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EOS789, a broad-spectrum inhibitor of phosphate transport, is safe with an indication of efficacy in a Phase 1b randomized cross-over trial in hemodialysis patients.

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Running title: Intestinal phosphorus absorption

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Abstract:

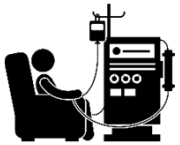
The treatment of hyperphosphatemia remains challenging in patients receiving hemodialysis. This Phase 1b study assessed safety and efficacy of EOS789, a novel pan-inhibitor of phosphate transport (NaPi-2b, PiT-1, PiT-2) on intestinal phosphate absorption in patients receiving intermittent hemodialysis therapy. Two cross-over, randomized order studies of identical design (ten patients each) compared daily EOS789 50 mg to placebo with meals and daily EOS789 100 mg vs EOS789 100 mg plus 1600 mg sevelamer with meals. Patients ate a controlled diet of 900 mg phosphate daily for two weeks and began EOS789 on day four. On day ten, a phosphate absorption testing protocol was performed during the intradialytic period. Intestinal fractional phosphate absorption was determined by kinetic modeling of serum data following oral and intravenous doses of ^{33}Phosphate (^{33}P). The results demonstrated no study drug related serious adverse events. Fractional phosphate absorption was 0.53 (95% confidence interval: 0.39,0.67) for placebo vs. 0.49 (0.35,0.63) for 50 mg EOS789; and 0.40 (0.29,0.50) for 100 mg EOS789 vs. 0.36 (0.26,0.47) for 100 mg EOS789 plus 1600 mg sevelamer (all not significantly different). The fractional phosphate absorption trended lower in six patients who completed both studies with EOS789 100 mg compared with placebo. Thus, in this Phase 1b study, EOS789 was safe and well tolerated. Importantly, the use of ^{33}P as a sensitive and direct measure of intestinal phosphate absorption allows specific testing of drug efficacy. The effectiveness of EOS789 needs to be evaluated in future Phase 2 and Phase 3 studies.

Key Words: hemodialysis, intestine, sodium-phosphate co-transporters, phosphorus absorption, phosphorus radiotracer

EOS789, a broad-spectrum inhibitor of phosphate transport, is safe with an indication of efficacy in a Phase 1b randomized cross-over trial in hemodialysis patients.

Two RCT crossover studies

Patients



- Adult HD patients (≥ 3 mo)
- Serum P > 4.9 mg/dL
- Discontinued P binders

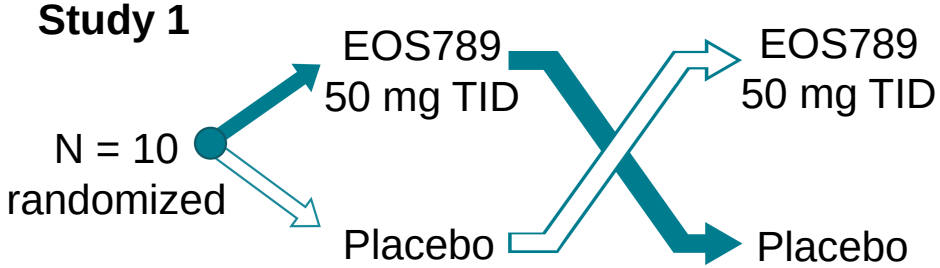
EOS789 novel inhibitor of NaPi-2b/PiT-1/PiT-2

14d Controlled diet

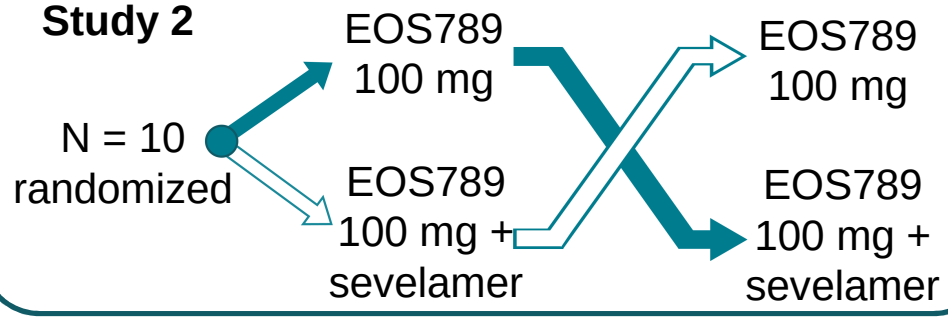
Safety & Pharmacokinetics

Intestinal P absorption by ^{33}P radiotracer

Study 1



Study 2



Results

No study drug related SAEs

Study drug well-tolerated

^{33}P radiotracer can be used for direct measurement of intestinal absorption in hemodialysis patients

CONCLUSION:

EOS789 was safe and well-tolerated in doses up to 100 mg thrice daily. Further studies are required to establish efficacy and optimize dose for reduction in intestinal P absorption.

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Hill Gallant et al., 2020

Introduction:

Hyperphosphatemia is associated with increased morbidity and mortality in patients with both chronic kidney disease (CKD) and with end-stage kidney disease (ESKD)[1-3]. Furthermore, elevated phosphorus/phosphate (P) directly induces vascular calcification and secondary hyperparathyroidism[4] and indirectly induces left ventricular hypertrophy mediated through fibroblast growth factor 23 (FGF23)[5-7]. These adverse effects of hyperphosphatemia have led to the administration of P binders to reduce serum P levels in patients undergoing dialysis. The Dialysis Outcomes and Practice Patterns Study indicates 88% of patients undergoing dialysis are prescribed binders, and yet the average P level remains above target [8]. Furthermore, in this same study, 45% of patients admitted to non-adherence of the binders [9]. Thus, alternative treatments to lower P are needed.

In patients with CKD, dietary P intake impacts serum P level, and P bioavailability varies by food source[10-12]. Although 24-hour urine studies have been considered surrogates of intestinal P absorption, we have shown from a secondary analysis of metabolic balance studies in CKD patients consuming a consistent controlled P diet that 24-hour urine P was unrelated to net intestinal P absorption from balance. Rather, 24-hour urine P was inversely related to whole-body P balance[13]. Thus, even in moderate CKD patients who still produce urine, the use of 24-hour urine P as a biomarker of intestinal P absorption may not be accurate. In patients undergoing dialysis, where urine output is negligible, serum P has been used to study drug efficacy. However, the removal of P with dialysis, the rebound of serum P post dialysis, diurnal variability of serum P levels, and bone turnover with uptake and release of P vary among patients and thus serum P is nonspecific in relation to intestinal absorption. To overcome these limitations, we adaptated the direct and sensitive gold-standard dual isotopic tracer that utilized both ^{32}P and ^{33}P isotopes[14, 15]. Here, we used oral and IV doses of ^{33}P radiotracer to assess intestinal fractional P absorption after an oral dose, followed by compartmental changes and total body removal with the IV dose. This approach parallels our previous work in intestinal calcium absorption in patients with CKD[16].

Intestinal P absorption includes both active (transcellular) and passive (paracellular) absorption. As recently reviewed, the major known and most studied intestinal P transporter is the sodium-P cotransporter NaPi-2b (SLC34) [17]. Studies in mice with CKD show that knocking out this transporter reduces hyperphosphatemia and bone loss[18, 19]. However, a NaPi2b inhibitor, ASP3325, did not show efficacy in inhibiting phosphrous transport in human healthy subjects and in patients undergoing dialysis[20]. Recent studies have also demonstrated the presence of both PiT-1 and PiT-2 (SLC20) in intestinal segments in humans[21], but their relative contribution to active P transport is unclear[22, 23]. Thus, it is possible that inhibition of PiT-1/2 may be required to significantly reduce active intestinal P transport in humans compared to inhibition of NaPi-2b alone.

