Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

Efficacy and Safety of Encaleret in Autosomal Dominant Hypocalcemia Type 1

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Supplementary Text

METHODS

TRIAL DESIGN

This was a single-site, open-label, dose-ranging, and dose-maintenance Phase 2b trial conducted at the National Institutes of Health (NIH), Bethesda, MD, USA.

The trial enrolled two cohorts over three periods: Period 1 (dose escalation, 5-day, inpatient, Cohort 1); Period 2 (dose adjustment, 5-day, inpatient, Cohorts 1 and 2); Period 3 (dose maintenance, 24 weeks, outpatient monitoring with three 1-day inpatient visits at 8, 16, and 24 weeks, Cohorts 1 and 2) [**Fig. S1**]. Serial blood and urine sampling were performed during inpatient visits with additional outpatient testing. Participants had the option of enrolling in a long-term extension (LTE; approximately 25 months) after Period 3.

PARTICIPANTS

Inclusion Criteria

Participants met the following criteria for inclusion during screening:

1. Able to understand and sign a written informed consent or assent form, which must be obtained prior to initiation of study procedures
2. Age ≥16 years
3. Postmenopausal women were allowed to participate in this study:
    a. Women were considered postmenopausal and not of childbearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g., age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least 6 weeks prior to start of the study. In the case of oophorectomy alone, only when the reproductive status of the woman was confirmed by follow-up hormone level assessment, was she considered not of childbearing potential
4. Body mass index (BMI) ≥18.5 to <39 kg/m^2
5. Had an activating variant of the calcium-sensing receptor gene (*CASR*) or had a first-degree relative with a documented *CASR* variant

6. Participants treated with thiazide diuretics were allowed to be enrolled if they were willing and able to discontinue thiazides for at least 5 half-lives prior to initiation of encaleret and during the study treatment period. When the thiazide was being used as an antihypertensive, alternative therapy was to be offered
7. Participants treated with strong cytochrome P450 (CYP) 3A4 inhibitors (including clarithromycin, telithromycin, nefazodone, itraconazole, ketoconazole, atazanavir, darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir) should have ideally, if clinically appropriate, discontinued these medications during the screening period for at least 5 half-lives prior to initiation of encaleret. Participants who needed to remain on strong CYP3A4 inhibitors were allowed to be enrolled if they were able to remain on their medications at stable doses throughout the trial
8. Participants treated with magnesium or potassium citrate supplements should have discontinued such treatment starting on Day -1 during Period 1 and Period 2 and might have been asked to discontinue treatment during Period 3 if the blood magnesium and urine citrate were within the normal ranges during Period 1 and Period 2

Exclusion Criteria

Participants who met any of the following criteria during screening were not eligible to participate in the study:

1. History of treatment with parathyroid hormone (PTH) 1–84 or 1–34 within the previous 3 months
2. History of hypocalcemic seizure within the past 3 months
3. Blood 25-OH vitamin D level <25 ng/ml
    a. If participants had a blood 25-OH vitamin D level <25 ng/ml at the screening visit, they were prescribed cholecalciferol or ergocalciferol supplementation. Once the 25-OH vitamin D level was >25 ng/ml, the participants were eligible to continue to the treatment phase of the study
4. Participants with hemoglobin (Hgb) <13 g/dl for men and <12 g/dl for women
    a. If participant had a low Hgb at the screening visit due to iron, B_{12}, or folate deficiency, they were prescribed supplementation. Once the Hgb level was >13 in men or >12 in women, the participants were eligible to continue to the treatment phase of the study.

5. Abnormal laboratory values which, in the opinion of the investigator, would have made the participant not suitable for participation in the study
6. Estimated glomerular filtration rate (eGFR) <25 ml/min/1.73 m^2 using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI; for participants <18 years old the Schwartz equation was to be used)
7. 12-lead resting electrocardiogram (ECG) with clinically significant abnormalities
8. Participants with positive hepatitis B surface antigen (HBsAg), hepatitis A immunoglobulin M (IgM), or human immunodeficiency virus (HIV) viral serology test results at the screening visit. Participants who were in complete remission from hepatitis C virus (HCV) as evidenced by sensitivity assay ≥12 weeks after completion of HCV therapy were allowed to participate in the study
9. Pregnant or nursing (lactating) women, where pregnancy was defined as the state of a female after conception and until the termination of gestation, confirmed by a positive serum human chorionic gonadotropin (hCG) laboratory test
10. Women of childbearing potential, defined as all women physiologically capable of becoming pregnant, unless they were using highly effective methods of contraception during dosing and for 3 months following the discontinuation of study treatment. Highly effective contraception methods include:
    a. Total abstinence (when this was in line with the preferred and usual lifestyle of the participant). Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal were not acceptable methods of contraception
    b. Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy) or tubal ligation at least 6 weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman had been confirmed by follow-up hormone level assessment
    c. Male sterilization (at least 6 months prior to screening). For female participants on the study, the vasectomized male partner should have been the sole partner for that participant
    d. Combination of the following (i+ii, i+iii, or ii+iii):
        i. Use of oral, injected, or implanted hormonal methods of contraception or other forms of hormonal contraception that have

comparable efficacy (failure rate <1%); for example, hormone vaginal ring or transdermal hormone contraception

ii. Placement of an intrauterine device (IUD) or intrauterine system (IUS)

iii. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository

11. Sexually active male participants who were unwilling to use a condom during vaginal intercourse while taking encaleret and for 3 months after the last dose of the study drug. Participants should have not fathered a child during active participation in the study starting with the first encaleret dose in Period 1 or Period 2 (for Cohort 2) until the end of study participation. Condoms were not required if the participant was vasectomized or if the participant's partner was not a woman of childbearing potential.
12. Hypersensitivity to any active substance or excipient of encaleret
13. History of drug or alcohol dependency within 12 months preceding the screening visit
14. History of thyroid or parathyroid surgery
15. Current participation in other investigational drug studies
16. Unwillingness to refrain from blood donation within 12 weeks prior to screening visit from the start of the study enrollment through 1 year after the last dose of the study drug

ENCALERET SULFATE DOSING

Dosing of encaleret sulfate in participants with autosomal dominant hypocalcemia type 1 (ADH1) in this trial (**Fig. S1A**) was informed by prior experience of both acute and chronic dosing of encaleret sulfate in the osteoporosis program that included exposures up to 1 year, primarily in postmenopausal women with presumed normal CaSR function.[1,2] The proposed starting dose was 30 mg in ADH1 because this is a dose proven to result in acute elevations in albumin-corrected blood calcium (cCa) in euparathyroid participants without an ADH1-associated *CASR* variant, and this is a dose previously demonstrated as safe and well tolerated.[1,2]

Encaleret sulfate was tested both as a once-daily (QD) and twice-daily (BID) dose. In the prior osteoporosis program, encaleret sulfate administered QD stimulated a transient increase in intact parathyroid hormone (iPTH) secretion within 2 hours and a second lower plateau level of iPTH (still elevated compared with baseline) for up to 12 hours at doses of 30 mg and above. Despite pharmacokinetic (drug exposure) and pharmacodynamic (increased iPTH levels) profiles that lasted less than 24 hours, some evidence for increased trough cCa (measured 24 hours after last dose) in the osteoporosis development program suggested that QD dosing and QD dose escalation should be tested in participants with ADH1. Calcitriol was discontinued 1–2 days prior to encaleret initiation, and calcium supplements were discontinued the night before the first encaleret dose. Participants were instructed to consume at least 1000 mg dietary calcium daily; supplements were permitted if this was not achieved.

Period 1

On Days 1–3, Cohort 1 participants underwent pre-breakfast daily dosing and protocol-specified dose-escalation of encaleret sulfate from the initial dose of 30 mg on Day 1, increasing to a maximum dose of 180 mg on Day 3. Based on the individualized responses to QD dose escalation an individualized dose of encaleret sulfate of up to 180 mg was chosen for BID administration on Days 4 and 5. BID dosing was given with water within 30 minutes prior to breakfast and dinner. After completion of Period 1, there was a washout period of at least > 8-12 weeks prior to enrollment in Period 2 during which time Cohort 1 received their usual doses of calcium and calcitriol.

Period 2

In Period 2, participants were administered encaleret sulfate BID with water within 30 minutes prior to breakfast and dinner. The initial starting dose in Period 2 of 180 mg BID was the dose in Period 1 at which all participants achieved normal cCa and 24-hour urinary calcium excretion (uCa). However, the 180 mg BID encaleret sulfate dose was found to be too high for most participants, and the starting dose was therefore decreased to 90 mg BID after the first four participants in Period 2. Serial blood and urine sampling occurred throughout Period 2 as illustrated by data collection points in **Figure 1, Figure S4** and **Figure S5**. cCa concentrations were monitored frequently throughout Period 2, and encaleret sulfate doses were up- or down-titrated as needed in 10, 30, or 60 mg

increments to target normal cCa concentrations. Time points shown for Period 2 in the **Figure 1** line graphs (except in Panel C) are as follows: for days 1 through 4, 15 minutes before the morning dose and 30 minutes, 2 hours, 4 hours, 8 hours, 11 hours, 13 hours, and 17 hours after the morning dose; and for day 5, 15 minutes before the morning dose and 1.5 hours, 4 hours, and 8 hours after the morning dose and 30 minutes, 1.5 hours, 4 hours, 8 hours, and 15 hours after the afternoon dose.

