the study or follow-up period. No clinically significant abnormalities of hematological parameters, clinical chemistry, urinalysis, or vital signs were reported. Particularly, there were no clinically significant changes in serum calcium levels. ECG monitoring was conducted repeatedly throughout the course of the trial, and no clinically sig-
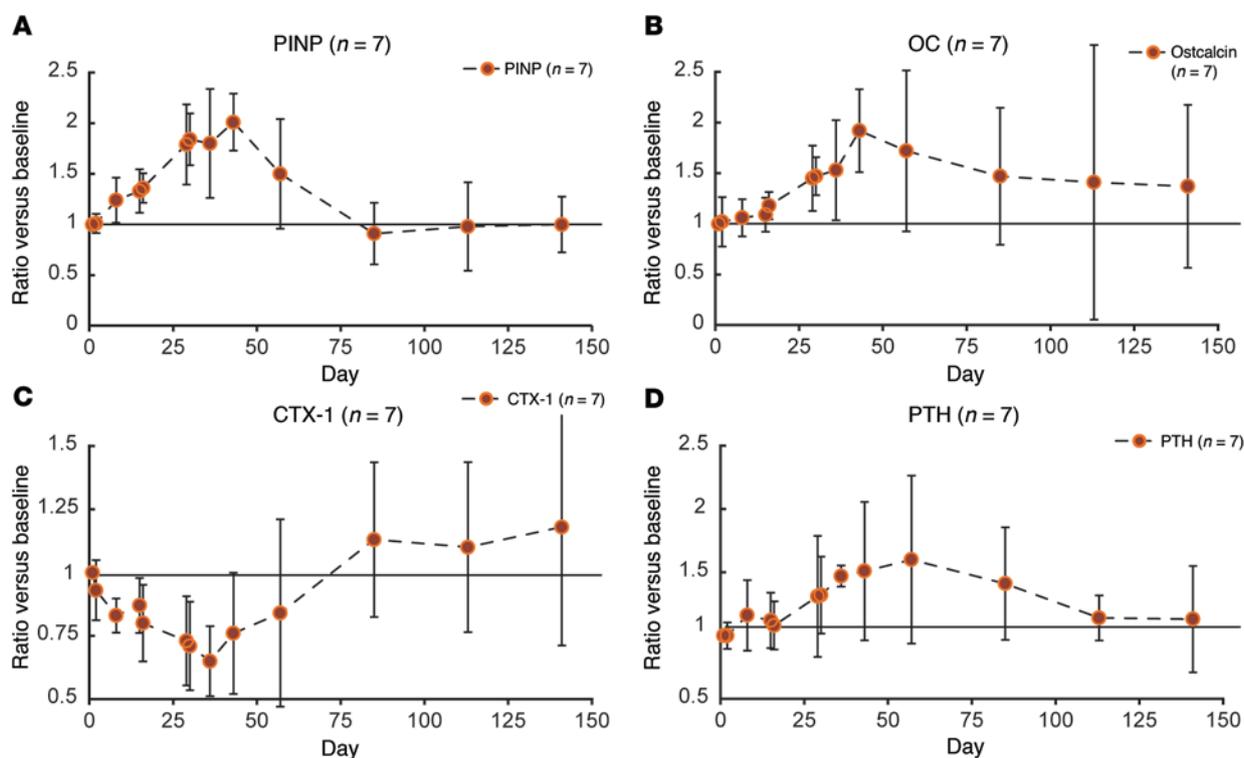
Figure 4. Bone turnover markers. Geometric mean plots of bone biomarkers. After infusion of BPS804, transient increases in bone formation markers (**A**) PINP (maximum 101% on day 43) and (**B**) OC (maximum 92% on day 43) were detected. (**C**) There was a concomitant transient decrease in the bone resorption marker CTX-1 (maximum –35% on day 36). (**D**) The bone formation biomarker PTH also showed an increase (maximum 60% on day 57) in the study.

nificant ECG changes were noted. In one patient, the QTcB interval was marginally prolonged on day 15 and day 30 by 459 ms and 463 ms, respectively (heart rate corrected QT interval [QTcB] normal range 354–448 ms), without clinical significance. All other ECG interval values were within normal ranges.