EOS789 is a novel inhibitor of NaPi-2b, PiT-1, and PiT-2. EOS789[24] inhibited sodium-dependent P uptake in *in vitro* cultured cells and inhibited sodium-dependent P uptake in brush border membrane vesicles prepared from rat small intestine. EOS789 inhibited intestinal P absorption in healthy rats in a dose dependent manner. Further, EOS789 given to hyperphosphatemic rats, reduced serum concentrations of P, FGF23 and intact PTH in a dose dependent manner[24]. In Phase 1 healthy human volunteer studies, there was a dose dependent increase in P content of feces with doses administered safety up to 100 mg TID (Hisada et al, ASN FR-PO144, 2019). Side effects included gastrointestinal symptoms, and a reversible skin rash observed only at the highest dose. The observed efficacy in the healthy volunteer study provided the basis for testing in patients undergoing hemodialysis. The starting dose for this study of 50 mg three times per day (TID) was selected to provide a slightly lower dose than the maximal safe dose with pharmacodynamic activity data in the healthy volunteer study.

We report the use of this dual ^{33}P radiotracer method in two Phase 1b, random-order, cross-over studies of identical design conducted in 10 patients per study with hyperphosphatemia receiving hemodialysis thrice weekly (**Figure 1**). The first study (Study 1) was a double-blinded study comparing 50 mg EOS789, a pan-phosphate transport inhibitor, to placebo TID with meals. The second study (Study 2) was unblinded and compared EOS789 100 mg TID with, versus without,

sevelamer carbonate 1600 mg TID with meals. The primary end points were the safety of EOS789, and the drug's efficacy in reducing intestinal P absorption measured directly with a radioisotope.

Results:

Safety Analyses:

The number of patients who reported at least one AE, and the number of events for all four arms is presented in **Table 1**. Serious Adverse Events (SAEs) occurred in 1 of 13 patients (7.7%, 2 events: lung abscess and osteomyelitis) treated with EOS789 50 mg TID and in 2 of 12 patients (16.7%, 3 events: cellulitis and heat stroke in 1 patient, and bacteremia in 1 patient) treated with EOS789 100 mg TID. AEs leading to discontinuation of study drug occurred in 1 of 13 patients (7.7%, 1 event: gastroenteritis) treated with EOS789 50 mg TID and in 1 of 12 patients (8.3%, 1 event: bacteremia) treated with EOS789 100 mg TID. The principle investigator (blinded to allocation) evaluated the available clinical information and determined unlikely causal relationship with study drug for all SAEs. A detailed list of AEs by organ system is provided in **Supplemental Table 1.**

Efficacy analyses:

The demographics and baseline laboratory tests are shown in **Table 2** and the patient allocation information in **Supplemental Figure 1**. Six patients completed both Study 1 and Study 2. The mean compliance rate of placebo/EOS789 treatments was a pill count of 95% to 97%. The percentage of patients with treatment compliance by pill count ≥ 80% in each group was 91.7% to 100.0%. The pharmacokinetic levels for EOS789 as a composite and by dose are shown in **Figure 2, and individual patient curves in supplemental Figure 2**. The Cmax and the AUC were estimated with the plasma concentration profile which was conducted for the period of 3 doses over 24 hours, corresponding to dosing at 0, 4 and 8 hours. The C_{max} for 50 mg TID, 100 mg TID, and 100 mg TID + sevelamer carbonate was 76.2 ± 24.2, 121 ± 20.3, and 188 ± 23.7 ng/mL, respectively; and mean ± SD of AUC from time 0 to 24 hours was 1190 ± 387, 1900 ± 342, and

2560 ± 119 h*ng/mL, respectively. The drug is highly protein bound (99.6% in humans), and thus, the removal rate with dialysis was negligible.

Mean [SEM] intestinal fractional P absorption in Study 1 for placebo was 0.53 [0.06] (95%CI: 0.39, 0.67) and for EOS789 50 mg TID was 0.49 [0.06] (95%CI: 0.35, 0.63) (difference of -0.04, 95%CI: -0.18, 0.10, p=0.52, **Figure 3A**); and in Study 2 was 0.40 [0.05] (95%CI: 0.29, 0.50) for EOS789 100 mg TID and 0.36 [0.05] (95%CI: 0.26, 0.47) for EOS789 100 mg TID + sevelamer carbonate (difference of -0.03, 95%CI: -0.13, 0.06, p=0.45, **Figure 3B**). Cross-over period and randomized order effects were tested in the ANOVA models and were not significant for either Study 1 or Study 2. The addition of potential confounders as covariates of peak plasma P, pre-dialysis plasma P, or pre-post dialysis plasma P drop did not alter the non-significant results. In a pre-planned secondary analysis of the N=6 patients who completed both Study 1 and Study 2, intestinal fractional P absorption was lower with EOS789 100 mg (0.44 [0.07]; 95%CI: 0.29, 0.59) compared with placebo (0.63 [0.07]; 95%CI: 0.48, 0.79) (difference of -0.19, 95%CI: -0.365, -0.03, nominal p=0.028) and trended lower compared with EOS789 50 mg (0.55 [0.07]; 95%CI: 0.40, 0.70) (difference of -0.11 95%CI: -0.28, 0.05, p=0.165) (**Figure 4)**. There was a strong correlation between the $1,25(OH)_2$-vitamin D levels and intestinal fractional P absorption in the patients from Study 1 in the placebo arm (n = 9 due to insufficient blood sample in one patient, $r^2 = 0.64$, p = 0.009; **Supplemental Figure 3A**). However, there was not a significant relationship in the $1,25(OH)_2$-vitamin D levels and fractional P absorption when all treatments were considered (n = 19, $r^2 = 0.16$, p = 0.09; **Supplemental Figure 3B**)

The mean change [SD] from baseline to Day 13 in serum P following treatment was −1.00 mg/dL [1.31] with placebo vs. −1.28 mg/dL [1.21] with EOS789 50 mg TID in Study 1 (Difference of -0.32; 95% CI: -1.03, 0.38, p = 0.32), and −1.46 mg/dL [1.34] with EOS789 100 mg TID vs. −2.71 mg/dL [1.73] with EOS789 100 mg TID + sevelamer carbonate in Study 2 (Difference of -1.47; 95%CI -2.07, -0.87, p< 0.001). The majority of study participants did not have daily fecal excretion during the inpatient stay limiting our evaluation of fecal data over the short two day collection

period. However, available data show that accumulated fecal excretion of P (N=8) trended lower in EOS789 50 mg TID treated subjects compared to placebo by −279 mg/day (95%CI: −883, 324; mean [SE]: 581[200] and 860[183] mg/d, respectively, p=0.33). In Study 2, accumulated fecal excretion of P (N=6) trended lower in EOS789 100 mg TID + sevelamer carbonate compared to EOS7789 100 mg alone by −226 mg/day (95%CI: −549, 97; mean[SE]: 552[92] and 777[112] mg/d, respectively, p=0.15). Urine collection was too sparse to evaluate or include in the absorption model. In Study 1, there were no significant differences in serum P, corrected Ca, Ca x P, intact PTH, or C-terminal FGF23. In Study 2, EOS789 100 mg TID + sevelamer carbonate lowered serum P, Ca x P, and serum intact PTH compared with EOS789 100 mg TID alone ($p<0.05$) (**Supplemental Table 2**).

