Safety and Efficacy of Oral Human Parathyroid Hormone (1-34) in Hypoparathyroidism: An Open-Label Study

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ABSTRACT

The standard treatment of primary hypoparathyroidism with oral calcium supplementation and calcitriol (or an analog), intended to control hypocalcemia and hyperphosphatemia and avoid hypercalciuria, remains challenging for both patients and clinicians. In 2015 human PTH(1-84) administered as a daily subcutaneous injection was approved as an adjunctive treatment in patients who cannot be well controlled on the standard treatments alone.

This open-label study aimed to assess the safety and efficacy of an oral human PTH(1–34) formulation as an adjunct to standard treatment in adult subjects with hypoparathyroidism. Oral hPTH(1-34) tablets (0.75 mg human hPTH(1-34) acetate) were administered four times daily, for 16 consecutive weeks and changes in calcium supplementation and alfacalcidol use, albumin-adjusted serum calcium (ACa), serum phosphate, urinary calcium excretion and quality of life

throughout the study were monitored. Of the 19 enrolled subjects, 15 completed the trial per protocol. A median 42% reduction from baseline in exogenous calcium dose was recorded (p=0.001), while median serum ACa levels remained above the lower target ACa levels for HypoPT patients (>7.5 mg/dL) throughout the study. Median serum phosphate levels rapidly decreased (23%; p=0.0003) 2-hours following the first dose and were maintained within the normal range for the duration of the study. A notable, but not statistically significant, median decrease (21%; p= 0.07) in 24-hour urine calcium excretion was observed between the first and last treatment days. Only four possible drug-related, non-serious adverse events were reported over the 16-week study, all by the same patient. A small but statistically significant increase from baseline quality of life (5%, p=0.03) was reported by the end of the treatment period.

Oral hPTH(1-34) treatment was generally safe and well tolerated and allowed for a reduction in exogenous calcium supplementation, while maintaining normocalcemia in adult patients with hypoparathyroidism.

Keywords: ORAL PARATHYROID HORMONE, HYPOPARATHYROIDISM, PARATHYROID-RELATED DISORDERS; CALCIUM/PHOSPHATE DISORDERS; PARATHYROID HORMONE

INTRODUCTION

Primary Hypoparathyroidism (HypoPT) is a rare mineral metabolism disorder, with a prevalence of 22 per 100,000 individuals [(1)], and is biochemically characterized by low serum calcium and low or undetectable parathyroid hormone (PTH) levels. The leading cause of HypoPT in adults is iatrogenic, typically secondary to excision or injury incurred during anterior neck surgery [(2)]. Less common etiologies include autoimmune disease, congenital absence, and genetic disorders resulting in defective biosynthesis or secretion of the hormone [(3)]. The characteristic

hypocalcemia in HypoPT is due to PTH levels insufficient to adequately mobilize calcium from bone, reabsorb filtered calcium from the distal nephron, or increase intestinal calcium absorption by stimulating renal 25-hydroxyvitamin D 1α-hydroxylase activity and subsequent 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) synthesis. Hyperphosphatemia develops due to the loss of the phosphaturic effect of PTH [4].

The standard treatment of HypoPT with oral calcium supplementation and calcitriol (or an analog), intended to control hypocalcemia and hyperphosphatemia and avoid hypercalciuria, remains challenging for both patients and clinicians. Loss of renal PTH action decreases renal tubular reabsorption of calcium and excretion of phosphate causing hypercalciuria and hyperphosphatemia respectively [5]. Thus, patients with chronic hypoparathyroidism have been found to have an increased risk of renal complications, such as nephrocalcinosis, nephrolithiasis, renal insufficiency and often ectopic calcification in other organs. Titration of calcium and calcitriol dose is often slow and imprecise and may require frequent dose adjustments. In addition, the intake of supplements multiple times throughout the day is inconvenient and often causes gastrointestinal intolerance [6]. PTH deficiency leads to low bone turnover and markedly altered microarchitectural and biomechanical properties of the skeleton that result in structural and dynamic skeletal defects which are not addressed by calcium supplements and active vitamin D therapy [7, 8]. Standard treatment is titrated to achieve blood calcium levels that are at the lower limit of normal to reduce the risk of hypercalciuria and ectopic calcification [9]. Patients have repeatedly reported quality of life problems such as altered mood and cognition, which may be caused by the relatively low blood calcium provided by standard treatment [10-12]. Replacement therapy with PTH has the potential advantages of reducing the high dose of calcium supplements, providing better correction of hypocalcemia and hyperphosphatemia, and

decreasing urine calcium. With these potential advantages, treatment of PTH deficiency with PTH is compelling.