Period 3

Initial outpatient doses of encaleret sulfate were carried over from Period 2 but were more flexibly timed. Period 3 included a titration phase of approximately 12 weeks and a maintenance phase of approximately 12 weeks for a total outpatient exposure to encaleret sulfate of 24 weeks. Encaleret sulfate continued to be up- or down-titrated as needed, usually adjusting by 10 or 30 mg increments, with up to 60 mg permitted to achieve normal cCa concentrations. The goal was to optimize the encaleret sulfate dose without calcitriol, targeting normal cCa and phosphorus concentrations, avoiding symptoms of hypo- or hypercalcemia, and minimizing the extent of hypercalciuria. No participants required calcitriol.

Dose-Limiting Toxicity

Dose-limiting thresholds for post-encaleret sulfate cCa levels were specified as part of the dosing and titration. Participants who achieved a post-dose cCa >10–10.5 mg/dl (Period 1) and cCa >10 mg/dl (Period 2) during the dose escalation did not proceed to higher encaleret sulfate doses. Participants with cCa values between 10 and 10.5 mg/dl during dose escalation in Period 1 had their doses increased by 30 mg only. Participants who achieved cCa >10 mg/dl in Period 2 had their dose reduced to a lower level. If blood cCa levels were >10.5 mg/dl, the encaleret sulfate dose could be held and restarted at a lower dose after blood cCa decreased to <10 mg/dl.

BIOCHEMICAL ASSESSMENTS

Routine blood and urine chemistries were performed at the National Institutes of Health (NIH) Clinical Center Department of Laboratory Medicine during screening and inpatient stays (Period 1, Period 2, and Period 3 Weeks 8, 16, and 24). Laboratory testing at other

visits was performed at local laboratories and some 24-hour urine collections were assessed at Litholink Corporation (Chicago, IL, USA).

Biochemical testing included iPTH (electrochemiluminescence immunoassay on Roche Cobas e601 analyzer; NIH Clinical Center, Bethesda, MD, USA), 1,25-$(OH)_2$ vitamin D (chemiluminescent immunoassay on DiaSorin Liaison XL; NIH Clinical Center), 25-hydroxyvitamin D (chemiluminescent immunoassay; NIH Clinical Center), serum collagen C-telopeptide (CTx; electrochemiluminescence immunoassay Elecsys Beta-CrossLaps Roche Diagnostics; Mayo Medical Laboratories Rochester, MN, USA), serum procollagen Type 1 N-propeptide (P1NP, competitive radioimmunoassay UniQ P1NP RIA Orion Diagnostica; Mayo Medical Laboratories), and 24-hour urine citrate excretion (enzymatic assay; Mayo Medical Laboratories).

Encaleret levels were measured by Worldwide Clinical Trials (Austin, TX, USA). Plasma samples were analyzed for encaleret concentration by a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method using a Sciex API 5000 LC-MS/MS (Applied Biosystems, Foster City, California) equipped with a high-performance liquid chromatography column. The method was validated over a range of 2.00 to 1000 ng/ml for each analyte based on the analysis of 0.100 ml of plasma. Human plasma samples containing encaleret, and internal standard encalaret-D6 were extracted using protein precipitation. Samples were chromatographed on a Kinetex 2.6 u F5, 3.0 x 100 mm column (Phenomenex, Torrance, CA, USA) with SecurityGuard ULTRA F5, 3.0 mm ID cartridge. Samples were eluted using a gradient between water/acetic acid/ammonium trifluoroacetate (1000:2.0:0.262, v:v:w) and acetonitrile/methanol/acetic acid/ammonium trifluoroacetate (500:500:2.0:0.262, v:v:v:w). The peak area of the m/z 514.2→239.2 encaleret product ion was measured against the peak area of the m/z 520.2→239.2 encaleret-D6 internal standard product ion. Quantitation was performed using a weighted 1/x2 linear least squares regression analysis, for each analyte, generated from calibration standards prepared on the day of extraction.

TRIAL ENDPOINTS

Primary endpoints in Periods 1–3 were adverse events (AEs), safety laboratory tests, vital signs, and ECGs (including Fridericia-corrected Q-T interval [QTcF]); Period 3 primary endpoints included albumin-corrected blood calcium (cCa) and 24-hour urinary calcium excretion (uCa) after 24 weeks of encaleret.

Secondary endpoints included: pharmacokinetics, blood intact PTH (iPTH), cCa (Periods 1 and 2); and for all Periods: creatinine, eGFR, 1,25-$(OH)_2$ vitamin D, magnesium, phosphorus; timed interval and/or 24-hour urine for magnesium, phosphorus [tubular maximum reabsorption of phosphate/glomerular filtration rate (TmP/GFR); tubular reabsorption of phosphorus (TRP)], creatinine, and citrate; serum bone turnover markers collagen cross-linked C-telopeptide (CTx) and procollagen Type 1 N-propeptide (P1NP). Urine citrate was included because previous studies evaluating PTH 1-34 replacement therapy in hypoparathyroidism demonstrated a significant decrease in urine citrate excretion that was associated with an increase in new or worsening renal calcifications.[3] Change from baseline to scheduled time points in nephrocalcinosis and/or nephrolithiasis as assessed by renal ultrasound was an exploratory endpoint. Ultrasounds were evaluated by radiologists not involved in the trial. An additional exploratory endpoint included bone mineral density (BMD; assessed by dual-energy x-ray absorptiometry [DXA], Hologic, Waltham, MA, USA).

Baseline values for blood parameters were measured 15 minutes prior to the first encaleret dose in Period 2, except for P1NP and CTx, for which baseline was 15 minutes pre-AM encaleret dose on Period 2 Day 5 (P2D5). Of note, bone turnover markers were collected prior to beginning encaleret and throughout Period 1 for Cohort 1 (n=6), with no change seen during the 5-day Period 1. To reduce the volume of blood collected during Period 2, bone turnover markers were tested only on the last day of Period 2, which included both cohorts 1&2 (n=13). Baseline values for 24-hour urine parameters were collected at screening, except for urine citrate for which baseline was Period 2 Day 1 (P2D1). See figure legends for details.

STATISTICAL ANALYSES

Pharmacokinetic and pharmacodynamic parameters were calculated for each participant and summarized by treatment. All data were descriptively analyzed. No formal statistical

tests were performed on efficacy variables; however, *post hoc* analyses were carried out comparing baseline values to those at the end of the 5-day inpatient (P2D5) and the 24-week outpatient (Period 3 Week 24 [P3W24]) periods. Baseline characteristics (Table S2) are presented as mean±SD or with counts and percentages. Dosing data (Figure S2) are presented as mean±SD. Remaining data are presented as mean and 95% CI. The reported CIs have not been adjusted for multiplicity and cannot be interpreted as hypothesis tests.

A sample size of up to 8 participants in Cohort 1 and up to 10 additional participants in Cohort 2 was selected based on several considerations, including exploration of dose range for safety and tolerability. To confirm definitive proof-of-concept on either iPTH and/or blood calcium levels, the proposed sample size of approximately 16 participants was considered sufficient, if effective doses were achieved. The sample size was not based on statistical testing of a formal powered hypothesis. Because ADH1 is a rare disease and the response of the first 13 participants enrolled was robust, enrollment was closed to additional subjects with the plan to proceed with a registrational Phase 3 trial.

No sensitivity analyses or subgroup analyses were performed given the single-site trial design. No adjustments for multiplicity were made.

TRIAL OVERSIGHT

The NIH Institutional Review Board approved the protocol. Written informed consent was obtained from participants. The trial was conducted in accordance with the Declaration of Helsinki and the International Council for Harmonisation Guideline for Good Clinical Practice and was registered with ClinicalTrials.gov (NCT04581629).

RESULTS

PARTICIPANTS

Thirteen adults, aged 21–60 years, with ADH1 due to nine different *CASR* variants were screened and enrolled. Ten of the participants were existing NIH patients known to the study team; the other three participants included a relative of an existing participant, a local patient referred by an endocrinologist, and an individual who learned of the study through a patient support group. While conducting this study, we performed a systematic review to better characterize the genetics and natural history of people with ADH1[4]. This review was utilized to describe the general population characterizes of people with ADH1 (**Table S1**).

Cohort 1 (n=6) participated in Period 1. Cohorts 1 and 2 (n=7) participated in Periods 2 and 3. All participants completed the periods in which they enrolled, entered the LTE (**Fig. S1B**), and were included in the efficacy and safety analyses. Baseline characteristics prior to Periods 1 and 2 were typical for ADH1 (**Table S2**), with low mean cCa and iPTH, and elevated uCa. Mean blood 1,25-$(OH)_2$ vitamin D and magnesium were near the LLN; blood phosphorus was at the upper limit of normal (ULN). Most participants (69.2%) had renal calcifications.

DOSING

Participants received predetermined escalating encaleret sulfate doses in Period 1 ranging from 30 mg QD to 180 mg BID (**Fig. S1A**). The evening dose on Day 5 was reduced (120 mg) in one participant due to cCa approaching the ULN. The mean±SD total daily encaleret sulfate dose on Period 1 Day 5 (P1D5) was 350.0±22.4 mg (range 120 mg BID to 180 mg BID). In Period 2, individually titrated doses of encaleret sulfate ranged from 10 mg to 180 mg BID with a mean±SD total daily encaleret dose of 187.7±128.2 mg on Period 2 Day 5 (P2D5). At the end of Period 3 Weeks 8 (P3W8), 16 (P3W16), and 24 (P3W24), the mean encaleret sulfate doses were 187.7±152.2 mg (P3W8; 10 mg QD to 180 mg BID), 168.5±138.0 mg (P3W16; 10 mg QD to 180 mg BID), and 171.5±140.1 mg (P3W24; 5 mg BID to 190 mg BID), respectively (**Fig. S2**). The median (range) dose was 70 mg (5–190 mg) BID at the end of Period 3. Participants did not take calcium or calcitriol while receiving encaleret treatment except for two participants who could not meet the

daily dietary calcium minimum of 1000 mg: one received 300 mg elemental calcium daily during Period 1 and one received 500 mg elemental calcium daily during Period 3.