Discussion:

EOS789, a NaPi-2b, PiT-1, and PiT-2 inhibitor, was shown to be safe and well tolerated in patients undergoing hemodialysis. We elected to start at the lowest possible dose of 50 mg TID in this Phase1b study given the primary end point was safety, and patients undergoing dialysis have many co-morbidities. That dose was not efficacious in reducing intestinal P absorption compared to placebo. However, a pre-planned secondary analysis of the six patients who were enrolled in both studies showed a nominally significant reduction in intestinal P absorption with EOS789 100 mg TID compared to placebo. Additional studies are required at higher doses of EOS789 and/or more patients to determine efficacy.

Interestingly, intestinal P absorption in patients receiving 100 mg TID of EOS789 without, versus with sevelamer at 1600 mg TID were not different. However, serum P was lower at day 13, and fecal P was greater with the addition of sevelamer. Unfortunately, we did not have a treatment arm with sevelamer alone, making interpretation of this finding difficult. In humans, the relative proportion of passive absorption versus active transport is difficult to study. Larsson et al. found that a NaPi-2b inhibitor, ASP3325, was effective in lowering P in rodents, but did not alter urine or fecal P excretion in healthy individuals[20]. In contrast, EOS789 in Phase 1 studies in healthy individuals

increased fecal P by 13.4 to 209 mg/day in doses investigated (Hisada et al, ASN FR-PO144, 2019, unpublished). This would suggest that pan-inhibition of NaPi-2b, PiT-1, and PiT-2 may be required. Paracellular absorption is also important in humans, as tenapanor, a gastrointestinal sodium/hydrogen exchanger 3 (NHE3) inhibitor which decreases paracellular P permeability and thus absorption [25], lowered serum P in dialysis patients[26]. Interestingly, we did find a relationship between $1,25(OH)_2D$ levels and intestinal P absorption in the placebo arm, but not when all treatments were considered. However, given the inter-individual variability in intestinal P absorption, and that most patients achieved normal levels with pharmacologic therapy of doxercalciferol, the role of vitamin D in intestinal transport assessed with this methodology needs to be tested by prospectively assessing absorption before and after calcitriol administration.

In the present study we demonstrate that the use of ^{33}P radiotracer can be used in patients on hemodialysis to directly measure intestinal P absorption. Isotope techniques were used frequently in the 1960s and 70s[27], but have been sparsely used since due to challenges including expense, subject burden, and/or need for radioactivity exposure. An accepted gold standard dual-isotope method for fractional P absorption requires use of both the low-energy beta emitter, ^{33}P, plus the high-energy ^{32}P, which is more hazardous. We adapted our previously published ^{45}calcium radiotracer technique to ^{33}P, where we mimicked a dual-isotope method using ^{33}P for both the oral dose and the IV dose, staggering these by a day to differentiate between the oral and IV administrations in the specimens. After the measurement of the ^{33}P in serum following the oral administration, any residual ^{33}P prior to the IV ^{33}P administration is included in the model. All subsequent radioactivity counts in samples are considered to be from the IV ^{33}P administration. This approach is possible because after one day, the detectable oral ^{33}P dose remaining is an order of magnitude lower than the radioactivity counts assessed following the IV ^{33}P dose (**Figure 5**). By containing the absorption test during the interdialytic period, we were able to account for IV clearance of the isotope for each subject individually to estimate intestinal fractional P absorption, despite differences in dialysis among patients and potential dialytic clearance.

A rigorous “gold-standard dual isotope study” would administer two isotopes on the same day and both isotopes would be measured from the same samples. Farrington et al[14] used oral ^{32}P and IV ^{33}P in patients with severe non-dialysis CKD (creatinine clearance of 6 ± 4 ml/min), controls, and transplant recipients. They found that an intial rapid phase of intestinal absorption was completed by around 3 hours, but absorption continued at a slower rate through 7 ½ hours, and that fractional absorption calculated from data through 24 hours was not different than calculations based on data only through 7 ½ hours. As shown in our Figure 5, we allowed for 25 hours between the oral and IV doses of ^{33}P, which based on the results reported by Farrington et al[14], the absorption of the oral ^{33}P isotope should have been completed. And, although the oral ^{33}P radioactivity counts were not back to baseline (similar to observations by Farrington), as described above, they were >10-fold less than the radioactivity counts following the IV ^{33}P dose. Thus, this order of magnitude difference between oral and IV ^{33}P radioactivity counts and the minimal additional absorption between 7 ½ and 24 hours, as described by Farrington, allows for the use of this single-isotope modified absoption testing protocol. Further, the gold-standard dual-istope method for P requires the use of ^{32}P, which is more hazardous and less acceptable now for human use, so even a validation study of our single isotope method against the dual-isotope method would be a challenge in regard to accepatable risk to patients. Wiegmann and Kaye[15] performed balance studies in hemodialysis patients using a single isotope (^{32}P) given two weeks apart, first by IV, then by oral administration. In this study, $^{45}Calcium$ was also used which precluded use of concurrent, administration of ^{33}P, which have similar engery peaks by scintillation counting. We surmise that the long duration between the IV and oral administrations was because the more hazardous ^{32}P was used; thus, the longer duration spaced out the radioactivity exposures to the patients. However, this long duration between IV and oral isotope administrations is not ideal for assessing fractional absorption, because of lack of steady-state over that time due to a variety of predicatble as well as unknown changing conditions – e.g. dialytic removal and flutuating dietary intake.

A strength of our study was the controlled diet designed to provide consistent amounts of macronutrients and key micronutrients daily and evenly by meal. Our rotating 3-day cycle menu introduced some variation which is a limitation in contrast to providing the exact same meals every day. However, use of cycle-menus are standard in controlled feeding and balance studies in humans to enhance participant recruitment and retention into these already-burdensome studies. The menus were designed to be consistent in macronutrients and key micronutrient and even similar in proportion of animal and plant sources of protein, which can affect P bioavailablity. Additionally, the same menu was given during the P absorption study in the CRC on both the oral ^{33}P and IV ^{33}P dose days. Additional limitations of our study was the sparse stool collections over the two-day inpatient period that precluded use of stool data in the kinetic model or measurement of stool P as a secondary measure of absorption, and that the two-day absorption test period is not long enough for estimation of the slow turnover pools in the kinetic model (e.g. bone turnover) – which would require a longer full metabolic balance study.

It is important to emphasize the multiple variables affecting serum P in patients on dialysis. While many dialysis patients are anuric, some patients, especially those on peritoneal dialysis retain residual renal function. Wang et al. demonstrated that anuric patients undergoing peritoneal dialysis had elevated serum P levels nearly twice as often as patients without residual renal function[28]. The removal of P with dialysis varies considerably and is affected by blood flow, dialysis duration, the pre-dialysis serum P, PTH level and ultrafiltration volume[29, 30]. Traditional thrice weekly hemodialysis leads to a rebound of serum P from extracellular stores, including bone, after dialysis but the timing of that rebound varies[31-33]. Recent studies demonstrated hemodiafiltration, compared to hemodialysis, leads to greater removal of P due to the expanded ultrafiltration[34] and nocturnal, compared to intermittent dialysis removes more P[35]. Increased bicarbonate dialysate may impact P removal[36], and flux of P out of bone may also vary depending on the bone turnover[29]. Finally, even in dialysis patients there is diurnal variation in serum P levels, complicating pre-dialysis measures across multiple dialysis shifts[20]. Thus, studying P homeostasis in dialysis patients is very difficult and direct

assessment using oral and IV radiotracer ^{33}P as demonstrated in the present study is a way to confirm true drug efficacy at the intended target site of the intestine.