In 2015, the US Food and Drug Administration approved a daily subcutaneous injection of the full-length human PTH molecule (hPTH (1-84)), synthesized through recombinant technology as an adjunct to calcium and calcitriol (or other calcitriol analogs) treatment in order to control hypocalcemia and hyperphosphatemia in patients with HypoPT. Currently, two human PTH (hPTH) analogs are clinically available for different indications: hPTH(1–34), the 1–34 N-terminal fragment of human PTH (teriparatide) for the treatment of osteoporosis, and hPTH(1–84) for HypoPT. Both the full length 84 amino acid hormone [13-16] and its fully active amino-terminal 34 amino acid peptide [17-24] have demonstrated salutary effects in HypoPT management and have lowered or eliminated supplemental calcium and active vitamin D requirements while maintaining serum calcium within the reference range. Presently, both hPTH(1-84) and hPTH(1-34) are only available in injectable subcutaneous forms, and while hPTH(1-84) is approved for the treatment of hypoparathyroidism, hPTH(1-34) is only approved for the treatment of osteoporosis. An oral formulation devoid of the injection-related complexities will likely have a positive impact on compliance and adherence, simplifying the patients' treatment regimen and their general quality of life.

Entera Bio is developing an oral formulation of hPTH(1-34) based on its novel drug delivery technology that facilitates absorption of proteins, which has achieved therapeutic relevant plasma concentrations and pharmacokinetics of the drug [23]. We report the results of a 16-week pilot study aimed to assess the safety, tolerability (rate of discontinuation), and efficacy (reduction of calcium supplementation) of an oral hPTH(1–34) formulation as an add-on therapy to conventional treatment in adults with HypoPT.

MATERIALS AND METHODS

Study drug

Oral hPTH(1-34) tested in the current study is based on the proprietary oral peptide delivery technology of Entera Bio Ltd.[24]. The technology consists of the novel excipients salcaprozate sodium and soybean trypsin inhibitor (SBTI), which facilitate absorption of the hPTH(1-34) peptide across the GI wall and protect the hPTH(1-34) peptide from proteolysis. Each tablet contained 0.75 mg hPTH(1-34) acetate, equivalent to 0.69 mg of the hPTH active moiety (formulation code EBP02). Tablets were provided by Entera Bio Ltd., Jerusalem, Israel.

Study design

The study was an open-label, multicenter pilot study, conducted in Israel between August 12, 2014 and June 21, 2015, in which Oral hPTH(1-34) was administered four times daily for 16 consecutive weeks for the treatment of hypocalcemia in patients with HypoPT. The study was conducted in accordance with the Good Clinical Practice Guidelines and the Declaration of Helsinki and registered in the US clinical trials database (Clinical trial # - NCT02152228). The Institutional Review Boards at each investigational site approved the protocol before study initiation. Written informed consent was obtained from patients before participation in the study.

The main inclusion criteria included males and females aged 18-80 years, body mass index (BMI) 18–30 kg/m^2, with HypoPT for more than 12 months, who, at the time of enrollment, were taking supplemental calcium with ≥1.0 g elemental calcium/day with correlate calcitriol analog dose and had 25-hydroxyvitamin D [25(OH)D] levels ≥ 20 ng/mL. Individuals were eligible for the trial after physical, cardiac and renal evaluations, as well as blood chemistry and hematology assessments were found to be within reference ranges. Abnormalities due to HypoPT, such as low serum calcium or high phosphate, were acceptable. Main exclusion criteria

included anemia, renal insufficiency (estimated glomerular filtration rate (eGFR) <40mL/min/1.73m^2), elevated liver associated enzymes, alcohol or drug abuse, positive serology test (HIV, HBsAg, HCV Ab) or active infections, nephrocalcinosis, concurrent drugs that might interfere with mineral metabolism, and pregnancy or planned pregnancy.

Treatment with calcium supplements and calcitriol analogs (generally alfacalcidol) were continued as prescribed by the physicians providing long-term care of each patient's HypoPT.

On treatment day 1, the subjects underwent a comprehensive evaluation, including physical exam, medical history, complete blood count and serum biochemical safety and efficacy analysis. Patients were then treated with three in-clinic doses of 0.75 mg Oral hPTH(1-34), delivered at four-hour intervals, at approximately 08:00, 12:00 and 16:00. The first dose was administered after an overnight fast and meals were supplied one hour post the first and second dose administrations. The fourth dose of the first treatment day was self-administered at home, after 20:00. Thereafter, the subjects were instructed to self-administer the study drug at home with 100 mL water, 4 times a day (prior to breakfast, lunch, dinner and at bedtime) for a treatment period of 16 weeks. The study drug was to be taken at least 30 minutes before eating or drinking, at least 30 minutes before consumption of calcium tablets or any other drug, and at least 1 hour after consumption of any food or drug. During the treatment period, the dose was titrated up to a maximum of 12 tablets a day (total daily oral hPTH(1-34) dose: 9 mg) by the Investigator, according to each subject's albumin adjusted serum calcium (ACa) and supplement treatment regimen. Subjects' ACa was measured weekly for the first 4 weeks, biweekly for the next 8 weeks, and once again at the end of the study. The decision to decrease or increase the doses of Oral hPTH(1-34), supplemental calcium, or alfacalcidol was made according to each

patients' pre-dose ACa levels according to dosing guidelines presented in Table 1 and adjusted according to the clinical judgment of the investigator.