Immunogenicity (i.e., the presence of anti-BPS804 antibodies) was positive in 7 serum samples from 3 patients among 30 samples from 8 patients in the screening assay; however, the antibody levels were below the lower LOQ (LLOQ; <250 ng/ml) in the confirmatory assay.

Discussion

Currently, specific medical treatment options for HPP are limited to bone-targeted enzyme replacement therapy (asfotase

Table 3. Adverse events

AE[A]	Total, N = 8 [n (%)]
Pain in extremity	3 (37.5%)
Arthralgia	2 (25.0%)
Bone pain	2 (25.0%)
Headache	2 (25.0%)
Myalgia	2 (25.0%)
Nasopharyngitis	2 (25.0%)
Toothache	2 (25.0%)

[A]There were 6 (75.0%) patients with AE(s). N, total number of patients; n, number of patients who experienced the AE.
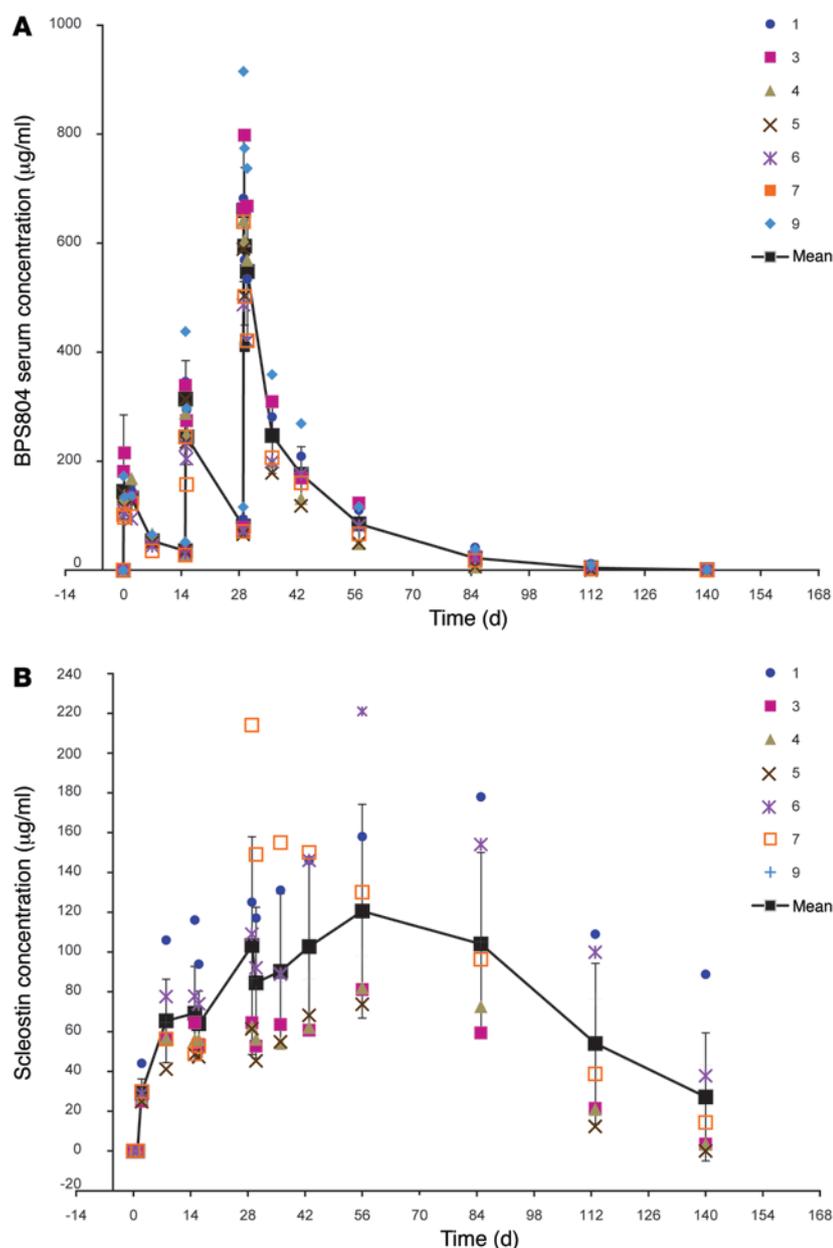
alfa, Strensiq, Alexion), approved for pediatric-onset HPP (22, 37). However, there is only limited evidence demonstrating its efficacy in adults with mild and/or late-onset forms of HPP, and in particular patients with adult onset of the disease are not covered by the label. Such patients, according to our experience, can develop secondary osteoporosis, bone marrow edema, and delayed fracture healing or difficulties with implant failure. Since these patients do not qualify for enzyme replacement therapy and — due to the pathophysiology of HPP — at the same time cannot appropriately be treated with common antiresorptives, there is an urgent need for identifying further bone-targeted treatment options.