Pharmacokinetics

The pharmacokinetic response to encaleret during Period 1 is shown in **Figure S3** and demonstrated a dose-proportional increase in plasma exposure. Overall, the pharmacokinetic data were highly consistent across patients when administered the same encaleret sulfate dose; therefore, differences in dose requirements across these 13 participants could not be explained by differences in encaleret absorption or metabolism.

EFFICACY

Encaleret treatment normalized mineral homeostasis in participants with ADH1 in Periods 1–3 (**Figs. 1**, **S4, S5**, and **S6**).

Baseline hypocalcemia corrected during Period 2 with mean cCa remaining normal throughout Period 3 (**Fig. 1 A1** and **A2)**. Mean iPTH, which was below the LLN at baseline, increased immediately with treatment and remained normal throughout Period 3 (**Fig. 1 B1** and **B2)**. Of note, the initial iPTH peaks on encaleret initiation in Period 2 decreased as encaleret dosing was individually optimized. Elevated mean uCa at screening decreased by P2D5 and was maintained through P3W24 (**Fig. 1 C1** and **C2**), remaining normal in 9 patients throughout Periods 2 and 3.

Mean blood phosphorus was near the ULN at baseline, decreasing by P2D5 and remaining normal throughout Period 3 (**Fig. 1 D1** and **D2**). Intermittent decreases in phosphorus below the LLN during Period 2 resolved as encaleret was titrated. Baseline Period 2 blood magnesium levels increased from LLN by P2D5 and were maintained at P3W24 (**Fig, 1 E1** and **E2**). Mean TmP/GFR and TRP%, which were near the ULN at baseline, decreased to below the LLN at the beginning of Period 2, and returned to the normal range by Period 3 (**Figs. S5A-B** and **S6A-B**). Baseline 1,25-$(OH)_2$ vitamin D levels increased from LLN by P2D5 and were maintained at P3W24 (**Fig. S5C and S6C**). Baseline mean 24-hour urine magnesium (uMg) was elevated, dropping below the LLN on P2D1 before returning to baseline, and ultimately re-equilibrating near the ULN during Period 3 (**Figs. S5D** and **S6E**). Mean eGFR remained unchanged and within the normal range (**Fig. S6D**). Mean citrate excretion decreased initially at P2D5 but was unchanged

from baseline at P3W24 (**Fig. S6F**). QTcF on ECG decreased from baseline throughout Periods 2 and 3 (**Fig S6G**). Neither the prevalence nor severity of nephrocalcinosis or nephrolithiasis on ultrasound changed over the course of the study (**Table S3**).

CTx and P1NP increased from baseline during Period 3; 9 participants remained in the age- and sex-appropriate normal ranges (**Fig. S7**). To allow for the aggregate visualization of the effects of encaleret treatment on bone turnover markers given the age- and sex-specific differences in normal ranges, the data are displayed as the ratio of the measured value relative to the ULN for each individual (**Fig**. **S7A and C**). Bone turnover markers are also shown as absolute values (**Fig**. **S7B and D**). Encaleret had clinically negligible short-term effects on mean BMD Z-scores measured by DXA (**Table S4**).

SAFETY

Encaleret was well tolerated with no safety signals of potential clinical concern observed. All participants experienced mild AEs; moderate AEs were reported by one participant in Period 1 and two participants in Period 3 [**Table S5**]. No serious AEs were reported. No treatment discontinuations, study withdrawals, or deaths occurred. Treatment-related hypophosphatemia (phosphorus <2 mg/dl) was noted in 2 (33.3%) participants in Period 1, eight (61.5%) in Period 2, and one (7.7%) in Period 3. Treatment-related hypercalcemia (cCa >10.2 mg/dl) was noted in one (7.7%) participant in Period 2 and five (38.5%) participants in Period 3. Participants were asymptomatic during all treatment-related AEs; cCa never exceeded 10.9 mg/dl. Treatment-related AEs were transient and resolved either spontaneously or with encaleret dose adjustment. There were no clinically meaningful changes in safety laboratory tests, vital signs, or other ECG intervals (data not shown). No participant experienced a seizure or fracture during the study.

DISCUSSION

Individually titrated encaleret was well tolerated and restored mineral homeostasis in adults with ADH1 through 24 weeks of treatment, as evidenced by normalization in iPTH, cCa, uCa, blood phosphorus, 1,25-$(OH)_2$ Vitamin D, and magnesium levels. This is the first trial to demonstrate clinical efficacy of a calcilytic in a human disease.

While conventional therapy for ADH1 can normalize cCa and improve neuromuscular symptoms, it usually exacerbates hypercalciuria, thus increasing the risk of renal complications.[4-6] This limitation of conventional therapy emphasizes the need for improved

treatment; that normal uCa levels were achieved with encaleret demonstrates the potential for encaleret to effectively treat ADH1.

Multiple tissues express the CaSR, however its primary physiological function is maintenance of mineral homeostasis.[7] This relative specificity makes the CaSR an ideal therapeutic target. Positive allosteric modulators of the CaSR (calcimimetics) have proven effective in treating primary and secondary hyperparathyroidism and were approved for use in 2004.[7-12] Because of their ability to increase PTH, calcilytics were initially investigated as a potential treatment for osteoporosis, but were ultimately abandoned, in part due to drug-induced hypercalcemia and hypercalciuria.[1,2,13,14] However, the PTH and calcium-raising effects suggest that they may be ideal for patients with ADH1. Animal and human proof-of-principle studies support this concept.[15-19]

Although encaleret absorption and metabolism were highly consistent across participants, the physiologic responses varied, as demonstrated by a wide, yet individually stable, range in doses needed to maintain eucalcemia and normal iPTH levels. While transient hypophosphatemia was noted in Periods 1 and 2 on higher encaleret doses, phosphate levels remained normal as doses were individualized. Moreover, the safety profile was favorable, with participants exhibiting mostly treatment-unrelated mild and transient AEs. There was no worsening in eGFR, nephrocalcinosis or nephrolithiasis after 24 weeks of treatment. This trial presented no safety signals of potential clinical concern compared with the prior clinical development program of encaleret in post-menopausal women with osteoporosis.[1,2]

In an earlier proof-of-concept trial, we demonstrated that the calcilytic NPSP795, administered by intravenous infusion over 4 days in five adults with ADH1, rapidly increased plasma PTH levels, consistent with this study.[19] However, there was marked variability in response to NPSP795 that was assumed to be due to the small number of participants representing only four *CASR* variants. Further, even at the highest levels of exposure, there was neither a sustained nor a statistically significant effect on renal calcium excretion or blood calcium with NPSP795. The lack of effect is likely explained by insufficient dosing of the drug coupled with its pharmacokinetic and pharmacodynamic properties. This is supported by evidence that four of the NPSP795 study participants, who later enrolled in the present study, responded robustly to encaleret.

A response to encaleret was observed in patients with nine different genetic variants, including those with variants that were minimally responsive to NPSP795, suggesting that encaleret may be effective across multiple genotypes. Notably, participants bearing the same *CASR* variant were related and had similar encaleret dosing requirements, suggesting either a direct genotype relationship to drug dose or other genetic factors. Unexpectedly, the participant who required the lowest dose of encaleret to maintain normal cCa was persistently hypercalciuric during Period 3. This individual, who had one of the highest UCa at baseline, also carries a *CASR* polymorphism (R990G) that has been associated with increased nephrolithiasis/hypercalciuria in otherwise healthy individuals[20] as well as increased sensitivity to calcimimetics in patients on hemodialysis.[21] These genotype-specific observations warrant further investigation.

In addition to conventional therapy with calcium and active Vitamin D, various PTH formulations have been studied in hypoparathyroidism.[22-24] PTH 1–84 was approved for the treatment of hypoparathyroidism, but studies specifically excluded patients with ADH1;[25] thus, it has not received approval by regulatory authorities for this condition. Some studies of PTH 1–34 included patients with ADH1.[22,24] One report described the effective treatment of three patients with ADH1 using subcutaneous injections of PTH 1–84,[26] and another reported the use of PTH 1–34 continuous subcutaneous infusion in six children with ADH1-associated hypocalcemic seizures.[27] In contrast, prior evaluation of off-label synthetic human PTH 1–34 administered 2–3 times daily as an injection or via continuous subcutaneous infusion in patients with ADH1 was unable to lower and normalize urine calcium in all patients.[22] One study with long-term PTH 1–34 treatment (14 years) of a patient with ADH1 showed that PTH treatment failed to prevent nephrocalcinosis and resulted in calvarial thickening with abnormal sclerotic and lytic changes.[28]

As expected in hypoparathyroidism, participants were in a low bone turnover state at baseline. CTx and P1NP were low, and BMD Z-scores were elevated at all anatomical sites, except the 1/3 distal radius. With increased PTH secretion on encaleret, bone turnover increased, consistent with other studies of PTH replacement in patients with hypoparathyroidism[22,24,29,30] and post-menopausal women with osteoporosis treated with encaleret.[1,2] In contrast to those studies where bone turnover markers exceeded the ULN, after 24 weeks of encaleret treatment bone turnover markers remained within the normal range for age, sex, and menopausal status in most participants. In three participants, bone

turnover markers were persistently above the ULN during Period 3; these participants did not have the highest PTH levels nor were they on the highest encaleret doses. While the cause for this is unknown, it is notable that two participants were fraternal twins with a potential genetic contributor, and the remaining participant with a different *CASR* variant, had mild hypogonadism. After 24 weeks of encaleret treatment, there were no noteworthy changes in BMD. Longer follow-up is needed to confirm these observations.