Based on previous Phase 1 studies and IRB approval, the maximal allowed dosage was 9.0 mg hPTH(1-34) acetate per day. Due to the heterogeneous patient population, their sensitivity to PTH, and their variable daily supplements, the extent of the adjustment was left to the investigators' discretion for how to best adjust in order to achieve the individual patient's target ACa levels. Follow up visits were performed at the end of weeks 1, 2, 3, 4, 6, 8, 10, 12, 14 and at the end of week 16, when treatment with Oral hPTH(1-34) was discontinued. Serum calcium, phosphate, albumin, and creatinine levels were evaluated at all visits (before and one-hour after self-administration of the study drug at the clinic) and medication dose adjustments were performed, according to the subjects' ACa levels. Individuals were contacted by a study coordinator during each week that did not include a clinical visit. Urinalysis and 24hr urine collection for calcium, creatinine, and phosphate was performed prior to treatment initiation and at the end of weeks 8 and 16. A follow-up/end of study (FU/EOS) visit was performed 7–14 days after the completion of the treatment phase or at early trial discontinuation.

Treatment compliance was monitored throughout the study by checking personal diaries and weekly patient status update calls. Compliance to treatment was calculated as the percentage of the tablets consumed by the subject from the prescribed amounts and defined as follows: >80% (good), 60-80% (satisfactory) and <60% (poor).

Quality of life (QoL) was assessed with the EQ-5D-5L™ (EuroQoL), a standardized instrument used to measure health-related quality of life assessments, which was completed by the subjects at each visit during the treatment phase. This instrument used a Visual Analog Scale (VAS, 1

being worst to 100 being best) to record patients' self-rated overall health. This instrument has been used to assess quality of life in patients with HypoPT [10].

Hypocalcemia-related symptoms were reported at each treatment visit, beginning the first visit after treatment was initiated.

Pharmacokinetic and pharmacodynamics tests following first two drug doses

Blood samples were collected following the two first drug administrations that took place on the first study day. To this end, samples were collected prior to and 10, 15, 20, 30, 45, 60, 90, 180, and 240 minutes following administration of each of the two doses. Blood samples were collected into spray-coated EDTA tubes, placed on ice and spun at 4°C, 2500 g, 10 minutes. The separated plasma was stored at -20 ºC until analysis. Analysis of hPTH(1-34) in human plasma was performed by Bioanalytical Facility, University of East Anglia (UK), using a validated commercial chemiluminescence-based immunoassay (IDS-iSYS hPTH(1-34) Boldon, UK) on a IDS-iSYS automated analyzer.

For pharmacodynamic analysis, blood samples collected on the first study visit prior to, 60 and 180 minutes following the first drug dose, as well as 60, 180 and 240 minutes following the second dose, were used to determine serum calcium, albumin and phosphate levels.

Data analysis

Pharmacokinetics, pharmacodynamics, and safety analyses included the data of all enrolled subjects (N=19), unless noted otherwise, all of whom completed the first study day. Evaluation of treatment compliance included all subjects that completed the 16-week trial (N=17). All other analyses, including the 16-week calculation of supplemental calcium and alfacalcidol dosing, serum ACa and phosphate concentrations, urine calcium excretion, quality of life evaluation, and hypocalcemia-related symptoms were performed on data collected from all patients completing

the study per protocol (N=15). For the two subjects not treated per protocol, calcium supplements were increased during the initial weeks of the study in order to increase ACa levels above baseline value instead of having the levels maintained.

Statistical analysis

For categorical variables, descriptive statistics included sample size, absolute and relative frequency for proportions and 95% confidence interval (if appropriate). For continuous variables, descriptive statistics included sample size, arithmetic mean, geometric mean, standard deviation, standard error, coefficient of variation (if appropriate), median, minimum and maximum and 95% confidence interval for means.