Considering the fact that besides substrate accumulation and deficient mineralization, HPP in these patients is also marked by low bone turnover, we hypothesized that osteoanabolic treatment with an anti-sclerostin monoclonal antibody might stimulate bone formation and regeneration and enhance bone mass, thereby improving bone stability in these patients. Therefore, we initiated this exploratory proof-of-concept study to investigate the safety and efficacy of BPS804 in adult HPP patients. The hypothesis incorporated the assumption that HPP is not only a syndrome of mineralization deficiency owing to substrate accumulation but also causes deficits in bone formation (1, 38). Accordingly, this approach primarily addresses the issue of reduced bone turnover rather than deficient mineralization due to substrate accumulation. Sclerostin is an inhibitor of osteogenic Wnt signaling, as discussed above (28, 29, 33). The efficacy of enabling bone formation by targeting sclerostin using highly specific monoclonal antibod-

Figure 5. BPS804 serum concentration– and total sclerostin concentration–time profiles. (A) BPS804 mean serum concentration–time profile (linear plot). Peak serum concentrations were 158, 314, and 679 μg/ml after i.v. administration of 5, 10, and 20 mg/kg BPS804, respectively, with a time to maximum concentration of 2 hours. **(B)** Total sclerostin serum concentration–time profile (linear plot). Since the assay captures both free and antibody complex–bound sclerostin, values increased following each dose of 5, 10, and 20 mg/kg BPS804. Arithmetic mean (SD) and serum concentrations by each patient at different time intervals are presented.

patients with healthy bone metabolism (39). We started by examining the absolute effects of *ALPL* stimulation, as very low ALP is a pathognomonic sign of HPP (40), and found that the increase in absolute ALP levels was well below that seen in healthy subjects. Similar to the results of anti-sclerostin treatment in osteoporosis, there was a broad individual range of therapeutic responses. However, if roughly calculated as a percentage change over baseline, the increase reached more than 100%. This may indicate that the molecular defects basically cannot be overcome by anti-sclerostin treatment, but bone formation can be enhanced despite the serious deficit in a central mineralizing enzyme, at least in patients with residual ALP activity. This notion is supported by the substantial stimulation of PINP, which is a surrogate for new bone formation, and indicates that inhibition of sclerostin signaling can allow for increased osteoblast activity even in a state of altered osteoblast function, and the fact that this stimulation translates into a considerable rise in BMD supports this hypothesis. However, in terms of bone turnover markers and presumably concerning BMD as well, the positive effect is only transient, suggesting that the mechanism might be beneficial to support bone formation and regeneration in well-defined critical periods. Due to the exploratory nature of this trial — focusing on safety, PD, and PK in a small number of HPP patients — it is premature to make a firm statement about long-term safety and whether the observed transient stimulation of bone formation will result in enhanced fracture resistance. Based on the case reports for teriparatide (rhPTH1-34, Forteo, Eli Lilly), one could assume that the response to bone anabolic treatment may vary among patients with HPP depending on their residual ALP enzymatic activity; this again might depend on the type of mutation and different mechanisms leading to decreased ALP activity, e.g. reduced quantity, defective catalytic activity, or instability of the enzyme. This again is associated with the localization of the mutation(s) within the different functional domains of the enzyme (41) and the question of a compound heterozygous constellation versus dominant-negative expressivity (17).