This study was limited by the open-label, single-arm design, and a small number of participants at a single-center. As our entire cohort was comprised of non-Hispanic white adults, we cannot say for certain that our findings are generalizable to children or individuals of other ethnicities or races. However, given what is known about the function of the CaSR, there is no reason to suspect that this medication would not be efficacious in most individuals with ADH1. Despite these limitations, the consistent biochemical and safety profile observed in all participants is highly encouraging. Future international, multi-center studies and continued follow-up in the long-term extension period will further inform the impact of encaleret treatment on renal calcifications and BMD, as well as its safety and efficacy in more diverse populations.

In conclusion, this trial represents a novel, molecularly targeted, precision medicine approach for the treatment of ADH1, restoring physiological mineral homeostasis including simultaneous normalization of blood and urine calcium. The consistent and sustained results from this Phase 2b trial establish a clinically meaningful efficacy, tolerability, and safety profile for encaleret as a potential treatment of individuals with ADH1.

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The authors assume responsibility for the accuracy and completeness of the data and analyses, as well as for the fidelity of the trial and this report to the protocol. All trial investigators involved in participant care and data acquisition are authors of this manuscript. The authors and the sponsor, BridgeBio Pharma Inc. affiliate Calcilytix

Therapeutics, Inc., participated in the trial design and collection, analysis, and interpretation of the data.

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A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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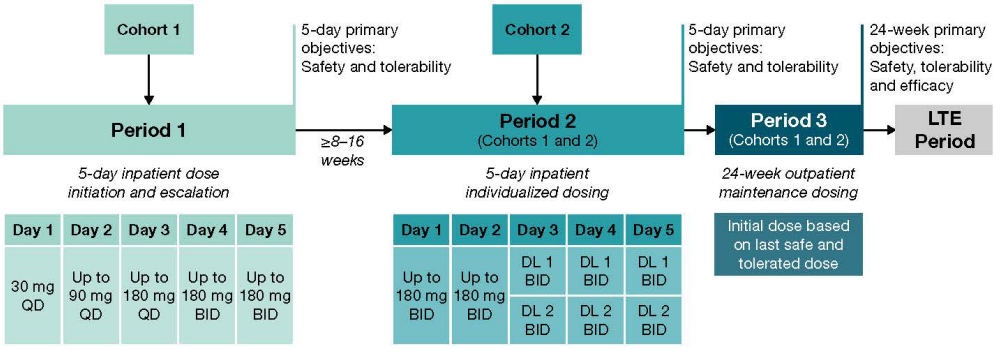
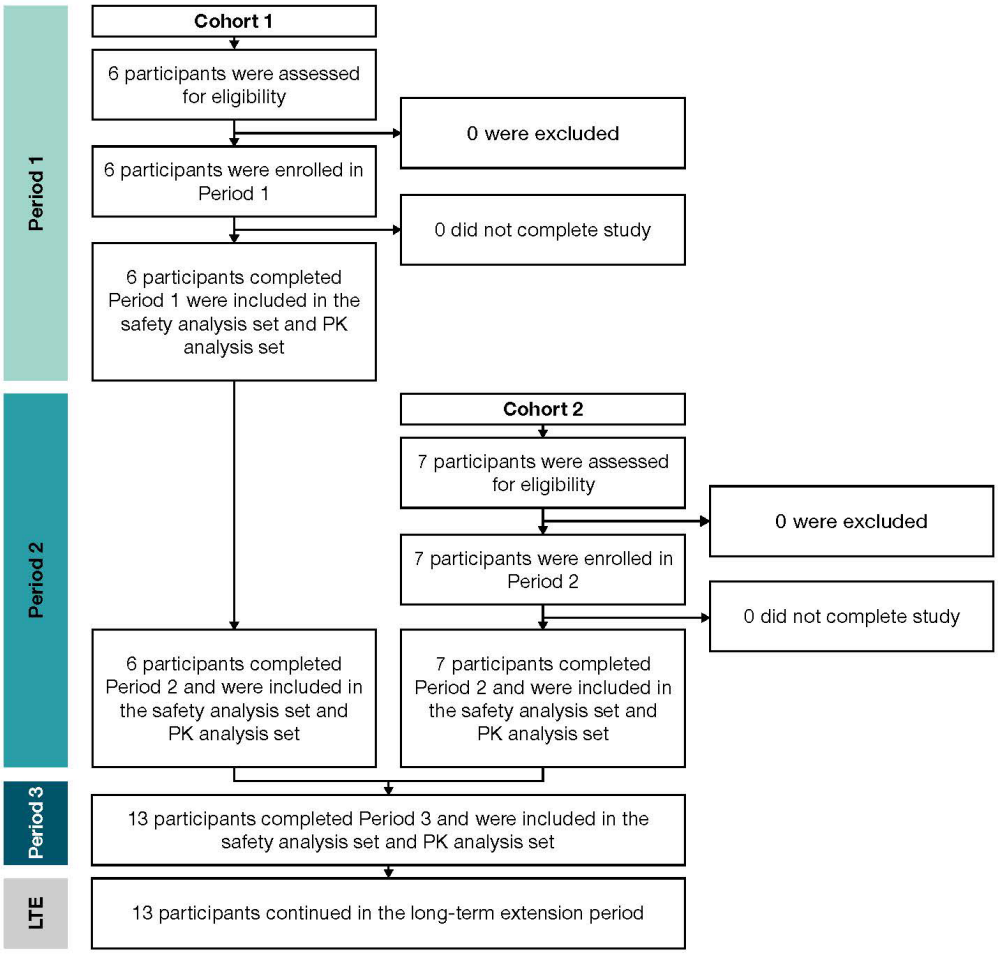
Supplementary Figures

Figure S1. Trial Design and Participant Enrollment

A Trial Design



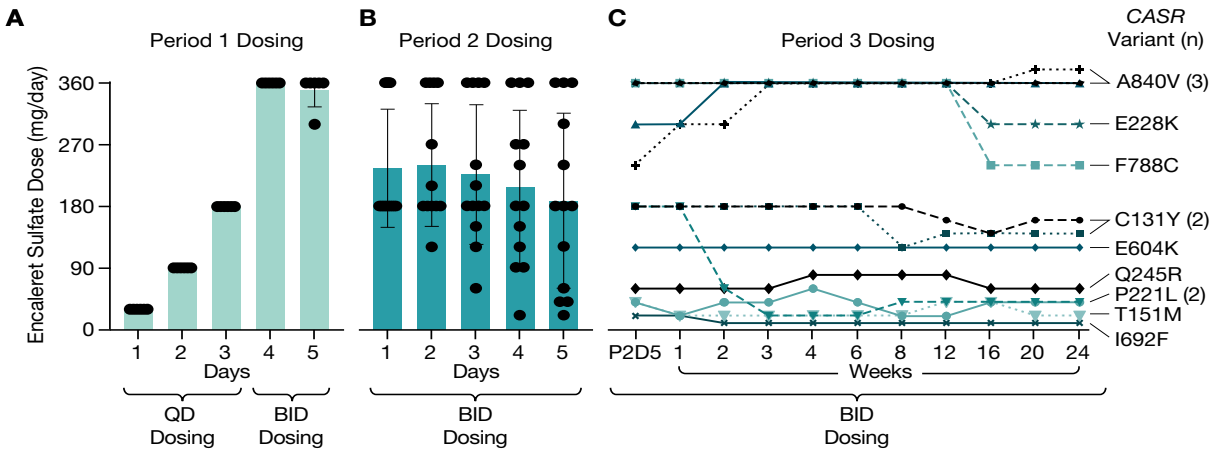
B Participant Enrollment



Panel A: Participants were assigned to Cohort 1 or 2. In Period 1, Cohort 1 participants received encaleret sulfate dosing of 30, 90, and 180 mg QD on Days 1–3, and up to 180 mg BID on Days 4–5. After completion of Period 1, Cohort 1 participants were eligible to enter Period 2, and additional encaleret-naïve participants could enter directly into Period 2 as Cohort 2. In Period 2, the encaleret sulfate BID starting dose was informed by results from Period 1, and the encaleret sulfate BID dose was titrated as needed with the goal of attaining a normal cCa level. Following Period 2, participants were eligible to enter Period 3, in which encaleret sulfate BID doses could continue to be titrated as needed to achieve a normal cCa. Participants had the option to enroll in a long-term extension period (approximately 25 months) following completion of Period 3. Panel B: All enrolled trial participants completed each of their assigned periods and continued in the long-term extension period. Period 1 (Cohort 1; n=6) occurred from 27 September to 6 December 2020. Cohort 1 and Cohort 2 (n=7) were enrolled in Period 2 (January 3 to August 1, 2021). The first Period 3 visit occurred January 9, 2021 with the last participant completing on January 24, 2022.

ADH1, autosomal dominant hypocalcemia type 1; BID, twice daily; cCa, albumin-corrected blood calcium; DL, dose level; LTE, long-term extension, PK, pharmacokinetic; QD, once daily.