Pharmacokinetic analysis

The following parameters were derived from a non-compartmental pharmacokinetic analysis by SAS Software package (SAS Institute Inc., Cary, NC, USA) using individual hPTH(1-34) concentration data: C_{max} - maximum plasma concentration, T_{max} - time to maximum plasma concentration, AUC_{last} - area under the plasma concentration-time curve (AUC) from time of administration up to last measurable concentration, calculated by linear trapezoidal summation, λz - elimination rate constant, determined by linear regression of the terminal points of the ln - linear plasma concentration - time curve, and $t_{1/2}$ - terminal elimination half-life, defined as $0.693/\lambda z$.

RESULTS

Subject disposition

Nineteen subjects with confirmed diagnoses of HypoPT for more than 1 year were enrolled in the study. Most of the subjects (16/19) were female. The median age of the subjects was 41.1 years (range 20.3 – 71.0 years), with a median body mass index (BMI) of 25.5 kg/cm^2.

Seventeen subjects completed the study, while two subjects withdrew on the first day of the study - one due to withdrawal of consent and one due to an unrelated serious adverse event (SAE) of hypercalcemia, which occurred prior to the first study drug administration and was only identified after administration of the first dose, when pre-dose lab results were received.

Reduction in supplemental calcium and alfacalcidol (or calcitriol) doses:

The mean daily doses of Oral hPTH(1-34) by week are summarized in Table 3. In accordance with the study protocol, supplemental calcium intake was reduced in parallel with the gradual increase in the daily Oral hPTH(1-34) dose (up to 9.0 mg) to maintain stable serum ACa concentrations. Calcium supplement requirements decreased consistently during the study (Table 4; Figure 1a) and was significantly lower than baseline from Week 4 until the end of the study. Median exogenous calcium doses decreased from 3.6 g at baseline to 2.2 g by week 16, equivalent to a 42% (p=0.0001) reduction from baseline requirements. By the end of the study, 40% of the patients achieved a ≥50% reduction in calcium intake and 73% of patients achieved a ≥30% reduction. While some decreases in the average alfacalcidol doses were observed throughout the study, mean alfacalcidol doses reduced 4.3% from 1.1 to 1.0 µg per day, which was not significantly different than baseline doses. Despite significant reductions in supplemental calcium doses, total serum ACa levels remained stable and above the lower target ACa levels for HypoPT patients (>7.5 mg/ dL[25]) (Figure 1b). At the start of the study, 20% of patients had ACa> 8.5 mg/dL, compared to 33% at the end of week 16.

Human hPTH(1-34) pharmacokinetics following Oral hPTH(1-34) administration

After administration of the first and the second doses, the respective median maximum plasma hPTH(1-34) concentrations were 47.9 pg/mL and 41.2 pg/mL (Table 5), which were reached at a median 20.0 min and 30.0 min, respectively, after dosing (Table 5). Similarly, the total

systemic exposure (AUC_{last}) was considerably greater after the first dose of Oral hPTH(1-34) administered after an overnight fast, in comparison to the second dose (Table 5) administered three hours after a standard meal. The mean terminal half-life of hPTH(1-34) was similar after both dose administrations (21.1 min and 27.5 min for the first and second doses, respectively).

Serum ACa and phosphate levels

Following the first two dose administrations with the starting dose of 0.75mg Oral hPTH(1-34) tablets on Day 1, there was no significant effect on serum ACa levels. With that said, an upward trend in serum ACa levels was observed three hours post-dose, following a transient decrease at one-hour post-dose. As shown in Figure 2, the effect of Oral hPTH(1-34) on serum ACa levels extended well beyond the duration of exposure to the drug, resembling an indirect pharmacodynamic model [(26)].

A more prominent effect was observed for serum phosphate levels, which rapidly decreased following the first Oral hPTH(1-34) dose, bringing the high baseline levels to within the reference range (2.5 – 4.5 mg/dL).

The phosphate levels continued to stay low after the second dose, remaining within the reference range for a total of at least 7 hours. The same rapid effect on phosphate levels was observed throughout the study; at each study visit, the median serum phosphate levels one-hour post-dose were significantly lower than pre-dose values (1-12%; $p \leq 0.04$) (Figure 4).

It should be noted, that one of the study participants was on a phosphate binder medication (sevelamer carbonate) at the start of the trial and therefore was not included in any of the serum phosphate analyses. Additionally, one subject who had hypercalcemic serum levels before any study drug administration was excluded from the day 1 serum ACa and phosphate analysis.

Urinary calcium levels

Mean (±SD) 24-hour urinary calcium excretion was 189.8 (±131.6) mg pre-treatment, 192.3 (±146.0) mg at the middle (end of week 8), and 140.5 (±82.4) mg at the end of the study (end of week 16). A non-significant change from baseline mean 24-hour urinary calcium was observed over the 16-week treatment period (26% p=0.07). 80% of the subjects had a decrease in urinary calcium levels by the end of the study. There were seven subjects who began the trial with calcium levels above the 24-hr urinary calcium reference range (>200 mg for females; >250 mg for males). These subjects had a mean decrease of 21% in comparison to their baseline levels. Notably, six of the seven subjects had a decrease in 24-hr urinary calcium levels, three of which decreased to reference range levels.

