Efficacy and Safety of Tenapanor in Patients with Hyperphosphatemia Receiving Maintenance Hemodialysis: A Randomized Phase 3 Trial

Geoffrey A. Block,[1] David P. Rosenbaum,[2] Andrew Yan,[2] and Glenn M. Chertow[3]

[1]Denver Nephrology Research Division, Denver Nephrology, Denver, Colorado; [2]Ardelyx Inc., Fremont, California; and [3]Division of Nephrology, Stanford University School of Medicine, Stanford, California

ABSTRACT

Background Guidelines recommend reducing elevated serum phosphate in patients with CKD. Tenapanor, a minimally absorbed inhibitor of gastrointestinal sodium/hydrogen exchanger 3 (NHE3), reduces paracellular phosphate transport.

Methods In this phase 3 randomized, double-blind trial, we randomly assigned patients with hyperphosphatemia receiving maintenance hemodialysis to receive twice-daily oral tenapanor (3, 10, or 30 mg [the latter down-titrated, if needed]) for 8 weeks. Patients were then rerandomized 1:1 to receive either their previously assigned dose or placebo for a 4-week 'withdrawal' period. We measured serum phosphate levels over the course of the trial. The primary end point was mean change in serum phosphate over the 4-week withdrawal period for the tenapanor group (using pooled data) versus the placebo group.

Results Of 219 patients randomized, 152 completed both study phases. During the initial 8-week treatment period, all three treatment groups experienced significant decreases in mean serum phosphate (reductions of 1.00, 1.02, and 1.19 mg/dl, corresponding to the 3, 10, and 30 mg [down-titrated] dose groups, respectively). Tenapanor also showed a significant benefit over placebo during the withdrawal period, with a mean increase of 0.85 mg/dl in the placebo group versus a mean increase of 0.02 mg/dl in the pooled tenapanor group. Adverse events were largely limited to softened stool and a modest increase in bowel movement frequency, resulting from increased stool sodium and water content, stemming from tenapanor's mechanism of action.

Conclusions Tenapanor significantly reduced elevated serum phosphate in patients with hyperphosphatemia receiving maintenance hemodialysis. Adverse effects were limited to those induced by its known mechanism of action, which increases stool sodium and water content.

J Am Soc Nephrol 30: 641–652, 2019. doi: https://doi.org/10.1681/ASN.2018080832

Reducing elevated serum phosphate in patients with ESRD receiving maintenance dialysis has been a therapeutic goal for nearly three decades.[1] Initial clinical concerns were focused on the contribution of serum phosphate to the development of secondary hyperparathyroidism and uremic pruritus; subsequently, these concerns were overshadowed by consistent observations demonstrating a monotonic relationship between serum phosphate concentration and cardiovascular risk in patients spanning the entire spectrum of kidney function.[2–5] Today, most nephrologists attempt to reduce serum phosphate as part of a comprehensive cardiovascular risk-reduction strategy.[6] Therapeutic interventions designed to accomplish this goal remain limited to phosphate binders

Received August 17, 2018. Accepted January 24, 2019.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Geoffrey A. Block, Denver Nephrology Research Division, Denver Nephrology, 130 Rampart Way Suite 175, Denver CO 80230. Email: geoff@goldenblocks.org

and reducing dietary phosphate intake. Frequent hemodialysis improves phosphate control, yet logistic and regulatory hurdles preclude adoption of this intervention at a population level.[7]

Surprisingly little is known about the physiology of phosphate homeostasis in patients with CKD or in healthy persons. Serum phosphate concentration is known to be influenced by circadian rhythm as well as by phosphate ingestion, absorption, and excretion.[8–11] Absolute intestinal phosphate absorption is dependent on both active transport and passive, paracellular transport. Active phosphate transport is thought to be saturated at relatively low intraluminal phosphate concentrations; thus, the bulk of net phosphate transport is thought to be driven by the concentration-dependent, nonsaturable, paracellular pathway.[12]

Tenapanor is a minimally systemically absorbed inhibitor of intestinal sodium/hydrogen exchanger 3 (NHE3) that reduces phosphate absorption in healthy persons and lowers elevated serum phosphate concentrations in patients receiving maintenance hemodialysis.[13–15] The effect of tenapanor on phosphate absorption is mediated by transiently increasing the intracellular proton concentration in cells lining the gastrointestinal lumen, a result of NHE3 inhibition, which induces a conformational change in tight junction proteins, thereby decreasing permeability to paracellular phosphate transport; this action has no apparent effect on the absorption of other ions (except sodium) or nutrients.[12] A consequence of intestinal NHE3 inhibition is that stool sodium and water content are increased, loosening stool consistency and increasing bowel movement frequency.[15–17] We conducted this phase 3 placebo-controlled, randomized clinical trial in patients receiving maintenance hemodialysis with hyperphosphatemia to test the safety and efficacy of tenapanor.