In summary, we present promising data for safety of a novel pan- inhibitor of sodium/phosphate co-transport in patients undergoing hemodialysis using a novel technique of oral and intravenous of ^{33}P radiotracer kinetic modeling. Future studies are required to determine efficacy of higher doses of EOS789, the role of EOS789 with diets of differing P content, and the potential mechanisms by which intestinal P absorption varies in patients with CKD. In addition, long term safety studies are needed to assess any adverse effects of pan-phosphate transporter inhibition.

Materials and Methods:

Study design: Two Phase 1b, random-order, cross-over studies of identical design were conducted in patients with hyperphosphatemia receiving hemodialysis thrice weekly (NCT02965053; Indiana University Institutional Review Board approval 1608100544). The first study (Study 1) was a double-blinded study comparing EOS789 50 mg to placebo TID with meals. The second study (Study 2) was unblinded and compared EOS789 100 mg TID with versus without sevelamer carbonate 1600 mg TID with meals. Twelve to 14 patients were recruited in each study to have a final number of 10 patients per study with all measurements from both treatments available for efficacy analyses. The first patient was consented in May 2017, and the last intervention for study 2 was July 2018. See **Supplemental Figure 1** for enrollment information. The primary endpoint for both study 1 and study 2 was safety and determined by adverse events and safety labs. Secondary efficacy endpoints for both studies were differences between the two treatments in 1) intestinal fractional P absorption, 2) serum P, serum Ca, serum Ca × P, serum intact PTH, and serum C-terminal FGF23 at Day 13, and 3) accumulated fecal excretion of P from Day 11 to 13. Plasma concentration and pharmacokinetics of EOS789 were determined in the first cross-over session of each study prior to the ^{33}P dosing (n = 6). The study schema is shown in **Figure 1**.

The complete list of inclusion and exclusion criteria are detailed in **Supplemental Table 3**. In brief, patients were 18 years or older, on P binders, and on thrice weekly hemodialysis for at least 3

months with no change in dialysis prescription or CKD-MBD drug treatments in the preceding 4 weeks. After informed consent, patients stopped their P binders for 15-19 days and underwent screening laboratory tests pre-dialysis. Those with serum P ≥ 7.0 mg/dL were eligible for the study and on Day 1 began the controlled study diet of 900 mg/d P. The study diet was designed by a registered dietitian bionutritionist using ProNutra software (Viocare, Inc., Princeton, NJ). The diet consisted of a 3-day cycle menu, with assurance that patients had the same diet during the oral and IV ^{33}P administration during each arm of each study while in the CRC. Meal composites were prepared, homogenized, and sent for analysis of minerals (Ca, P, Mg, Na, K) by inductively coupled plasma-optical emission spectrometry (ICP-OES) and protein content by the Dumas method (Covance Laboratories, Madison, WI). Nutrient content of for each meal of the 3-day cycle menu is in **Supplemental Table 4**. All study meals were prepared in a metabolic kitchen, with ingredients weighed to 0.1g accuracy and precision. For Days 1-9, patients received their study diets as pack-out meals. Diet began on Day 1, and on Day 4, subjects underwent pre-randomization visit with baseline blood biochemistries. Those with serum P < 10 mg/dL and increase in serum P by ≥ 0.5 mg/dL were randomized 1:1 by the Indiana University Investigational Pharmacy to receive study drug (EOS789 50 mg or placebo in Study 1, and EOS789 100 mg with or without 1600 mg sevelamer carbonate in Study 2). Patients continued the diet for a total of 13-14 days and study drug for 10 days.

After 7 days of study drug, on Day 10 of each treatment, patients were admitted to the Indiana University Clinical Research Center (CRC) for pharmacokinetic testing. On Day 11, blood was drawn and patients underwent their regular hemodialysis treatment. On returning to the CRC, they began the ^{33}P absorption test protocol (see below). On Day 13, patients had their final ^{33}P blood drawn pre-dialysis and were discharged home. On Day 28, subjects had a follow-up safety visit to either begin the second sequential treatment or complete the study. Thus, wash out between the two arms was 28 days, chosen to account for more than 5 half-lives of the study drug. Pill counts were used to assess study drug compliance, and uneaten diet was estimated from returned checklists or review of the meal tray when in the CRC. Electrocardiograms were done in both studies, and echocardiography

in Study 2 for safety purposes. No changes in dialysis prescription (other than ultrafiltration) occurred during each study. Adverse events were monitored by a safety committee.

Biochemistries: Serum Ca, P and other safety biochemistries were analyzed in the Indiana University Pathology Laboratories. Intact PTH was measured by Alpco Intact PTH ELISA (1-84), and C-terminal FGF23 was measured by Quidel Corporation human FGF-23 (C-terminal) ELISA Kit. $1,25(OH)_2$-vitamin D levels were measured at baseline of each period by Extraction/Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) at Mayo Clinic, Rochester, MN.

Intestinal Fractional P Absorption (Figure 5 and Supplemental Figure 4): Intestinal fractional P absorption was determined from oral and IV administration of ^{33}P radiotracer. The method used mimicked the double-isotope method using a single isotope by administering the oral isotope on the first day and the IV isotope on the second day. On Day 11 after dialysis, baseline blood was drawn, then 10 microcurie (μCi) ^{33}P (^{33}P-orthophosphate, Perkin Elmer) was administered orally with lunch (meal contained ~300 mg P) followed by 6 hours of blood draws (time points: 15 and 30 minutes, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 6 hours post-administration of ^{33}P). Patients were asked to void urine and defecate prior to the oral administration of ^{33}P. The 24-hour post-oral ^{33}P blood sample was obtained on Day 12 before the IV ^{33}P administration. On Day 12, 10 μCi ^{33}P was then given by IV (1 hour after lunch, or 25 hours after oral ^{33}P), after a baseline blood draw (time point: 0, prior to IV ^{33}P), followed by 6 hours of blood draws at time points: 15 and 30 minutes, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 6 hours post-administration of IV ^{33}P). On Day 13, the final, 24-hour post-IV dose blood sample for ^{33}P was collected. During the 48-hour inpatient study, all feces and urine (if patient was not anuric) was collected. Fecal samples were homogenized, ashed in a muffle furnace (Thermolyne Sybron Type 30400, Dubuque, IA, USA), and diluted with 2% nitric acid for mineral analysis (Optima 4300DV, Perkin Elmer, Shelton, CT)[37]. Urine samples were acidified with 1% (volume/volume) concentrated hydrogen chloride (HCl) at the time of aliquoting, and diluted with 2% nitric acid for mineral analysis by ICP-OES. ^{33}P activity was measured in serum, urine, and feces by liquid scintillation counting (Tri-Carb 2910 TR Liquid Scintillation

Analyzer, Perkin Elmer, Waltham, MA). ^{33}P tracer data in serum (% of dose/mL) and total P in serum (mg/dL) were used for kinetic modeling to determine fractional P absorption. Urine and fecal ^{33}P and total P data were obtained in some patients over the 48 hours but were too sparse to be included in the modeling results. The ^{33}P data were analyzed by compartmental modelling using a general equation-solving software (WinSAAM) with the percent absorption calculated as the fraction of P moving into blood vs. moving down the intestinal tract (**Supplemental Figure 4**). The model structure was determined from previous studies in healthy humans[38]; an example of the serum ^{33}P curves following oral and intravenous ^{33}P administration is shown in **Figure 5**.