Safety

During the 120-day study period, 18 of the 19 patients had at least one adverse event. A total of 199 AEs were reported. The vast majority of AEs (195/199, 98.0%) were deemed unrelated to the study drug. The four AEs considered drug-related (mild nausea, moderate back pain, moderate headache and moderate upper abdominal pain) were reported by a single subject who withdrew consent on Day 1 after expressing fear of participating in the study. These AEs are unlikely related but, as this could not be confirmed by repeated dosing, they were recorded as 'possibly related'. Aside from the one patient that was withdrawn from the study due to hypercalcemia on Day 1 prior to receiving the first dose of the study, there were no other incidences of hypercalcemia adverse events, and no patient had serum ACa greater than 9.42 mg/dL over the course of the treatment period. The most commonly observed adverse events were gastrointestinal disorders, including abdominal pain, nausea, and diarrhea (reported by 37%, 32% and 26% of the patients, respectively), nasopharyngitis (32%) and muscle spasms

(26%). There were no clinically significant changes in blood chemistry and hematology measurements, physical findings, vital signs or ECG measurements.

Quality of life

QoL dimensions assessed using the VAS score of this instrument found generally good QOL at study start (80 (60 – 100); median (range); maximum score of 100) which increased at the first week of treatment. This improvement was maintained throughout the study, with a final QoL VAS score of 85 (60 – 100) (p=0.03) at week 16.

Hypocalcemia-related symptoms

Hypocalcemia-related symptoms were monitored throughout the study, starting from the end of week 1, and the total number of hypocalcemia-related symptoms/signs reported, decreased from a total of 13 (paresthesias, muscle cramps, emotional instability, anxiety, muscle weakness, and Chvostek sign) in 5 patients at the end of week 1 to 4 (paresthesias, anxiety, and hypotension) observed in 4 patients at the end of the study.

Compliance with Study Medication

All 17 subjects completing the study showed good compliance (>80%). One subject showed 'Satisfactory – Poor' (<80%) compliance up until week 5, but his compliance improved to 'Good' thereafter. The average compliance per subject for the entire study duration was 95.6% ± 4.7% (mean ± SD).

DISCUSSION

The treatment objective of hypoparathyroidism is to increase serum calcium to within 0.5 mg/dL of the lower limit of the reference range, but not into the reference range to reduce the risk of nephrolithiasis and ectopic calcification including nephrocalcinosis. [27] The standard therapy of HypoPT with oral calcium supplements and calcitriol analogs increases serum calcium but is

limited since it does not increase renal tubular calcium reabsorption or increase phosphate excretion. Hormonal replacement therapy with both hPTH(1-34) and PTH(1-84) have shown that this objective can be attained and that calcium homeostasis can be maintained at a steady level by PTH without hypercalciuria and at the same time reducing supplemental calcium requirements and improving quality of life of patients [9] [30-32] . The main differences between the commercial formulations of hPTH(1-34) and PTH(1-84) is the longer elimination half-life and longer time to peak concentration for the full-length molecule resulting in a longer pharmacologic effect following a single subcutaneous injection [31,32]. Both hPTH(1-34) (Forteo) and PTH(1-84) (Natpara®) require parenteral administration because oral delivery and absorption of hPTH(1-34), and of polypeptides in general, is hindered by extensive proteolysis inside the gastrointestinal tract, limited absorption due to their molecular mass and their hydrophilic nature [33]. The oral route of administration is the most common, safest and convenient method of dispensing a drug [34]. Thus, an oral preparation of hPTH(1-34) which is easy to administer and has the potential to provide hPTH(1-34) throughout the day without the need for multiple injections will likely have a major impact on compliance, adherence, therapeutic impact and quality of life for patients in need of this chronic treatment. Entera Bio has developed an oral formulation of hPTH(1-34) based on a novel drug delivery technology that facilitates absorption of proteins, which has achieved biologically relevant plasma concentrations of the drug [23]. In order to obtain similar systemic exposure, the dose of Oral hPTH(1-34) administered in this study is significantly higher than the dose of commercially available subcutaneous PTH due to the lower absolute bioavailability of the oral formulation.