Considering this aspect for the participants of this trial, there was a tendency toward lowering ALP enzymatic activity in those patients carrying two mutations, i.e., patients 1, 4, 5, and 9 all had baseline ALP values <20 U/l. However, following dosing, there was no obvious difference in the extent of the ALP increase when comparing compound heterozygously affected patients with those harboring only a single mutation. Moreover, there was no correlation between baseline ALP/BSAP activity and the extent of ALP/

ies has already been demonstrated in preclinical and phase I/IIB clinical trials and is now being studied in phase III trials (36).

The present study provides the first clinical evidence to our knowledge for the treatment of adult HPP patients with an anti-sclerostin antibody. In this study, 3 single ascending doses (5, 10, and 20 mg/kg) of BPS804, an anti-sclerostin monoclonal antibody, consistently elevated ALP and BSAP enzymatic activity, increased levels of bone formation markers and LS-BMD, and reduced the bone resorption marker CTX-1 in adult HPP patients. These results are qualitatively similar to what was observed in

BSAP increase following treatment. From a clinical perspective, there was no observation of a more severe phenotype in those patients with two mutations as compared with those with one mutation. Although baseline BMD values did not correlate with genotype or residual ALP activity, BMD accrual upon treatment was highest in those compound heterozygous patients with lowest ALP and highest PLP baseline values (nos. 1, 4, and 5) and substantial PLP decrease upon BPS804 administration, suggesting that inhibition of sclerostin might also be effective in severely affected patients with particularly low residual ALP activity (≤12 U/l). Assuming that PLP decline upon BPS804 administration in these patients accompanies a decrease in the mineralization inhibitor PPi, this could suggest that besides addressing bone formation via the Wnt signaling pathway, BPS804 might have the potential to directly modify compromised mineralization in HPP.

Although still preliminary, this trend appears distinct from what was previously described in case studies reporting osteoanabolic treatment with teriparatide in HPP patients, where treatment efficacy was most pronounced in patients with relevant residual ALP activity (23, 40, 42). Hence, it remains to be investigated in larger trials whether the genotype and/or a dominant-negative activity of mutant proteins are predictive of the efficacy of the treatment.

The HPP-associated disease biomarkers PEA and PLP, as expected, showed large inter- and intra-patient variability. This might provide an explanation why a marked reduction in PLP upon treatment was only seen in those 2 patients with excessively increased baseline values, while in the others, a potential moderating effect could have been lost in the noise of physiological fluctuations of this parameter in HPP patients. Of note and consistent with what might have been expected, these exaggerated PLP baseline levels and the accentuated decline were seen in those 2 participants with particularly low baseline BSAP activity. The observed lack of a consistent PEA response to BPS804 treatment is understandable, considering that targeting canonical Wnt signaling alone is sufficient to increase bone cell–derived ALP activity. In humans, at least 3 circulating BSAP isoforms with different glycosylation patterns can be distinguished. As shown by a previous study assessing the enzymatic properties of these BSAP isoforms, their catalytic activity toward PEA is essentially undetectable, and PEA is not an endogenous substrate of BSAP isoforms (43).

The maximum mean increase in bone formation markers and simultaneous decrease in bone resorption markers with BPS804 were found to be consistent with the results shown by other anti-sclerostin antibodies in patients with osteoporosis (44, 45). Mean increases in LS-BMD of 3% on day 85 and 3.9% at EoS were observed. These results are similar to the efficacy shown by another anti-sclerostin antibody, romosozumab, in a similarly short treatment period in patients with osteoporosis, although this comparison has several limitations (36, 45). Future studies will be needed to demonstrate whether long-term treatment in larger HPP patient cohorts will result in improvements in clinical outcomes such as accelerated bone healing, reduced implant failures, and ultimately fewer fragility fractures.