Statistical Analyses: This was a pilot study with a goal of having 10 patients complete both arms in each study to assess safety and efficacy of phosphorus absorption, with 12-14 patients recruited to ensure 10 completions. This sample size provides 80% power to detect adverse events (AEs) of true proportion of 16.7%. The safety population was defined as the 14 (Study 1) and 12 (Study 2) patients who received any study treatment and had at least one safety assessment visit. Safety was determined by the summary of AEs, vital signs, clinical laboratory tests, ECGs, and echocardiograms. AEs were summarized by Medical Dictionary for Regulatory Activities (MedDRA) system. Drug relatedness was assessed by the Principal Investigator (blinded to treatment allocation) after reviewing all data available from each event. Efficacy analyses were conducted only on the patients that had complete data set (n=10 for each study). Demographic and baseline characteristics, and exposure to study treatment were summarized by treatment for each study. The efficacy outcomes of interest were analyzed using an analysis of variance (ANOVA) mixed model for crossover designs by SAS software (v9.4 Cary, NC) with fixed effects for order, period, and treatment, and random effects for subject nested within order. In exploratory analyses, peak plasma P, pre-dialysis P, and pre-post dialysis drop in plasma P were examined as covariates in the ANOVA models of treatment differences in intestinal fractional P absorption. In a secondary analysis, data from subjects who completed both Study 1 and Study 2 (N=6) were analyzed for differences in intestinal fractional P absorption among placebo, 50 mg EOS789, and 100 mg EOS789 by an

ANOVA mixed model with fixed effects for treatment and random effects for subject. Two-sided statistical significance was set at α=0.05.

The pharmacokinetic analyses calculated individual and mean plasma EOS789 concentration versus time data were tabulated and plotted by dose level. The plasma PK of EOS789 was summarized by estimating total exposure (area under the curve [AUC]), maximum concentration (C_{max}). Calculation of EOS789 removal ratio during a single hemodialysis session was performed. All plasma samples for the PK analyses were collected only for the first treatment arm in each study.

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Author contributions: SMM, KHG, NH, YO, and TK designed the study, SMM, KHG, ES, LT, RNM,SD carried out the human subject interventions, SMM, KHG, MEW, NH, JS, NF, YM, and TK analyzed the data, KHG and NH, JS made the figures, KHG and SMM drafted the manuscript, and all authors edited and approved the final version of the manuscript.

Supplementary Material:

1. Supplemental Figure 1: Consort Diagram of Recruitment
2. Supplemental Figure 2: Individual pharmacokinetic dose curves
3. Supplemental Figure 3: Relationship of vitamin D to phosphorus absorption
4 Supplemental Figure 4: Kinetic Model
5. Supplemental Table 1: Number of Patients with AEs by Event (Safety Population)
6. Supplemental Table 2: Biochemical Measures
7. Supplemental Table 3: Inclusion/Exclusion Criteria
8. Supplemental Table 4: Study Diets

References:

1. Kestenbaum B. Mineral metabolism disorders in chronic kidney disease. *JAMA* 2011; **305:** 1138-1139.

2. Block GA, Klassen PS, Lazarus JM*, et al.* Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol* 2004; **15:** 2208-2218.

3. Palmer SC, Hayen A, Macaskill P*, et al.* Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. *JAMA* 2011; **305:** 1119-1127.

4. Chen NX, Moe SM. Pathophysiology of Vascular Calcification. *Curr Osteoporos Rep* 2015; **13:** 372-380.

5. Rodelo-Haad C, Rodriguez-Ortiz ME, Martin-Malo A*, et al.* Phosphate control in reducing FGF23 levels in hemodialysis patients. *PLoS One* 2018; **13:** e0201537.

6. Kates DM, Sherrard DJ, Andress DL. Evidence that serum phosphate is independently associated with serum PTH in patients with chronic renal failure. *American Journal of Kidney Diseases* 1997; **30:** 809-813.

7. Floege J, Kim J, Ireland E*, et al.* Serum iPTH, calcium and phosphate, and the risk of mortality in a European haemodialysis population. *Nephrol Dial Transplant* 2011; **26:** 1948-1955.

8. Lopes AA, Tong L, Thumma J*, et al.* Phosphate binder use and mortality among hemodialysis patients in the Dialysis Outcomes and Practice Patterns Study (DOPPS): evaluation of possible confounding by nutritional status. *Am J Kidney Dis* 2012; **60:** 90-101.

9. Fissell RB, Karaboyas A, Bieber BA*, et al.* Phosphate binder pill burden, patient-reported non-adherence, and mineral bone disorder markers: Findings from the DOPPS. *Hemodial Int* 2016; **20:** 38-49.

10. Moe SM, Zidehsarai MP, Chambers MA*, et al.* Vegetarian compared with meat dietary protein source and phosphorus homeostasis in chronic kidney disease. *Clin J Am Soc Nephrol* 2011; **6:** 257-264.

11. Karp H, Ekholm P, Kemi V*, et al.* Differences among total and in vitro digestible phosphorus content of plant foods and beverages. *J Ren Nutr* 2012; **22:** 416-422.

12. Karp H, Ekholm P, Kemi V*, et al.* Differences among total and in vitro digestible phosphorus content of meat and milk products. *J Ren Nutr* 2012; **22:** 344-349.

13. Stremke ER, McCabe LD, McCabe GP*, et al.* Twenty-Four-Hour Urine Phosphorus as a Biomarker of Dietary Phosphorus Intake and Absorption in CKD: A Secondary Analysis from a Controlled Diet Balance Study. *Clin J Am Soc Nephrol* 2018; **13:** 1002-1012.

14. Farrington K, Mohammed MN, Newman SP*, et al.* Comparison of radioisotope methods for the measurement of phosphate absorption in normal subjects and in patients with chronic renal failure. *Clin Sci (Lond)* 1981; **60:** 55-63.

15. Wiegmann TB, Kaye M. Malabsorption of calcium and phosphate in chronic renal failure: 32P and 45Ca studies in dialysis patients. *Clin Nephrol* 1990; **34:** 35-41.

16. Hill KM, Martin BR, Wastney ME*, et al.* Oral calcium carbonate affects calcium but not phosphorus balance in stage 3-4 chronic kidney disease. *Kidney Int* 2013; **83:** 959-966.

17. Marks J. The role of SLC34A2 in intestinal phosphate absorption and phosphate homeostasis. *Pflugers Arch* 2019; **471:** 165-173.

18. Schiavi SC, Tang W, Bracken C*, et al.* Npt2b deletion attenuates hyperphosphatemia associated with CKD. *J Am Soc Nephrol* 2012; **23:** 1691-1700.

19. Sabbagh Y, O'Brien SP, Song W*, et al.* Intestinal npt2b plays a major role in phosphate absorption and homeostasis. *J Am Soc Nephrol* 2009; **20:** 2348-2358.