This study demonstrated the safety and tolerability of Oral hPTH(1-34) administered four times daily for 16-weeks to patients with HypoPT. Treatment was associated with decreases in serum

phosphate, while serum ACa levels remained stable throughout the study. No drug-related serious adverse events were reported and most of the adverse events were not considered study drug-related. Oral hPTH(1-34) adjunct to conventional HypoPT treatment with supplemental calcium and alfacalcidol led to a statistically significant decrease in supplemental calcium requirements from week 4 until the end of the 16-week treatment period (42% p=0.001). In 40% of the patients, a 50% or more reduction from baseline calcium intake was achieved and in 73% of patients, the reduction was at least 30%. The four-week delay in reduction of calcium requirements was expected, as the study was designed with a gradual titration of the study drug dose. Supplemental calcium dose was significantly reduced while total serum ACa levels remained stable during the study.

In contrast to the significant reduction in calcium requirements, the average calcitriol analog (alfacalcidol) doses did not change significantly. These results can be attributed to the study design, that targeted reduction of supplemental calcium, and its short duration. According to the study protocol, dose adjustments in calcitriol analogs were only to be implemented after reaching an adequate reduction of calcium supplement intake.

The pharmacokinetic profile of the 0.75 mg dose of Oral hPTH(1-34) measured on the first treatment day was characterized by rapid absorption and elimination (Table 5). The overnight fast preceding the first dose may explain the slightly higher hPTH(1-34) absorption as compared to the second dose, which was administered three hours after a standard meal (Table 5). Following both the first and second dose, median plasma hPTH(1-34) concentrations (on a molar basis) reached the normal levels of endogenous hPTH(1-84) (adjusting for relative molecular weight). The main reason for the different pharmacokinetic profiles of the Oral hPTH(1-34) and injectable hPTH(1-84) is the prolonged release from the injection site of the latter, which results

in the significantly longer apparent plasma half-life (21.1- 27.5 min versus ~3h, respectively) [25] [34].

Following the first 0.75 mg Oral hPTH(1-34) dose on the first study day no significant effect was observed for serum ACa levels (Figure 2), while serum phosphate levels decreased to below the upper limit of the reference range approximately one hour after dosing (Figure 3). The pharmacodynamic effect on phosphate levels following administration of the first two study doses lasted at least 7 hours. Because of the limited number of blood sampling time points during the first study day, and not repeating measurements after the doses increased from 0.75 mg to 2.25 mg later in the study, the complete pharmacokinetic and pharmacodynamic profile of the novel oral formulation of hPTH(1-34) was not obtained and additional investigation of the drug's pharmacodynamics is required.

In addition, similar to the pharmacodynamic profile shown on the first study day, at each of the subsequent study visits, serum phosphate levels rapidly decreased to within the reference range one hour after dosing. The median baseline serum phosphate levels measured at each treatment visit were consistently lower than the baseline levels at the study initiation.

Mean urinary calcium levels decreased over the 16-week period of the study by 26%; this change was gradual and most prominent in the final 8 weeks of treatment. This decrease paralleled the gradual increase in the Oral hPTH(1-34) dose (near the maximum of 9 mg in all subjects by week 9) and the decrease in median supplemental calcium dose (from 3.6 g at week 1 to 2.4 g at week 8 to 2.2 g at week 16). Further studies with multiple timed serum and urine calcium evaluations in a controlled clinical setting would be required to determine the contribution of Oral hPTH(1-34) and supplemental calcium to urinary calcium excretion.

A small but statistically significant increase from baseline in quality of life was reported by patients. The limited increase may be attributed to both the relatively high QoL VAS score at the start of the study and the questionnaire used which did not address the specific symptoms of HypoPT. Additionally, the number of patients with symptoms related to hypocalcemia (such as paresthesias, muscle cramps, emotional eating, instability, anxiety, and fatigue) appeared to decrease by the end of the study (13 patients at week 1 versus 4 patients by the end of week 16).

Significant limitations of the study included the small sample size and the short duration of the study. As it was a pilot study, it did not include blinded concurrent treatment with placebo, and there was no run-in period in which standard treatment with supplemental calcium and calcitriol analogs was optimized according to a protocol prior to the start of Oral hPTH(1-34). Other study limitations were the limited pharmacodynamic time points on the first day of the study, and the at-home urine collections as opposed to collection in a controlled clinical setting. Additionally, because there was no placebo control group, quality of life results and reported hypocalcemia symptoms should be interpreted with caution, as the placebo effect may have contributed to the study outcome. Despite these limitations, the study findings of the Oral hPTH(1-34) effect on serum ACa and phosphate levels, which enabled statistically significant reductions in oral calcium requirements, were clinically significant.

An additional drawback of the study was the high number of tablets patients were required to take - up to three tablets taken four times a day, as at the time of the study, the tablets were only available at a single strength. It should be noted, however, that the tablets administered in the study were significantly smaller in size than the calcium supplements that these patients are accustomed to taking regularly (~130 mg vs ~1500 mg). Additionally, there were no compliance issues related to the number of tablets or dose administered.