The results of target capture assessed by the serum sclerostin levels indicated a lag time of 2–4 weeks between the PK effect and the sclerostin target capture effect, and the PD effect was maximal at the 20 mg/kg dose compared with the 10 mg/kg dose. These

observations were similar to data from an unpublished clinical study conducted in healthy postmenopausal osteopenic women (ClinicalTrials. gov NCT01406548).

All 3 doses of BPS804 up to 20 mg/kg were well tolerated, with only mild to moderate AEs reported in 6 of 8 patients allocated to intervention. One patient experienced 2 SAEs, both of which were deemed unrelated to the study drug. There was no immunogenicity finding of clinical significance in the present study. During the course of the trial, no clinically significant changes in serum calcium levels were apparent. Calcium is an important component of the bone matrix, and, during bone formation, circulating calcium is actively incorporated in its mineral form within the bone matrix, removing it from the bloodstream. A previous study with a different anti-sclerostin antibody reported a mild, transient asymptomatic decrease in the mean serum calcium levels following dosing (36, 39). Overall, BPS804 was safe and well tolerated in HPP patients, although this needs to be confirmed in studies with a larger number of patients and long-term administration of the study drug.

There are limitations to this trial. As this is an exploratory, uncontrolled proof-of-concept study (phase IIA) intended to provide information on safety and tolerability as well as early, preliminary efficacy data, it included only a small sample size of 8 patients with heterogenic *ALPL* mutations all treated with BPS804 and therefore insufficient power for statistical significance testing versus baseline and no possibility for comparison against a control group. Although this trial focused on surrogate parameters and having PPi levels would have been desirable, this could not be included in the trial, since there still is no certified assay for evaluating PPi levels in human samples.

From a clinical perspective, we cannot at this point substantiate any significant improvements or changes in disease-associated symptoms and manifestations over the 6-month follow-up period. Even though some patients subjectively reported an improvement in their general health condition, owing to the open-label design of the trial, we cannot quantify to what extend this is a psychological consequence of intensified care and attention associated with the trial procedures.

In conclusion, short-term treatment with the fully human anti-sclerostin monoclonal antibody BPS804 increased ALP and BSAP enzymatic activity in *ALPL*-deficient adult HPP patients. BPS804 administration increased bone formation markers and BMD and suppressed bone resorption markers in HPP, similar to what has been reported for such a treatment modality in osteoporosis. Importantly, these results demonstrate that bone formation can be substantially enhanced even in this state of *ALPL* deficiency, which results in deficient osteoblast function because of alterations of mineralization, purinergic signaling, and bone formation. Keeping in mind the preliminary nature of these data and the inevitable need for carefully planned additional work to evaluate their clinical significance, these results support the hypothesis that osteoanabolic stimulation by sclerostin inhibition might be effective in adult patients with HPP, even in those with considerably low residual ALP activity. Accordingly, this strategy deserves further consideration in terms of establishing additional treatment options for patients with HPP, especially in challenging clinical situations such as osteoporosis or fragility fracture healing. Thus, this trial constitutes a foundation for

further inquiries to address potential concerns associated with sclerostin inhibition–based treatment strategies (46) and to collect more information on clinically meaningful parameters, e.g., bone histomorphometry and fracture risk reduction, in order to eventually assess whether this approach could be clinically meaningful for HPP patients.

Methods

Study participants. Male and female patients 18–75 years of age with a previously established clinical diagnosis of HPP, confirmed by *ALPL* gene mutation, normal serum calcium levels ≥8.5 to ≤10.2 mg/dl, and serum vitamin D levels ≥10 ng/ml were enrolled in this study. Key exclusion criteria were history of concomitant diseases related to bone metabolism; previous treatment with any antiresorptive medication or with the osteoanabolic drug teriparatide (rhPTH1-34, Forteo, Eli Lilly) within the last 6 months before the first dosing of study drug; and hypersensitivity to any monoclonal and polyclonal antibodies.

Study design. This was an open-label, single-center, intra-patient dose-escalating phase IIA study in adult patients with HPP. The study medication, i.e., anti-sclerostin monoclonal antibody BPS804, was provided by Novartis Pharma.