20. Larsson TE, Kameoka C, Nakajo I*, et al.* NPT-IIb Inhibition Does Not Improve Hyperphosphatemia in CKD. *Kidney Int Rep* 2018; **3:** 73-80.

21. Ichida Y, Hosokawa N, Takemoto R*, et al.* Significant Species Differences in Intestinal Phosphate Absorption between Dogs, Rats, and Monkeys. *J Nutr Sci Vitaminol (Tokyo)* 2020; **66:** 60-67.

22. Candeal E, Caldas YA, Guillen N*, et al.* Intestinal phosphate absorption is mediated by multiple transport systems in rats. *Am J Physiol Gastrointest Liver Physiol* 2017; **312:** G355-G366.

23. Candeal E, Caldas YA, Guillen N*, et al.* Na+-independent phosphate transport in Caco2BBE cells. *Am J Physiol Cell Physiol* 2014; **307:** C1113-1122.

24. Tsuboi Y, Ohtomo S, Ichida Y*, et al.* EOS789, a novel pan-phosphate transporter inhibitor of multi-phosphate transporters, is effective for the treatment of chronic kidney disease-mineral bone disorder. *Kidney Int* 2020; 98: 343-354

25. King AJ, Siegel M, He Y*, et al.* Inhibition of sodium/hydrogen exchanger 3 in the gastrointestinal tract by tenapanor reduces paracellular phosphate permeability. *Sci Transl Med* 2018; **10**.

26. Block GA, Rosenbaum DP, Yan A*, et al.* Efficacy and Safety of Tenapanor in Patients with Hyperphosphatemia Receiving Maintenance Hemodialysis: A Randomized Phase 3 Trial. *J Am Soc Nephrol* 2019; **30:** 641-652.

27. Leggett RW. Proposed biokinetic model for phosphorus. *J Radiol Prot* 2014; **34:** 417-433.

28. Wang AY, Woo J, Sea MM*, et al.* Hyperphosphatemia in Chinese peritoneal dialysis patients with and without residual kidney function: what are the implications? *Am J Kidney Dis* 2004; **43:** 712-720.

29. Elias RM, Alvares VRC, Moyses RMA. Phosphate Removal During Conventional Hemodialysis: a Decades-Old Misconception. *Kidney Blood Press Res* 2018; **43:** 110-114.

30. Rafik H, Aatif T, El Kabbaj D. The impact of blood flow rate on dialysis dose and phosphate removal in hemodialysis patients. *Saudi J Kidney Dis Transpl* 2018; **29:** 872-878.

31. Sugisaki H, Onohara M, Kunitomo T. Dynamic behavior of plasma phosphate in chronic dialysis patients. *Trans Am Soc Artif Intern Organs* 1982; **28:** 302-307.

32. Minutolo R, Bellizzi V, Cioffi M*, et al.* Postdialytic rebound of serum phosphorus: pathogenetic and clinical insights. *J Am Soc Nephrol* 2002; **13:** 1046-1054.

33. Viaene L, Meijers B, Vanrenterghem Y*, et al.* Daytime rhythm and treatment-related fluctuations of serum phosphorus concentration in dialysis patients. *Am J Nephrol* 2012; **35:** 242-248.

34. Svara F, Lopot F, Valkovsky I*, et al.* Phosphorus Removal in Low-Flux Hemodialysis, High-Flux Hemodialysis, and Hemodiafiltration. *ASAIO J* 2016; **62:** 176-181.

35. Zupancic T, Ponikvar R, Gubensek J*, et al.* Phosphate Removal During Long Nocturnal Hemodialysis/Hemodiafiltration: A Study With Total Dialysate Collection. *Ther Apher Dial* 2016; **20:** 267-271.

36. Bertocchio JP, Mohajer M, Gaha K*, et al.* Modifications to bicarbonate conductivity: A way to increase phosphate removal during hemodialysis? Proof of concept. *Hemodial Int* 2016; **20:** 601-609.

37. Hill KM, Martin BR, Wastney ME*, et al.* Oral calcium carbonate affects calcium but not phosphorus balance in stage 3-4 chronic kidney disease. *Kidney Int* 2012.

38. Hill Gallant KM, Choi MS, Stremke ER*, et al.* Evaluation of a Radiophosphorus Meethod for Intestinal Phosphorus Absorption Assessment in Humans. *J Bone Miner Res* 2018; **33**.

Table 1: Adverse Events (AE)

Arm	Number (percent) of patients with at least one AE	Number of overall AE events	Number (percent) of patients with AE considered related to EOS789	Number of AE events considered possibly related to EOS789
Study 1 placebo (n=13)	7 (53.8%)	9 events	2 (15.4%)	2 events (restless legs syndrome, abnormal liver function tests)
Study 1 EOS789 50 mg tid (n=13)	6 (46.2%)	12 events	1 (7.7%)	1 event (asthenia)
Study 2 EOS789 100mg tid (n=12)	6 (50.0%)	10 events	1 (8.3%)	3 events (nausea, diarrhea, headache)
Study 2 EOS789 100 mg tid + sevelamer carbonate 1600 mg tid (n=11)	7 (63.6%)	9 events	4 (36.4%)	4 events (nausea, hypercalcemia, hyponatremia, bundle branch block right)

Table 2 Subject Demographics and Baseline Biochemistries

	Study 1 (n = 10)	Study 2 (n = 10)
Age, yr	52.3 ± 10.0 (median 52.0, range 41-69)	56.7 ± 14.3 (median 55.0, range 38-85)
Age Groups, n (%)		
30 - 39	0	1 (10.0%)
40 – 49	4 (40.0%)	3 (30.0%)
50 – 59	4 (40.0%)	2 (20.0%)
60 – 69	2 (20.0%)	2 (20.0%)
> 70	0	2 (20.0%)
Sex, n (%)		
Male	4 (40.0%)	2 (20.0%)
Female	6 (60.0%)	8 (80.0%)
Ethnicity, n (%)		
Non-Hispanic or Latino	10 (100.0%)	10 (100.0%)
Race, n (%)		
Black or African American	8 (80.0%)	9 (90.0%)
White	2 (20.0%)	1 (10.0%)
BMI, kg/m^2	31.4 ± 10.8 (median 28.0, range 20.7-53.3)	32.0 ± 9.5 (median 30.2, range 20.6-52.5)
Tobacco use history, n (%)		
Never	5 (50.0%)	5 (50.0%)
Current	3 (30.0%)	3 (30.0%)
Previous	2 (20.0%)	2 (20.0%)
Dialysis Vintage, yr ± SD	5.24 ± 4.37	3.48 ± 2.27
On VDRA, n (%)	9 (90.0%)	9 (90.0%)
On Calcimimetic, n (%)	3 (30.0%)	1 (10.0%)
Serum P, mg/dL		
Screening	5.4 ± 1.3	5.8 ± 1.1
After binder washout	6.8 ± 1.7	8.0 ± 2.1
After 3 days diet, Baseline (pre-drug treatment)- first arm	6.8 ± 1.2	7.2 ± 1.8
Baseline Serum Ca, mg/dL	8.9± 0.98	9.5 ± 0.9
Baseline Serum intact PTH, pg/mL	529 ± 604 (median 398)	354 ± 228 (median 339)
Baseline C-terminal FGF23 (RU/mL)	37,085 ± 40,633 (median 15,781)	30,888 ± 40,794 (median 14,941)
Baseline 1,25 vitamin D, pg/mL *Reference range males-18-64 pg/mL and females 18-78 pg/L*	27.1 ± 13.9* Range (< 8 to 51)	35.7 ± 21* Range (< 8 to 83)

Values are mean ± SD unless otherwise indicated.