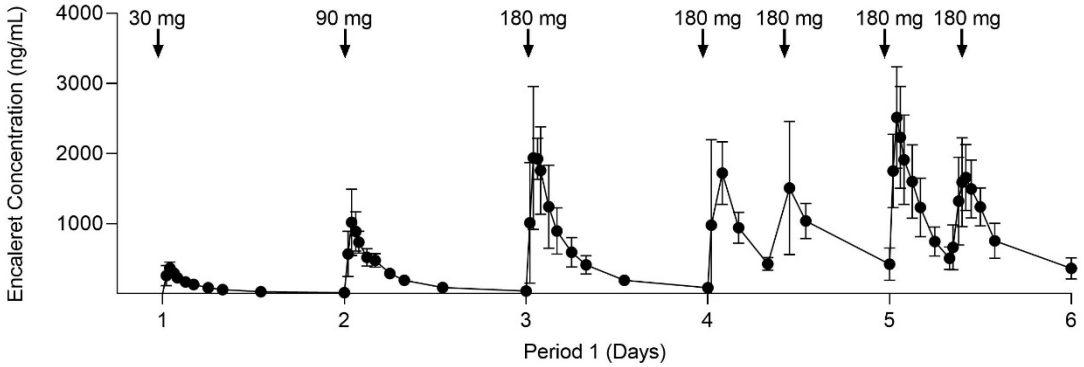
Figure S2. Encaleret Sulfate Dosing



Period 1 consisted of dose escalation (mean±SD) [Panel A]; Period 2 individualized dose titration (mean±SD) [Panel B]; Period 3 individual participant dosing (Panel C). Of note, all the participants with the same variant were related, thus there may have been other genetic modifiers contributing to similar dosing requirements.

BID, twice daily; *CASR*, calcium-sensing receptor gene; P2D5, Period 2 Day 5; QD, once daily; SD, standard deviation.

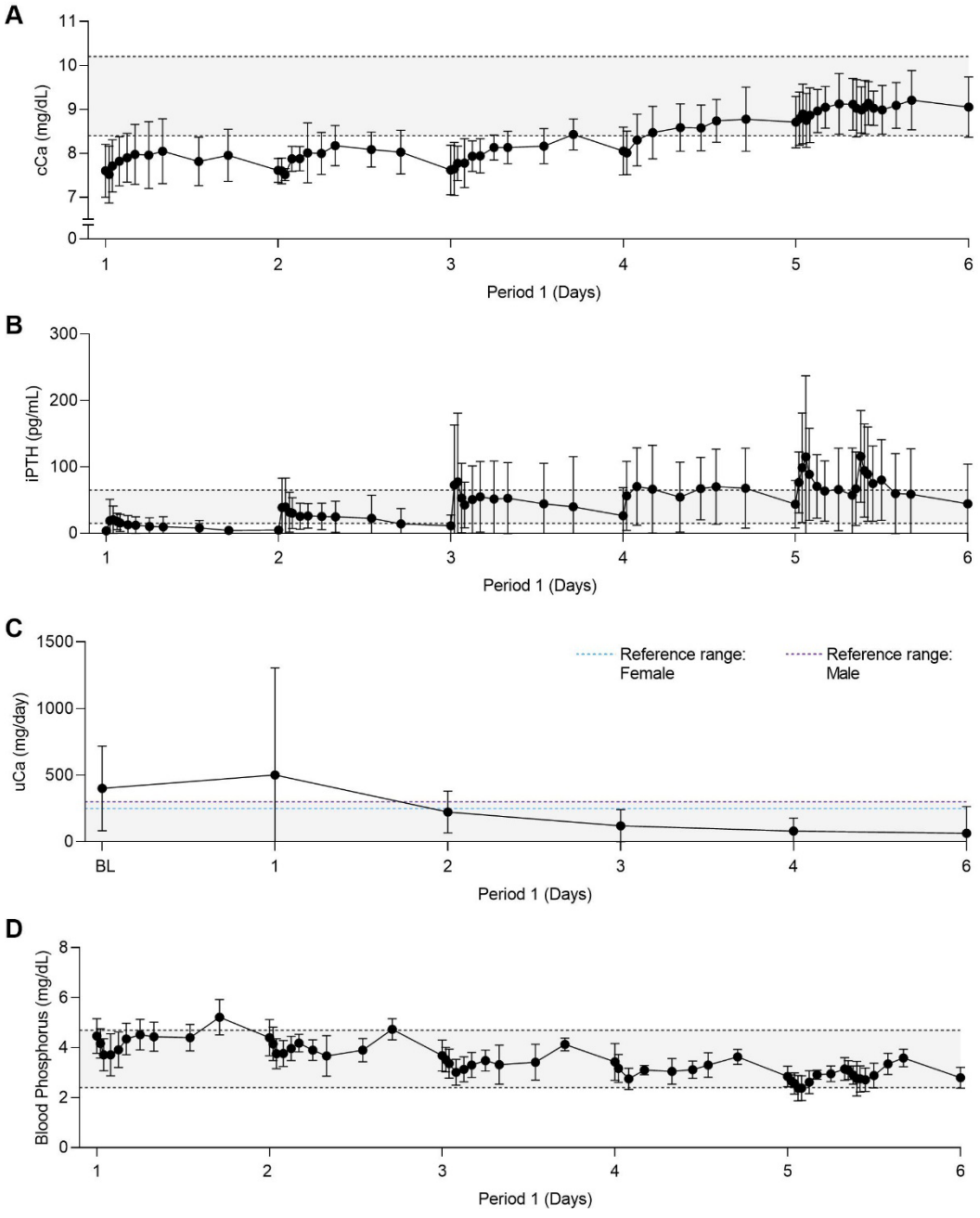
Figure S3. Period 1 Plasma Encaleret Concentrations

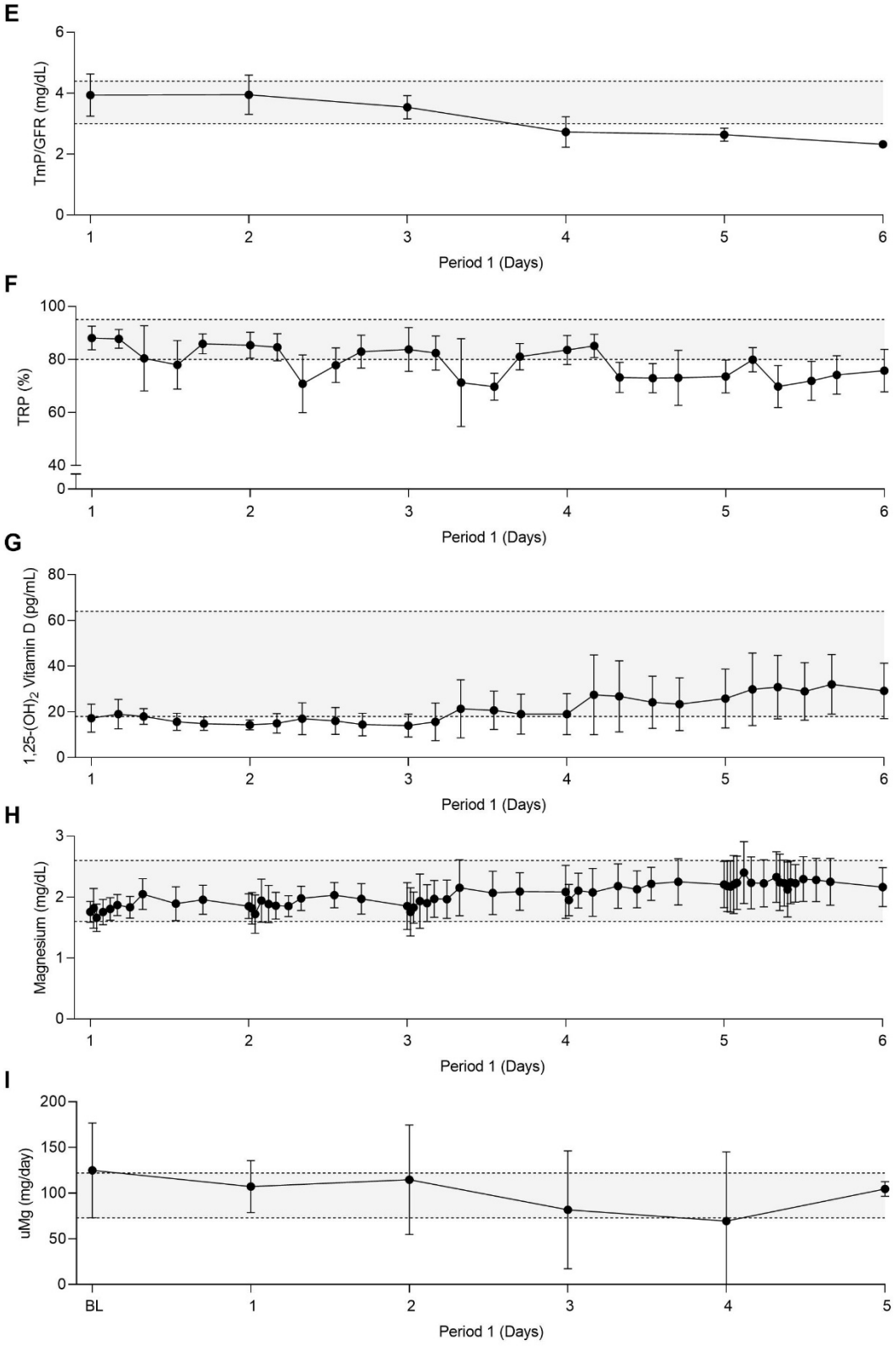


Time points shown are Day 1–3: 15 min pre-dose, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 13 h post-dose, Day 4: 15 min pre-AM dose, and 30 min, 2 h, 4 h, 8 h, 11 h, and 13 h post-AM dose, Day 5: 15 min pre-AM dose, 30 min,1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, and 8 h post-AM dose, and 30 min, 1 h 1.5 h, 2 h, 3 h, 4 h, 6 h, and 15 h post-PM dose. Graph and error bars displayed are mean and 95% CI.