After screening and baseline assessments, patients underwent pre-dose safety evaluations and received i.v. administration of 5 mg/kg BPS804 on day 1, followed by a 2-week safety review. If patients completed this review without any significant safety concern, they received 10 mg/kg i.v. BPS804 on day 15, followed by another 2-week safety review period. Similarly, patients completing the second safety review period without any concern arising received 20 mg/kg i.v. BPS804 on day 29 and were followed for 16 weeks (Figure 2). During the first 5 weeks following the last dose, there were weekly visits on days 36, 43, 50, 57, and 64. For patients whose laboratory values were within the normal ranges and judged safe by the investigator or whose out-of-range laboratory values were judged clinically insignificant by the investigator, additional follow-up visits occurred on days 85, 113, and 141 (study completion day).

For safety reasons, drug administration was started with a single patient (patient 1) and conducted in a sequential manner. The second and third patients (patients 2 and 3) did not receive the first dose of BPS804 until 24 hours after the second dose (i.e., 10 mg/kg) was administered to the previous patient. The remaining patients received the first dose of 5 mg/kg 24 hours after administration of the second dose (i.e., 10 mg/kg) to patient 3.

Study objectives and assessments. The primary objectives of the study were to assess the safety and tolerability of BPS804 when administered as multiple, dose-escalating i.v. infusions and to assess the PD effects and preliminary efficacy on the activity of serum TNSALP and BSAP. The secondary objectives were to assess the PD effects on plasma PLP, PEA, and serum PTH; to assess the potential immunogenicity and PK profile of BPS804; and to assess target capture by measuring serum sclerostin concentrations (free/bound). Serum bone turnover markers including PINP, OC, and CTX-1 were measured.

LS-BMD was assessed at screening, on day 85, and at the EoS visit 16 weeks after the last dosing using a GE Lunar Prodigy Advance DXA machine.

PK parameters assessed were C_{max}, time to reach peak plasma concentrations (T_{max}), $t_{1/2}$, exposure of study drug as area under the curve extrapolated to infinity (AUC_{last}), and area under the plasma concentration-time curve over the dosing interval.

Safety assessments included all AEs and SAEs (with their severity and relationship to study drug), physical examinations, ECGs, vital signs, standard clinical laboratory evaluations (i.e., hematology, blood chemistry, and urinalysis), calcium homeostasis, and pregnancies. Safety evaluations were performed on day 1, during the safety review period after the first and second doses, and during the first 5 weeks and after the last dose on days 36, 43, 50, 57, and 64.

Sample collection. To measure relevant biomarkers such as serum TNSALP, BSAP, HPP-associated disease biomarkers (PLP, PEA), bone metabolism biomarkers (PINP, OC, CTX-1, PTH), and sclerostin, we collected blood samples from patients in the fasting condition on days 1 (pre-dose); 2, 8, and 15 (before the second dose); 16 and 29 (before the third dose); and 30, 36, 43, 57, 85, 113, and 141. The blood samples for anti-BPS804 antibody for immunogenicity assessment were obtained before dosing on days 1 and 29 and during ambulatory visits on days 85 and 141. For PK assessments, the blood samples were collected on days 1 (pre-dose and 2 and 8 hours post-dose); 2, 8, and 15 (pre-dose and 2 hours post-dose); 16 and 29 (pre-dose and 2 and 8 hours post-dose); and 30, 36, 43, 57, 85, 113, and 141.

A MicroVue bone ALP enzyme immunoassay (Quidel) was used to measure the BSAP enzymatic activity, with a LLOQ of 4.95 U/l. PLP and PEA were determined in human plasma using a liquid chromatography–tandem mass spectrometry method validated by Eurofins Medinet (Denver) with reporting limits of 1.00 and 200 ng/ml, respectively. Sclerostin concentrations were measured using a validated assay, the homogenous AlphaLISA assay (PerkinElmer), with an LLOQ of 2.137 ng/ml. The assay detects both free and antibody complex–bound sclerostin. Anti-BPS804 antibodies were measured using a validated AlphaLISA assay. Samples were screened for a positive or negative signal, and, for the screening-positive samples, the specificity for BPS804 was analyzed in a confirmatory assay, with an LLOQ of 250 ng/ml. Elecsys electrochemiluminescence sandwich-based immunoassay (Roche Diagnostics Ltd.) was used for PINP, OC, CTX-1, and PTH, with lower detection limits of 5 ng/ml, 0.5 ng/ml, 10 pg/ml, and 1.2 pg/ml, respectively. The BPS804 concentrations were measured using a validated homogenous AlphaLISA assay, with an LLOQ of 0.49 μg/ml.