In conclusion, this study is the first to report on the use of an oral hPTH(1–34) formulation in the treatment of patients with HypoPT as part of Entera Bio's EB612 development program. As an add–on therapy in this patient population, Oral hPTH(1-34) achieved significant systemic exposure, exhibited a promising pharmacodynamic profile, was generally safe and well tolerated and was effective in reducing calcium supplement requirements. Further long-term, placebo-controlled studies are necessary to confirm the reported findings and to assess whether Oral hPTH(1-34) can serve as a new therapy for the treatment of HypoPT.

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DISCLOSURE

PS, HG, GB, ARo, ARa, and MB are employees of Entera Bio Ltd. SIS and YC received research grants from Entera. SIS received consulting fees from Entera Bio Ltd. JCYT and WDF received funding support from Entera Bio Ltd to perform the analysis. NSK, MG, and AS have no conflicts of interest to disclose.

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Authors' roles: Study design: SIS, YC, WF, PS and HG. Study conduct: SIS, YC, NSK, MG, AS, PS, HG, GB, ARo, ARa, and MB. Data collection: SIS and YC. Data analysis: JCYT and WF. Data interpretation: SIS, YC, HG, GB, and ARo. Drafting manuscript: EA, HG, GB and

ARo. Revising manuscript content: SIS, WF, YC. Approving final version of manuscript: SIS, WF, YC, HG, GB. HG and GB take responsibility for the integrity of the data analysis.

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TABLES

Table 1: *Recommended changes to calcium (Ca) supplements and alfacalcidol or hPTH(1-34) dosages in accordance with patient's albumin adjusted serum Ca (ACa)*

ACa (mg/dL)	**Recommended Action**
< 8	Increase hPTH(1-34) dose by 2 tablets daily. Increase Ca supplements/alfacalcidol, appropriately.*
8 - 8.6	Increase hPTH(1-34) dose by 1 tablet daily. Maintain Ca supplements and alfacalcidol doses.
8.6 - 9.2	Decrease Ca supplements by 500 mg daily.
9.2 - 9.8	Decrease Ca supplements by 500mg daily or – if not receiving calcium – decrease alfacalcidol appropriately.*
9.8 - 10.4	Decrease Ca supplements by 500-1000mg daily or – if not receiving calcium – decrease alfacalcidol supplementation appropriately.* If not on Ca or alfacalcidol, reduce hPTH(1-34) by a single tablet.
> 10.4	Decrease as for 9.6 -10.4 mg/dl and may require stopping hPTH(1-34) therapy transiently until values return to normal.

Albumin-adjusted Calcium (ACa) calculated as Serum Calcium (total) + [0.8 x (4.0-serum albumin)]

Table 2: *Baseline demographics and clinical parameters*

Characteristic	**Study Population (N= 19)**
Gender,	**n (%)**
Male	3 (15.8)

Female	16 (84.2)
Age, mean ± SD (years)	44.6 ± 16.1
Weight, mean ± SD (kg)	73.6 ± 17.3
BMI, mean ± SD (kg/cm^2)	26.6 ± 4.4
BMI, median (range), (kg/cm^2)	25.5 (19.8 – 33.8)
Etiology HypoPT, n (%)	
Acquired	13 (68.4)
Autoimmune	5 (26.3)
Hereditary	1 (5.3)
Supplemental calcium dose, mean ± SD (range), (g/d)[a]	3.7±2.4 (1.2-10.8)
Alfacalcidol dose, mean ± SD (range), (µg/d) [a]	1.1±0.7 (0.25-2)
Serum ACa, mean ± SD (mg/dL)[a, b]	8.0±0.57
Serum phosphate, mean ± SD (mg/dL)[a] (range)	5.0±0.84 (3.7-7.0)

[a] N=15 (patients completed the study per protocol)

[b] Target ACa levels for HypoPT patients are >7.5 mg/dL

SD= standard deviation, HypoPT= Primary Hypoparathyroidism, ACa – Albumin-adjusted Serum Calcium

Table 3: *Average daily dose of hPTH(1-34) acetate during each week over the course of the 16-week treatment period*

	Mean (mg)	**SD**	**Range (mg)**
Week 1	3.0	0.0	(3.0 – 3.0)
Week 2	4.9	1.4	(3.0 – 6.0)
Week 3	7.2	2.5	(3.0 – 9.0)

Week 4	7.4	2.2	(3.8 - 9.0)
Week 5	7.4	2.2	(3.0 - 9.0)
Week 7	8.2	1.7	(3.0 - 9.0)
Week 9	8.7	0.6	(7.5 - 9.0)
Week 11	9.0	0.0	(9.0 - 9.0)
Week 13	9.0	0.0	(9.0 - 9.0)
Week 16	9.0	0.0	(9.0 - 9.0)