Significance Statement

Phosphate binders are currently the only medications available to reduce elevated serum phosphate in patients with ESRD receiving hemodialysis. Tenapanor, a minimally absorbed inhibitor of gastrointestinal sodium/hydrogen exchanger 3 (NHE3), acts via a non–phosphate-binding mechanism, reducing paracellular phosphate transport in the intestine. The authors found that tenapanor significantly lowered elevated serum phosphate in patients receiving hemodialysis, resulting in a mean reduction of 1.0–1.2 mg/dl over 8 weeks. Tenapanor also showed a significant benefit over placebo in patients rerandomized to either continue tenapanor treatment or receive a placebo for 4 weeks. Adverse effects were largely limited to softening of stool and more frequent bowel movements. By targeting paracellular phosphate transport's substantial contribution to net phosphate absorption in the gut, tenapanor has the potential to improve management of mineral bone disorder in CKD.

METHODS

Study Design

The trial (Clinicaltrials.gov identifier NCT02675998) was conducted at 41 sites in the United States between January 20, 2016 and January 6, 2017 in accordance with the Declaration of Helsinki, International Conference on Harmonization, and Good Clinical Practice guidelines. The protocol and all amendments were approved by an independent ethics committee or institutional review board. All participants provided written informed consent.

The trial was originally designed as a double-blind, dose-ranging phase 2 study with the primary end point being the change in serum phosphate from baseline to the end of the 8-week randomized treatment period (RTP). After trial initiation, the US Food and Drug Administration (FDA) informed the sponsor that a previous phase 2 study[13] was sufficient for dose range finding and proposed conversion to a phase 3 trial incorporating a 4-week, double-blind, placebo-controlled, randomized withdrawal period (RWP); the protocol was amended on March 3, 2016, when 22 patients were enrolled (Figure 1). The primary end point was amended to the between-groups (pooled tenapanor versus placebo) difference in the mean change in serum phosphate from the end of the RTP to the end of the RWP (or the end point visit for this period) in a protocol amendment dated May 27, 2016, when 94 patients were enrolled. A responder analysis of serum phosphate change in the RWP was also requested by the FDA, which was performed among patients who experienced at least a 1.2-mg/dl decrease in serum phosphate during the RTP. All protocol amendments were made before any efficacy data had been analyzed. Details of key protocol changes are provided in the Supplemental Material.

Adults (aged 18–80 years) with ESRD who had been on maintenance hemodialysis for at least 3 months, who were receiving at least three doses of phosphate-binding medication per day, and who had serum phosphate concentrations of 4.0–7.0 mg/dl (inclusive) were eligible. Vitamin D and/or calcimimetic therapy were required to have been stable for at least 4 weeks before screening. After the 1–3-week washout period, patients must have had an increase in serum phosphate of at least 1.5 mg/dl, with an absolute value from 6.0 mg/dl to $<$10.0 mg/dl, to be eligible for randomization. Exclusion criteria included parathyroid hormone $>$1200 pg/ml, serum phosphate $>$10.0 mg/dl at any time in the previous 3 months, serum bicarbonate $<$18 mmol/L on two consecutive measurements, diarrhea/loose stool ($\geq$3 bowel movements/day on two or more days or any stool of Bristol Stool Form Scale [see Supplemental Material][18] $\geq$6 during the week before randomization), and life expectancy $<$6 months.

On day 1, eligible patients were randomly assigned to one of three parallel tenapanor regimens—3- or 10-mg fixed dose twice a day, or 30 mg twice a day which could be down-titrated during the first 4 weeks of the RTP in a step-wise fashion to 20, 15, 10, or 3 mg twice a day on the basis of gastrointestinal tolerability—in a 1:1:1 ratio using a computer-generated randomization schedule and a block size of 3. Study site staff and patients were blinded to treatment assignment. To preserve blinding, all patients were asked about tolerability at each study visit. Tenapanor was formulated as round, 9-mm diameter, plain, white, film-coated tablets irrespective of dose. Patients took two tablets