AM, morning; CI, confidence interval; PM, afternoon

Figure S4. Effect of Encaleret on Mineral Homeostasis in Period 1 (Dose Escalation)

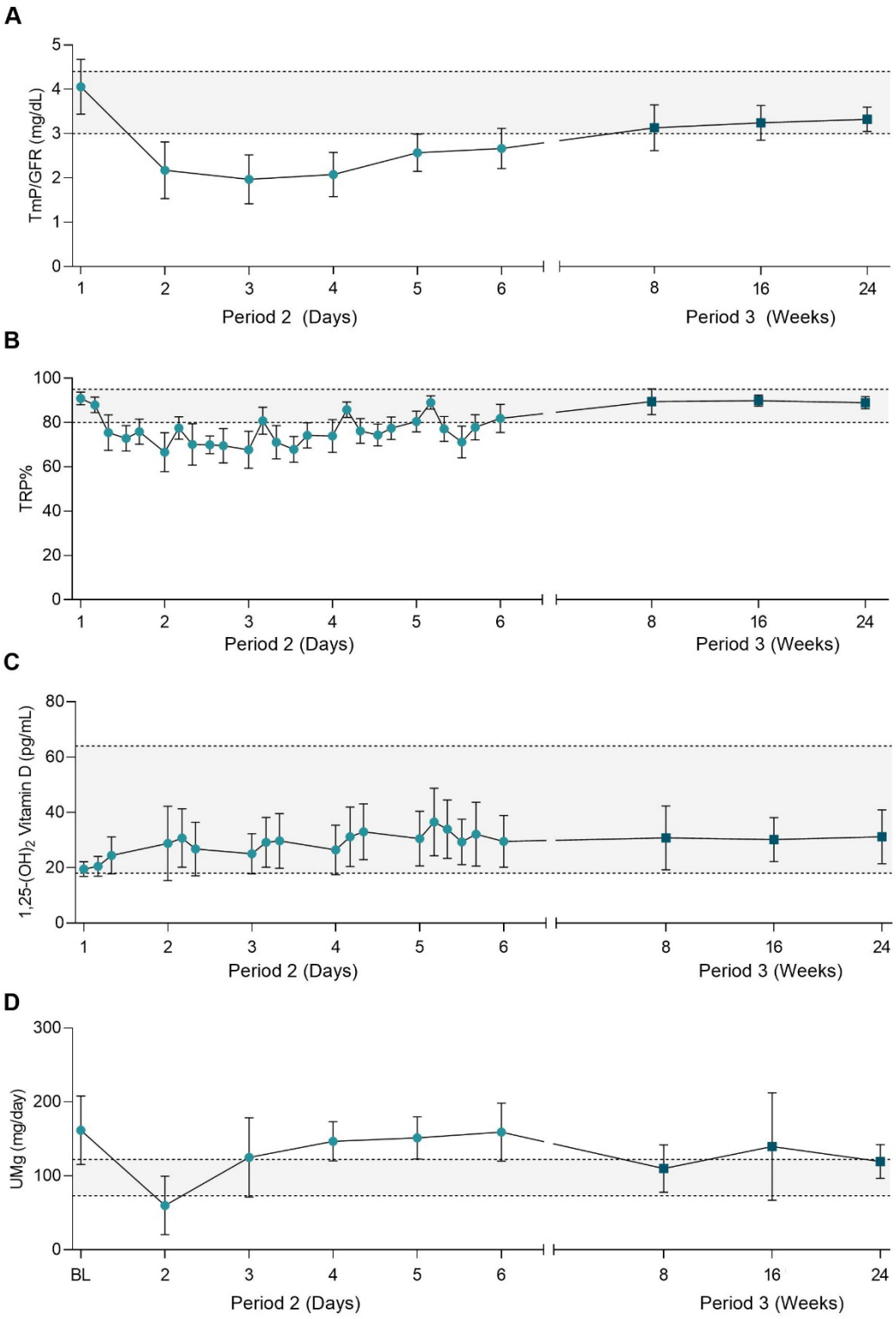



E
TmP/GFR (mg/dL)
Period 1 (Days)
F
TRP (%)
Period 1 (Days)
G
1,25-(OH)2 Vitamin D (pg/mL)
Period 1 (Days)
H
Magnesium (mg/dL)
Period 1 (Days)
I
uMg (mg/day)
BL
Period 1 (Days)

Time points shown in Panels A, B, D, and H are Day 1–3: 15 min pre-AM dose, 30 min, 1 h, 1.5 h (Panel B only), 2 h, 3 h, 4 h, 6 h, 8 h, 13 h, and 17 h post-dose; Day 4: 15 min pre-AM dose, and 2 h, 4 h, 8 h, 11 h, 13 h, and 17 h post-AM dose; Day 5: 15 min pre-AM dose, 30 min,1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, and 8 h post-AM dose, and 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 15 h post-PM dose. For Panels C and I, time points shown are 24-hour collections during the screening visit (BL) and initiated prior to the AM dose on Days 1–5. For Panel E, values were calculated using pre-AM dose samples. For Panel F, values were calculated using timed urine samples collected at −15 min (Day 1 only) and 0–4 h, 4 h, 8–13 h, 13–17 h, and 17–24 h relative to the morning dose. For Panel G, time points are Day 1–4: 15 min pre-AM dose, and 4 h, 8 h, 13 h, and 17 h post-AM dose; Day 5: 15 min pre-AM dose, 4 h, 8 h post-AM dose, and 4 h, 8 h, and 15 h post-PM dose. Graphs and error bars displayed are mean and 95% CI. Normal ranges are indicated by the dashed lines.

AM, morning; BL, baseline; cCa, albumin-corrected blood calcium; CI, confidence interval; iPTH, intact parathyroid hormone; PM, afternoon; TmP/GFR, tubular maximum reabsorption of phosphate/glomerular filtration rate; TRP, tubular phosphorus reabsorption; uCa, 24-hour urinary calcium excretion; uMg, 24-hour urinary magnesium excretion.

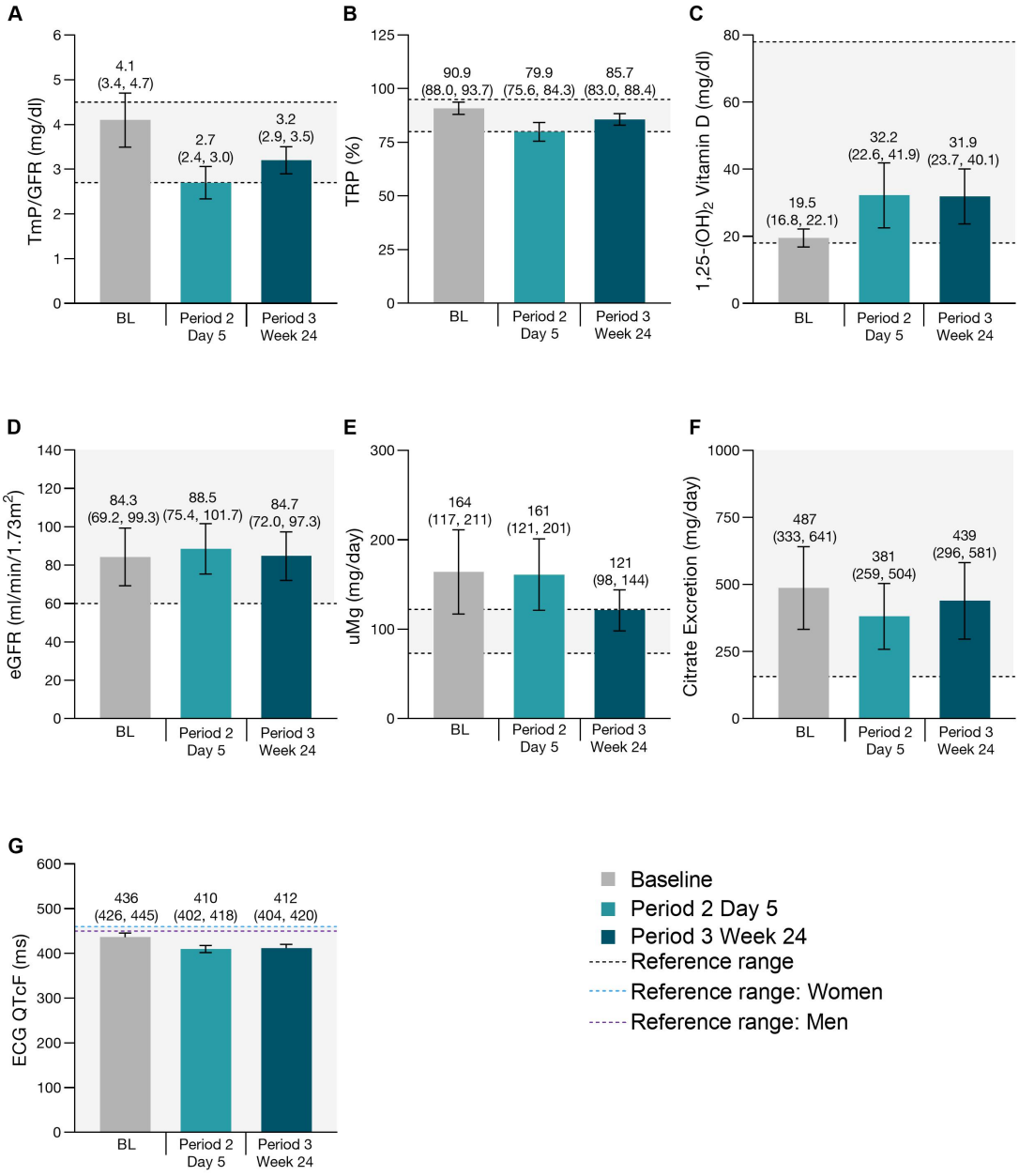
Figure S5. Effect of Encaleret on Additional Pharmacodynamic Measures in Dose Adjustment and Maintenance Periods (Period 2 and Period 3)



Panel A: TmP/GFR samples were collected fasting 15 min pre-AM dose for each study day. Panel B: TRP was calculated pre-AM dose (Periods 2 and 3) and at 0–4 h, 4–8 h, 8–13 h, 13–17 h, and 17–24 h post-AM dose (Period 2). Panel C: 1,25-OH_2-Vitamin D was measured P2D1–4: 15 min pre-AM dose, and 4 h and 8 h post-AM dose; P2D5: 15 min pre-AM dose, and 4 h and 8 h post-AM dose and 4 h, 8 h, and 15 h post-PM dose; Period 3: pre-AM dose. Panel D: uMg excretion time points shown are 24-hour collections during the screening visit (BL) and initiated prior to the AM dose during Periods 2 and 3. Graphs and error bars displayed are mean and 95% CI. Normal ranges are indicated by dashed lines.