N=15

Table 4: *Summary of daily supplemental calcium dose (± SD) during each week over the course of the 16-week treatment period.*

	Mean (g)	**SD (g)**	**Median (g)**
Week 1	3.7	2.4	3.6
Week 2	3.6	2.4	3.3
Week 3	3.4	2.4	3.0
Week 4	3.0	2.1	3.0
Week 5	3.0	2.1	2.4
Week 6	2.9	2.1	2.4
Week 7	2.8	2.1	2.4
Week 8	2.7	2.2	2.4
Week 9	2.6	2.1	2.4
Week 10	2.5	2.1	2.4
Week 11	2.4	2.1	2.4
Week 12	2.4	2.1	2.4

Week 13	2.4	2.1	2.4
Week 14	2.4	2.1	2.4
Week 15	2.4	2.1	2.4
Week 16	2.3	2.1	2.2

N=15

Table 5: *Main pharmacokinetic parameters measured for the first two doses of Oral hPTH(1-34) 0.75 mg on Day 1 of the study*

Pharmacokinetic Parameter	**Dose 1 (N=19)**	**Dose 2 (N=19)**
C_{max} median (range), (pg/mL)	47.9 (14.5 - 427.2)	41.2 (5.6 - 213.4)
T_{max} median (range), (minutes)	20.0 (10.0 - 45.0)	30.0 (10.0 - 90.0)
AUC_{last} median (range), (pg/L*hours)	70.9 (7.6 – 638.9)	52.6 (2.4 – 746.8)
$T_{1/2}$ mean ± SE (minutes)	21.1 ± 2.9[a]	27.5 ± 5.3[a]

[a]N=18

SE=standard error

FIGURES

Figure 1. Change from baseline daily calcium supplement requirements and serum ACa levels. A*. Box plot represents the percent of baseline supplemental calcium doses for subjects (N=15) treated with Oral hPTH(1-34) at the beginning of each treatment week (16 weeks). Bold line represents the median values. Whiskers represent the minimum and maximum values. At weeks 4-16, the percent change from baseline supplemental calcium doses was statistically significant ($p \leq 0.006$). P values for percent change from baseline for each visit were calculated using Wilcoxon signed-rank test.* ***B****. Box plot represents serum ACa levels (N=15) at each study visit (the last of which occurred at the beginning of week 17). Bold line represents the median values. Whiskers represent the minimum and maximum values.*

Figure 2. Median and Individual Serum Albumin adjusted Calcium (ACa) and Mean hPTH(1-34) levels following each of the first two doses of Oral hPTH(1-34). *Following the first and second dose of 0.75 mg hPTH(1-34) on Day 1, in the clinic, blood samples were collected at predetermined intervals for hPTH(1-34) and ACa analysis. The median (bold line) ACa profile (N=18) is superimposed on the geometric mean (dashed line) drug pharmacokinetic profile (N=19). See Table 5 for complete pharmacokinetic data analysis. One subject was omitted from the ACa analysis due to their pre-dose hypercalcemic levels.*

Figure 3. Median and Individual Phosphate and Mean hPTH(1-34) levels following each of the first two doses of Oral hPTH(1-34). hPTH(1-34). *Following the first and second dose of 0.75 mg hPTH(1-34) on Day 1, in the clinic, blood samples were collected at predetermined intervals for hPTH(1-34) and serum phosphate analysis. The median (bold line) phosphate profile (N=17) is superimposed on the geometric mean (dashed line) drug pharmacokinetic profile (N=19). See Table 5 for complete pharmacokinetic data analysis. Two subjects were*

omitted from the serum phosphate analysis, one due to pre-dose hypercalcemic levels and the second was on a phosphate binder medication. The grey dashed line represents the upper level of the reference range for serum phosphate in adults (4.5 mg/dL).

Figure 4. Serum phosphate levels following Oral hPTH(1-34) administration. *At each treatment visit over the 16-week treatment period, blood sampling for serum phosphate analysis was performed before and 1-hour after Oral hPTH(1-34) administration. Box plot represents the serum phosphate levels at T=0 and T=60 minutes post-dose at each study visit (the last of which occurred at the beginning of week 17). Whiskers represent the minimum and maximum values. The grey dashed line represents the upper level of the reference range for serum phosphate in adults (4.5 mg/dL). The bold line shows the change in median phosphate levels from T=0 to T=60 per visit.* $p \leq 0.04$ *when comparing pre-dose to one-hour post-dose values at each study visit. P values were calculated using t test for paired samples.*