VDRA = Vitamin D receptor analogue

* for values < 8, the number 8 was used to calculate the mean ± SD

Figure Legends:

Figure 1: Schematic of study design.

Each study consisted of two sequences (arms) of 13-14 days duration with a two week wash out between sequences. During the sequence, subjects had study diet for the entire duration and study drug began on Day 4. The subjects were hospitalized in the Clinical Research Center on Day 9 (first sequence for PK) or Day 10 (second sequence) of each study. Patients underwent hemodialysis prior to admission, and as the final event during the admission such that all of ^{33}P studies were done between dialysis treatments.

Figure 2: Pharmacokinetic (PK) analyses of EOS789.

Mean ± SD plasma EOS789 concentration at steady state (on Day 10). PK sampling points were pre-morning dose, and 1, 2, 4, 6, 8, 12, 24 hours post-dose. Doses were given at 0, 4, and 8 hours. Only the first sequence was used for the PK sampling with n = 5 to 7 per group. Individual PK curves for each dose are shown in Supplemental Figure 2.

Figure 3: Intestinal fractional P absorption by ^{33}P.

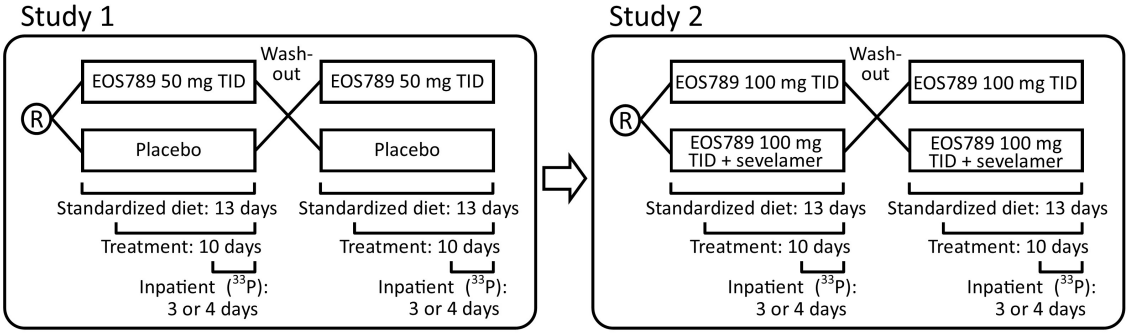
Fractional P absorption in individual patients in Study 1 (A: comparing placebo versus EOS789 50 mg TID with meals) and Study 2 (B: comparing EOS789 100 mg versus EOS789 100 mg with sevelamer TID with meals). Each line represents one patient, EOS789-50= EOS789 50 mg TID, EOS789-100 = EOS789 100 mg TID, EOS789-100 + Sev = 100 mg EOS789 + 1600 mg sevelamer TID. P = phosphorus/phosphate.

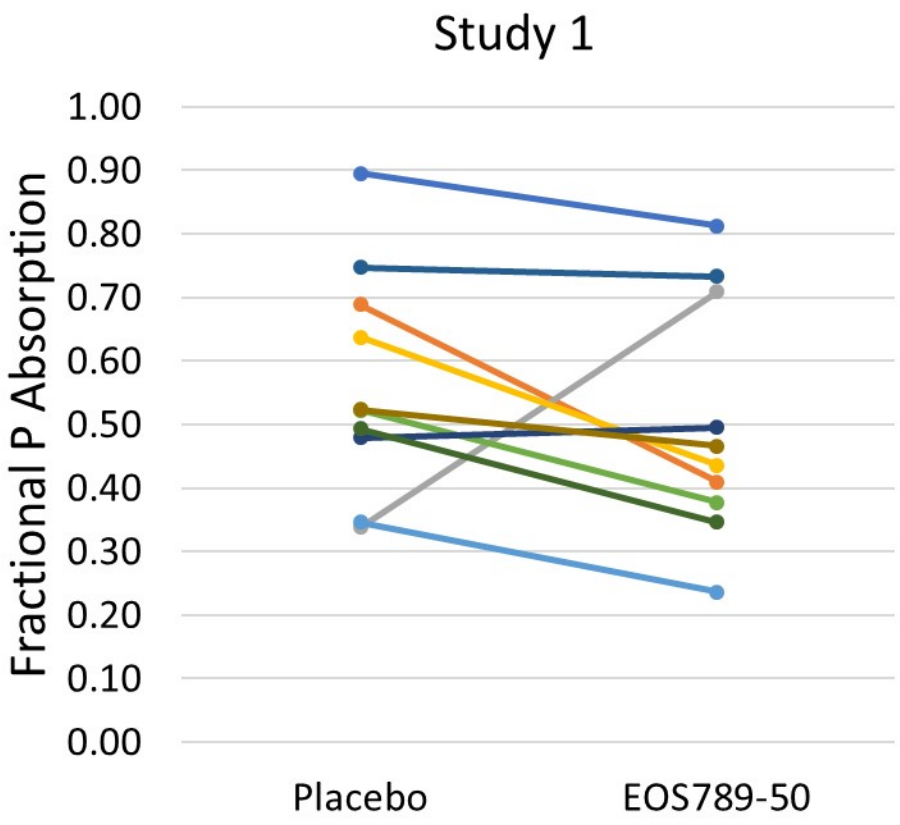
Figure 4: Dose efficacy of EOS789 in intestinal fractional P absorption.