AM, morning; BL, baseline; CI, confidence interval; P2D1–4, Period 2 Days 1–4; P2D5, Period 2 Day 5; PM, afternoon; TmP/GFR, tubular maximum reabsorption of phosphate/glomerular filtration rate; TRP, tubular phosphorus reabsorption; uMg, 24-hour urinary magnesium excretion.

Figure S6. Effect of Encaleret on Additional Pharmacodynamic Variables at the End of Dose Adjustment (Period 2) and Maintenance (Period 3) Periods Compared with Baseline

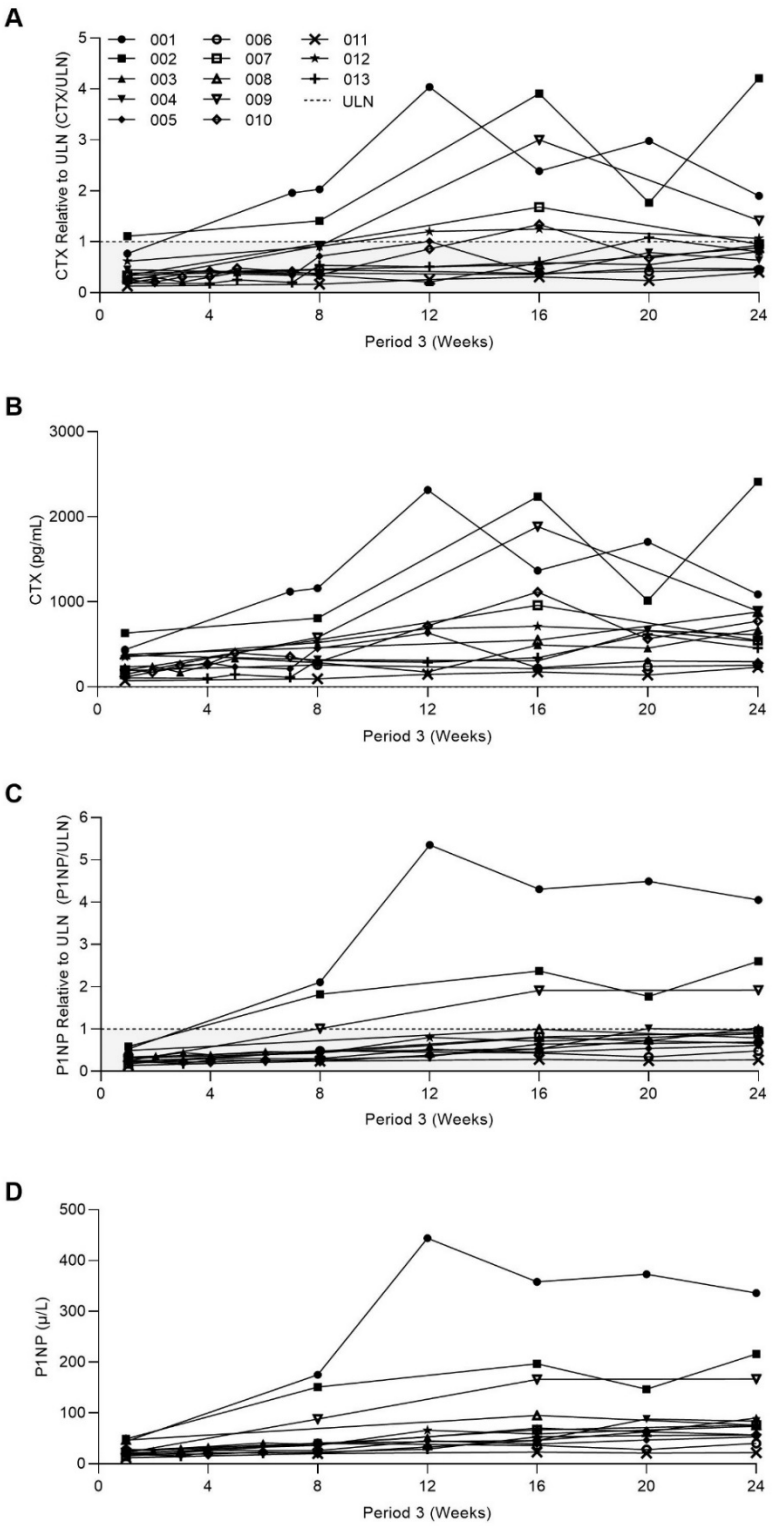


Panel A: TmP/GFR measured prior to first encaleret dose in P2BL was compared with 15 min pre-AM dose on P2D5 and P3W24. Panel B, Panel C, and Panel D: BL was measured

prior to first encaleret dose and was compared with the average of serial measures over 24 hours on P2D5 and P3W24. Panel E: 24-hour uMg excretion at screening visit (BL) was compared with P2D5 and P3W24. Panel F: Citrate excretion during the first 24 hours of encaleret (BL) was compared with P2D5 and P3W24. Panel G: ECG QTcF at screening visit (BL) compared with P2D5 and P3W24. Data displayed as mean and 95% CI.

BL, baseline; CI, confidence interval; ECG QTcF, electrocardiogram Fridericia-corrected Q-T interval; eGFR, estimated glomerular filtration rate; P2D5, Period 2 Day 5; P3W24, Period 3 Week 24; TmP/GFR, tubular maximum reabsorption of phosphate/glomerular filtration rate; TRP, tubular reabsorption of phosphate; uMg, urinary magnesium.

Figure S7. Effect of Encaleret on Bone Turnover Markers



Individual serum CT_X (Panel A, Panel B) and serum P1NP (Panel C, Panel D) during Period 3. Baseline values were collected on Period 2 Day 5. CT_X and P1NP are presented as individual participant data relative to ULN for age, sex, and menopausal status in Panels A and C, respectively. Measures shown for Weeks 8, 16, and 24 are pre-AM dose of encaleret; other time points are variable.

AM, morning; CT_X, serum collagen C-telopeptide; P1NP, serum procollagen Type 1 N-propeptide; ULN, upper limit of normal.

Supplementary Tables

Table S1. Representativeness of Study Participants

Disease under investigation	Autosomal dominant hypocalcemia type 1 (ADH1)
Sex and gender	ADH1 affects men and women equally as it is inherited in an autosomal dominant manner
Age	ADH1 is a genetic condition that is present at birth. Because it is a rare disease, data for age of diagnosis is limited. A recent systematic review of the medical literature[4] found that median age at diagnosis of a hypocalcemia-related disorder was 4 years with a range of 0-66 years. The median age at diagnosis of ADH1 was 25 years with a range of 0-77 years.
Race or ethnic group	Data on race or ethnicity in this rare disease is limited, but ADH1 is presumed to have the same chance of occurrence across all races and ethnicities because it is an autosomal dominant condition.
Geography	A recent systematic review of the medical literature identified patients with ADH1 reported in North America, Europe, Asia, and Australia. While cases of ADH1 from South America or Africa were not identified in the literature search, patients with ADH1 are expected to be found in all geographies because it is an autosomal dominant condition with variants that can occur *de novo*.
Other considerations	The diagnosis of ADH1 in various countries is dependent on regular use of genetic testing in patients with non-surgical forms of hypoparathyroidism.
Overall representativeness of this trial	The participants in the present trial demonstrated a slightly higher ratio of women (61.5%) to men (38.5%) than expected based on autosomal dominance inheritance pattern. Because this is the first Phase 2 trial assessing encaleret in ADH1, study enrollment was limited to adults. Therefore, the age distribution in the study population is not reflective of the total ADH1 patient population. All participants in this study were non-Hispanic white and from the United States. Baseline biochemical characteristics and treatment of the trial participants were consistent with those reported in the medical literature for ADH1. Eight of the nine *CASR* variants present in this study population have been reported in other patients with ADH1.