Statistics. A sample size of at least 6 patients completing the trial was considered for this study, assuming that the probability of not observing an AE would be 2% when the true incidence rate of AE was 50%. With the sample size of 6 patients and 0.04 variability of the intra-individual contrast obtained in a previous study, the power of a 1-sample *t* test (2-sided, α = 0.05) was greater than 99% for BSAP enzymatic activity. Patients who received at least 1 dose of the study drug were included in the safety analysis set. All patients with evaluable PD parameter data were included in the PD analysis set. The PD parameters were summarized by time point using descriptive statistics along with geometric mean. Individual and geometric mean time profiles were presented. Summary statistics including geometric mean were also presented for the ratio versus baseline. Statistical analysis was performed on the basis of the log-transformed data. For exploratory end points (e.g., LS-BMD) per protocol, no statistical analyses were performed.

For the primary end points BSAP and ALP, a comparison versus study baseline was performed by calculating an average ratio from baseline to days 2–29 following the third infusion. This calculation

included assessments performed on days 30, 36, 43, and 57 and additionally day 50 for ALP enzymatic activity. This average ratio from baseline, RAUC, was calculated for each patient as standardized AUC on the log scale and then divided by the actual length of the time interval. For reporting, the RAUC was back-transformed to the original scale to obtain a weighted geometric average of the ratio from baseline per patient. A Bayesian analysis was performed on the RAUC on the log scale using a noninformative prior for model parameters. The quoted 37% and 30% median increase for ALP and BSAP refer to the median of the resulting posterior distributions. It was considered a sign of efficacy if the resulting posterior probability of the average ratio from baseline being larger than 1 was at least 90%. This criterion was met for both parameters. The posterior probability was also calculated for the average ratio from baseline being larger than 0.7, 1.3, 1.54, and 2.

All the PK parameters were estimated by a noncompartmental method and were derived using Phoenix WinNonlin, version 6.2 (Certara). Descriptive statistics of the PK parameters included mean, SD, coefficient of variance, and minimum and maximum. Median or range values were provided for T_{max}.

Study approval. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, and it received approval from the Ethics Committee of the Medical Faculty at the University of Würzburg, Germany. All participants provided written informed consent before participating in any study procedures. The study was registered with ClinicalTrials.gov (NCT01406977).

Author contributions

LS conducted the trial and contributed to designing the study, acquiring data, analyzing data, and writing the manuscript. As corresponding author, LS confirms that he had full access to all the data in the study and has final responsibility for the decision to submit for publication. JB contributed to conducting experiments, acquiring data, analyzing data, and revising the manuscript. SH contributed to designing the study, providing trial conduct oversight, analyzing data, providing reagents, and revising the manuscript. CH contributed to analyzing data and writing the manuscript. EK contributed to acquiring data, analyzing data (genetic testing), and revising the manuscript. BK contributed to designing the study and analyzing data (statistical analysis). YH contributed to designing the study, analyzing data (PK/PD), and revising the manuscript. SC contributed to designing the study, analyzing data (PK/PD), and revising the manuscript. MAV contributed to designing the study, analyzing data (biomarker), and revising the manuscript. BB contributed to designing the study, analyzing data (imaging), and revising the manuscript. RR contributed to designing the study, providing medical oversight, analyzing data (safety, laboratory values), and revising the manuscript. UJ contributed to designing the study, providing medical oversight, acquiring data, analyzing data, and writing the manuscript. FJ contributed to designing the study, acquiring data, analyzing data, and writing the manuscript.

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