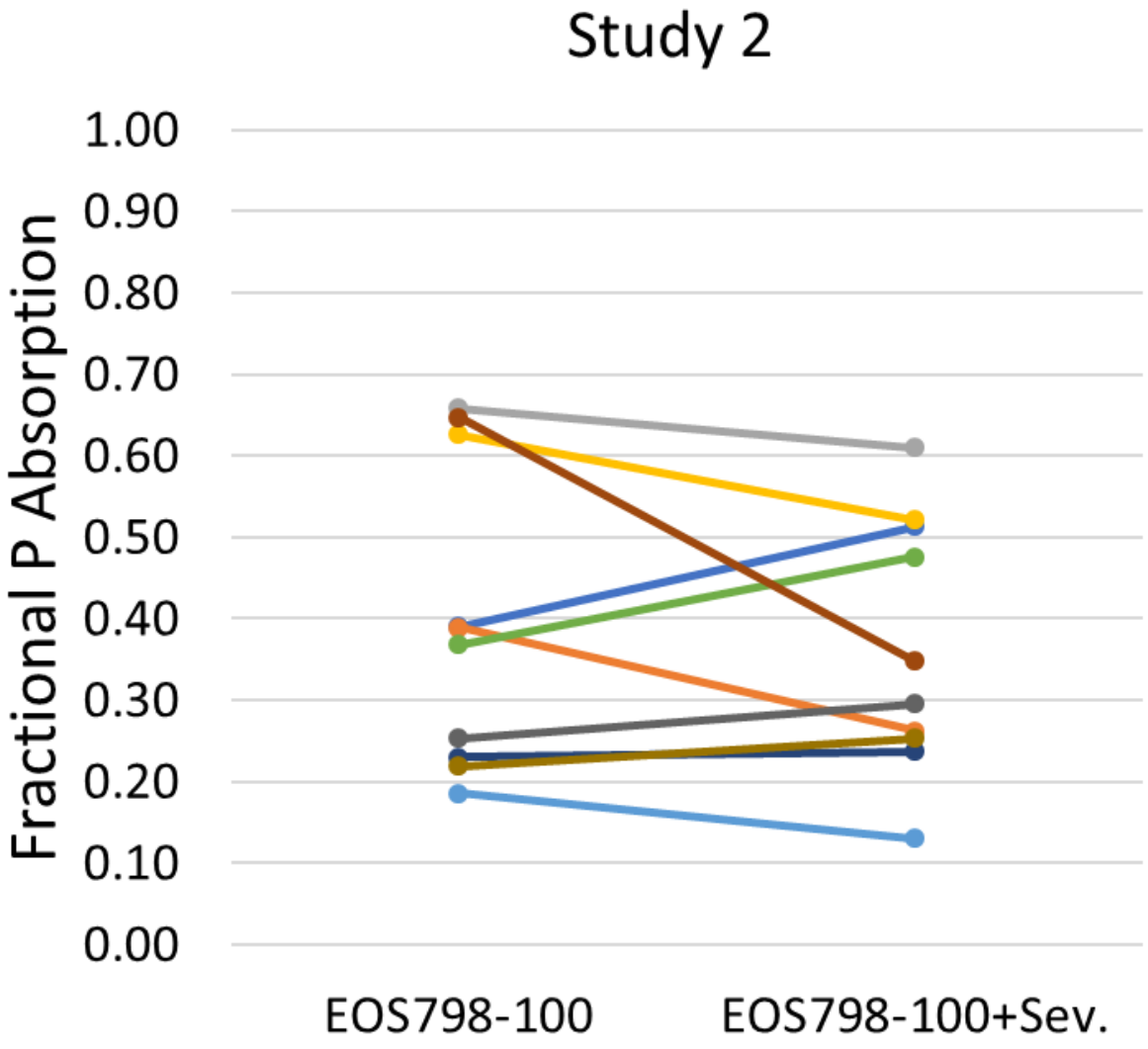
In a planned secondary analysis, data from the six patients who completed both studies were analyzed to generate a dose response comparing placebo, EOS789-50 = 50 mg TID, and EOS789-

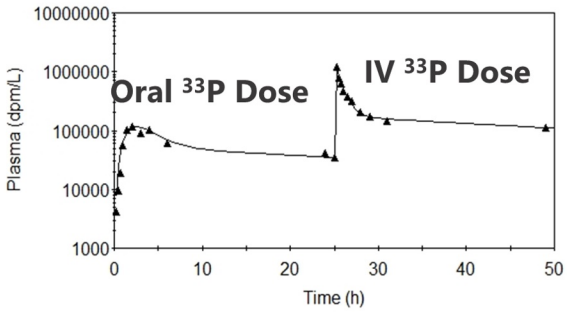
100 = EOS789 100 mg TID. There was a significant difference between placebo and EOS789 100mg TID ($p = 0.028$).

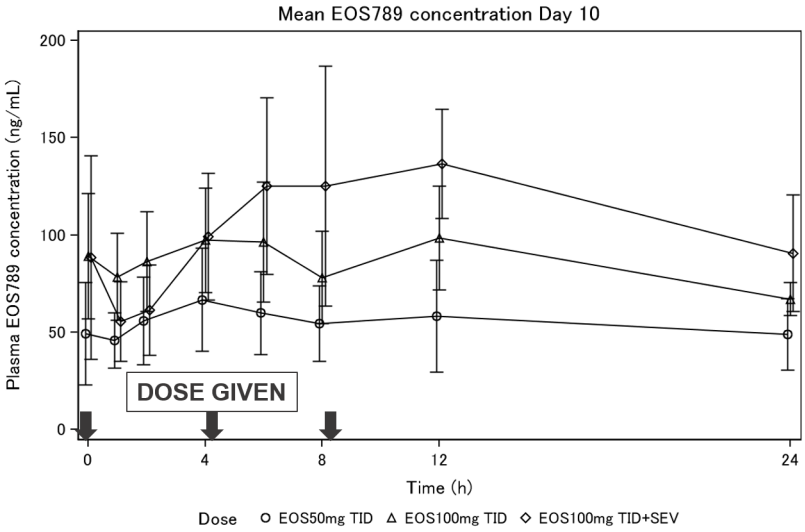
Figure 5. **Example of assessment of intestinal fractional P absorption using a single isotope**. ^{33}P was used to assess fractional P absorption. Staggering the oral and IV ^{33}P istotpe administration by one day allows for the use of this single low energy beta-emitter isotope. Kinetic modeling of the ^{33}P radioactivity counts by liquid scintillation counting of plasma samples allows for calculation of intestinal fractional P absorption. DPM = disintegrations per minute.

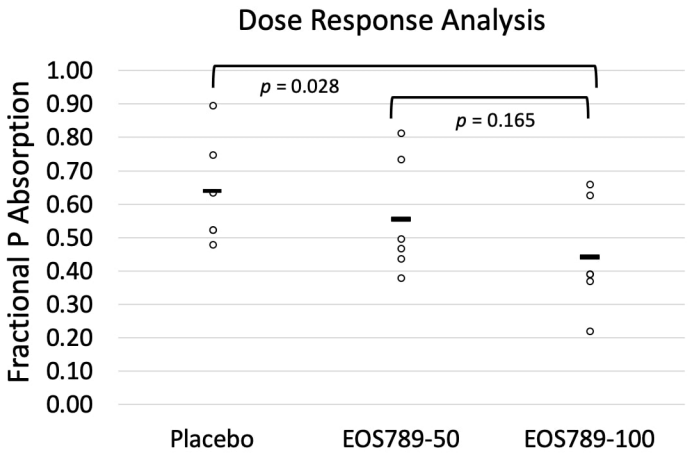

Study 1
Wash-out
EOS789 50 mg TID
EOS789 50 mg TID
R
Placebo
Placebo
Standardized diet: 13 days
Standardized diet: 13 days
Treatment: 10 days
Treatment: 10 days
Inpatient (^{33}P): 3 or 4 days
Inpatient (^{33}P): 3 or 4 days
Study 2
Wash-out
EOS789 100 mg TID
EOS789 100 mg TID
R
EOS789 100 mg TID + sevelamer
EOS789 100 mg TID + sevelamer
Standardized diet: 13 days
Standardized diet: 13 days
Treatment: 10 days
Treatment: 10 days
Inpatient (^{33}P): 3 or 4 days
Inpatient (^{33}P): 3 or 4 days


Study 1
Fractional P Absorption
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Placebo
EOS789-50


Study 2
Fractional P Absorption
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
EOS798-100
EOS798-100+Sev.


10000000
1000000
100000
10000
1000
Plasma (dpm/L)
Oral 33P Dose
IV 33P Dose
0
10
20
30
40
50
Time (h)


Mean EOS789 concentration Day 10
Plasma EOS789 concentration (ng/mL)
200
150
100
50
0
DOSE GIVEN
0
4
8
12
24
Time (h)
Dose ○ EOS50mg TID △ EOS100mg TID ◇ EOS100mg TID+SEV


Dose Response Analysis
Fractional P Absorption
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
p = 0.028
p = 0.165
Placebo
EOS789-50
EOS789-100