Table S2. Baseline Demographic and Clinical Characteristics of Cohorts 1 and 2*

Characteristic	Cohort 1† n=6	All Participants (Cohorts 1 and 2†) n=13	Reference Range
Median age (range) — years	39.5 (22–60)	36.0 (21–60)	–
Female sex — n (%)	3 (50.0)	8 (61.5)	–
White race — n (%)	6 (100.0)	13 (100.0)	–
Median BMI (range) — kg/m^2	33.0 (26.8–34.4)	31.2 (23.7–38.9)	–
CASR variant (protein)‡ — n (%)	6 (100.0)	13 (100.0)	–
A840V	1 (16.7)	3 (23.1)	
C131Y	2 (33.3)	2 (15.4)	
P221L	2 (33.3)	2 (15.4)	
Q245R	0	1 (7.7)	
E228K	0	1 (7.7)	
E604K	1 (16.7)	1 (7.7)	
F788C	0	1 (7.7)	
I692F	0	1 (7.7)	
T151M	0	1 (7.7)	
Renal calcifications — n (%)	3 (50.0)	9 (69.2)	–
Nephrocalcinosis	3 (50.0)	8 (61.5)	
Nephrolithiasis	0	3 (23.1)	
iPTH§— pg/ml	3.7±4.2	6.1±7.9 [n=12]¶	15–65
cCa§ — mg/dl	7.6±0.6	7.1±0.4	8.4–10.2
uCa‖ — mg/day	435±254	384±221	*Men:* <300; *Women:* <250
Blood phosphorus§ — mg/dl	4.5±0.7	4.5±1.1	2.4–4.7
TmP/GFR** — mg/dl	3.9±0.7	4.1±1.0	2.72–4.45††

Characteristic	Cohort 1[†] n=6	All Participants (Cohorts 1 and 2[†]) n=13	Reference Range
Tubular phosphorus reabsorption[‡‡] — %	88±4	91±5	80-95
Blood magnesium[§] — mg/dl	1.8±0.2	1.7±0.2	1.6–2.6
uMg[‖] — mg/day	125±49	164±78	73–122
24-hour citrate excretion[‖] — mg/day	638±486	487±255	157–1191
Serum 1,25-$(OH)_2$ vitamin D[§] — mg/dl	17.2±5.0	19.5±4.4	18–78
Serum 25-OH vitamin D — ng/ml	46.7±17.5	44.6±10.1	20–50
eGFR[§] — ml/min/1.73 m^2	83.4±19.7	84.3±24.9	≥60
Mean SOC medication (range)			–
Elemental calcium[§§] — mg/day	2433 (800–4800)	2120 (750–4800)	
Calcitriol — µg/day	0.8 (0.5–2.0)	0.7 (0.2–2.0)	
Cholecalciferol — IU/day	2540 (1000-3200)	3050 (1000–7200)	
Bone turnover markers — n		n=7	[¶¶]
CTx — pg/ml	265±117	253±111	
P1NP — µg/l	36±10	34±10	
DXA anatomical site Z-score[‖‖]			-2 to +2
Total body BMD	2.7±0.8 [n=5]	2.1±1.4 [n=11]	
AP lumbar (L1–L4) spine			
BMD	2.7±1.1 [n=6]	2.6±1.5 [n=12]	
TBS	0.3±0.7 [n=5]	0.7±1.1 [n=11]	
Total hip BMD	2.4±1.1 [n=6]	2.1±1.3 [n=13]	
1/3 distal radius BMD	0.2±0.8 [n=6]	0.3±1.0 [n=13]	

Characteristic	Cohort 1[†] n=6	All Participants (Cohorts 1 and 2[†]) n=13	Reference Range
ECG QTcF — ms	433±13	435±16	*Male:* <450; *Female:* <460

ADH1, autosomal dominant hypocalcemia type 1; AP, anterior–posterior; BMD, bone mineral density; BMI, body mass index; *CASR*, calcium-sensing receptor gene; cCa, albumin-corrected blood calcium; CTx, serum collagen cross-linked C-telopeptide; DXA, dual-energy X-ray absorptiometry; ECG QTcF, electrocardiogram Fridericia-corrected Q-T interval; eGFR, estimated glomerular filtration rate; iPTH, intact parathyroid hormone; IU, International Unit; P1NP, serum procollagen Type 1 N-propeptide; SD, standard deviation; SOC, standard of care; TBS, trabecular bone score; TmP/GFR, tubular phosphorus reabsorption/glomerular filtration rate; TRP, tubular reabsorption of phosphate; uCa, 24-hour urinary calcium excretion; uMg, 24-hour urinary magnesium excretion.

*All values are mean±SD unless otherwise stated.

[†]For Cohort 1, the last non-missing assessment prior to the first dose in Period 1 was summarized. Otherwise, the last non-missing assessment prior to the first dose in Period 2 was summarized.

[‡]Participants with the same activating variant of the *CASR* gene were related. Participant with the I692F variant also carried a *CASR* R990G polymorphism.

[§]Measurements were taken pre-dose Day 1 Period 1 (Cohort 1) or Period 2 (Cohort 2).

[¶]One participant was removed from this calculation due to variable presence of heterophile antibodies which can interfere with the iPTH assay.

[‖]24-h urine assessments at baseline were collected during the screening visit on SOC except for 24-h urine citrate baseline which was collected at Period 2 Day 1.

**TmP/GFR was calculated using the following equation: blood phosphorus – ([urine phosphorus / urine creatinine] x blood creatinine). Fasting pre-dose samples were used for the calculation.

[††]Range is for men and women across 25–65 years of age.

[‡‡]TRP was calculated using the following equation: (1 – [urine phosphorous x blood creatinine] / [blood phosphorous x urine creatinine]) x 100.

[§§]Calcium supplement doses at baseline are inclusive of calcium from multivitamins.

[¶¶]CTx: *Men*: 93–630 pg/ml (31–50 years), 35–836 pg/ml (51–70 years); *Women*: 25–573 pg/ml (pre-menopausal), 104–1008 pg/ml (post-menopausal), P1NP: *Adult men*: 22–87 µg/l; *Adult women*: 19–83 µg/l (pre-menopausal), 16–96 µg/l (post-menopausal). Baseline for CTx and P1NP were collected on Period 2 Day 5.

[‖‖]Some DXA Z-score data are not available for two participants due to surgical hardware.

Table S3. Nephrocalcinosis and Nephrolithiasis at Screening and the End of Period 3.

Parameter, n (%)	Screening Visit (n=13)	P3W24 (n=13)
Nephrocalcinosis	8 (61.5)	8 (61.5)
Nephrolithiasis*	3 (23.1)	3 (23.1)

P3W24, Period 3 Week 24.

*One subject had a pre-enrollment history of a stable nephrolith seen on prior imaging that was missed at screening but was identified at P3W24 and was unchanged. The other 2 subjects with nephrolithiasis also had nephrocalcinosis.

Table S4. BMD Z-Scores by DXA Anatomical Site at Screening and the End of Period 3

DXA Anatomical Site [n]	Screening Visit Z-Score†,‡	P3W24 Z-Score†,‡
Total body BMD [11]	+2.1 (1.1, 3.0)	+2.0 (1.1, 2.9)
AP lumbar (L1–L4) spine		
BMD [12]	+2.6 (1.6, 3.5)	+2.3 (1.3, 3.4)
TBS [11]	+0.7 (-0.1, 1.4)	+1.0 (0.1, 2.0)
Total hip BMD [13]	+2.1 (1.4, 2.9)	+2.0 (1.2, 2.7)
1/3 distal radius BMD [13]	+0.3 (-0.3, 0.9)	+0.4 (-0.1, 1.0)

AP, anterior–posterior; BMD, bone mineral density; CI, confidence interval; DXA, dual-energy X-ray absorptiometry; P3W24, Period 3 Week 24; TBS, trabecular bone score.

†Data presented as mean and 95% CI.

‡Some Z-score DXA data are not available for two participants due to surgical hardware.

Table S5. Summary of Adverse Events.

Parameter n (%)	Period 1 N=6	Period 2 N=13	Period 3 N=13
Number of participants with any AE*	6 (100.0)	11 (84.6)	13 (100.0)
Number of participants with any AE by severity[†,‡]			
Mild	6 (100.0)	11 (84.6)	13 (100.0)
Moderate	1 (16.7)	0	2 (15.4)
Severe	0	0	0
Number of AEs by severity[§]			
Overall	19	14	64
Mild	18 (94.7)	14 (100.0)	62 (96.9)
Moderate	1 (5.3)	0	2 (3.1)
Severe	0	0	0
Number of participants with any SAE*	0	0	0
Number of participants with treatment-related AE[†,‡]	2 (33.3)	8 (61.5)	6 (46.2)
Hypophosphatemia	2 (33.3)	8 (61.5)	1 (7.7)
Hypercalcemia	0	1 (7.7)	5 (38.5)
Most common AEs*[,‡,¶]			
Hypercalcemia	0	1 (7.7)	5 (38.5)
Hypocalcemia	1 (16.7)	1 (7.7)	4 (30.8)
Nausea	0	0	4 (30.8)
Upper respiratory tract infection	0	0	3 (23.1)
Vulvovaginal mycotic infection	0	0	3 (23.1)
Constipation	0	1 (7.7)	2 (15.4)
Pyrexia	0	0	2 (15.4)
Arthralgia	0	0	2 (15.4)

Parameter n (%)	Period 1 N=6	Period 2 N=13	Period 3 N=13
Cough	0	0	2 (15.4)
Hypertension	0	0	2 (15.4)
Headache	1 (16.7)	1 (7.7)	2 (15.4)
Hypophosphatemia	2 (33.3)	8 (61.5)	1 (7.7)
Pharyngitis	1 (16.7)	0	1 (7.7)
Iron deficiency	1 (16.7)	0	0
Blood pressure decreased	1 (16.7)	1 (7.7)	0

AE, adverse event; SAE, serious adverse event.

*Participants who experience ≥1 AEs or SAEs were counted only once.

†Participants are counted only once within a particular severity grade or relatedness category.

‡Percentages are based on number of participants (N) in each period.

§Percentages are based on number of AEs reported for each period.

¶Listed in descending order of incidence in Period 3 are preferred terms for events that were reported for at least 10% of participants in any period.

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