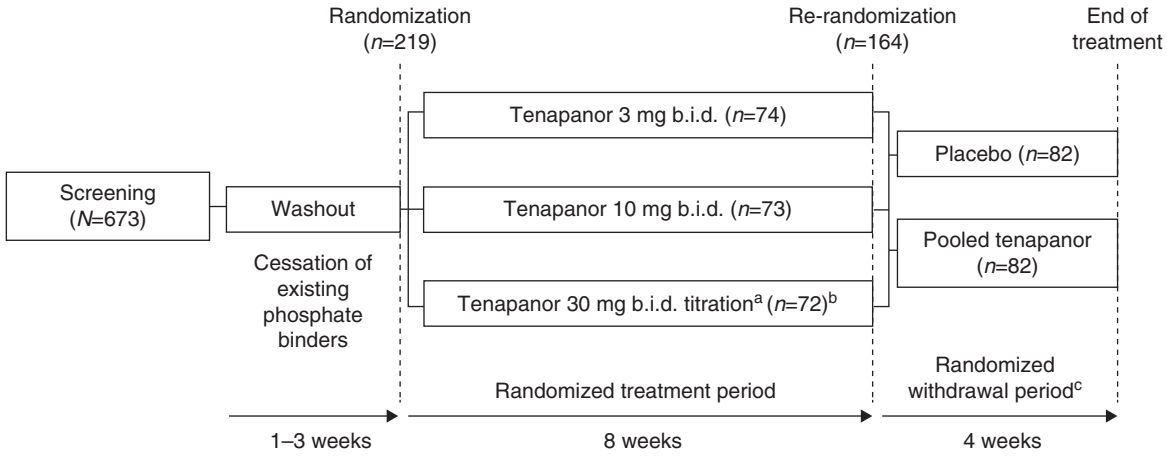


Figure 1. Study design. [a]Patients initially receiving tenapanor 30 mg twice a day were allowed to down-titrate weekly (stepwise 30 → 20 → 15 → 10 → 3 mg twice a day) during the first 4 weeks of the RTP, on the basis of gastrointestinal tolerability. Mean final dose of tenapanor in this group at the end of the RTP was 24.4 mg twice a day for the safety and ITT analysis sets, and 22.8 mg twice a day for the secondary efficacy analysis set. [b]One patient did not receive any dose of study drug and was excluded from analyses. [c]The study was initiated on January 20, 2016 as an 8-week randomized dose range–finding study; the RWP was added to the protocol in an amendment dated March 3, 2016, when 22 patients were enrolled. Patients randomized to tenapanor in the RWP remained on their previous dose from the RTP. Mean exposure during the RTP was 48 days in all three treatment groups, and during the RWP was 26 days for patients receiving placebo and 27 days for patients receiving tenapanor. *bid*, twice daily.

in the morning before breakfast and two tablets in the evening before dinner; patients did not take tablets at the meal before dialysis and instead took them before another meal on that day (see Supplemental Table 1 for dosing regimens).

All randomized patients entered the 8-week RTP. At the end of the RTP, patients were rerandomized 1:1 to either remain on their previously assigned dose of tenapanor or to receive matching placebo and entered a 4-week RWP. We pooled data from all three tenapanor groups in the RWP.

Study Assessments

Study visits included a screening visit, 1–3 postwashout visits, a randomization visit, weekly visits at weeks 1–4, and every other week thereafter. All study assessments occurred after a short dialysis interval and predialysis. Serum parathyroid hormone and fibroblast growth factor 23 (FGF23) were assessed using intact assays (Roche Diagnostics, Indianapolis, IN and Kainos Laboratories, Tokyo, Japan, respectively). To assess tolerability, all patients used an electronic diary (phone) to record daily bowel habits (frequency and stool form, using the Bristol Stool Form Scale[18]) for the entire study. Safety assessments included physical examination, vital signs, laboratory tests, 12-lead electrocardiograms, and adverse event recording. We assessed adherence to study medication by pill count. Full details of study assessments are provided in the Supplemental Material.

Study Outcomes and Statistical Analyses

Separate safety analysis sets were analyzed in the RTP and RWP, each of which included all patients who received at least one dose of study treatment in the respective period and was used for the analysis of all safety outcomes. The intention-to-treat (ITT) analysis set for efficacy assessments included all patients who received at least one dose of study treatment and had at least one postbaseline serum phosphate assessment during the RTP. Key efficacy outcomes were the change in serum phosphate from baseline to the end of the 8-week RTP for each tenapanor group (the original primary end point) and the change in serum phosphate from the end of the RTP to the end of the RWP (or the end point visit for this period) for the pooled tenapanor and placebo groups (the revised primary end point). The proportion of patients with serum phosphate <5.5 mg/dl at each visit during the RTP was a secondary efficacy outcome. The secondary analysis requested by the FDA, change in serum phosphate from the end of the RTP to the end of the RWP among responders, was assessed using an efficacy analysis set composed of all patients who completed the RTP and achieved at least a 1.2-mg/dl reduction in serum phosphate from baseline to the end of the RTP.

Continuous efficacy variables were assessed using an analysis of covariance model with investigator site and treatment group as fixed factors and baseline value as a covariate, with change from baseline to each assessment as the dependent variable. We log-transformed FGF23 data before inference testing owing to the highly skewed distribution and, as such, we report ratios of the geometric means to aid interpretability.

Further details of the study outcome measures and statistical analyses are provided in the Supplemental Material.

RESULTS

Patient Disposition and Baseline Characteristics

Of 673 patients screened, 219 patients were randomized to one of three tenapanor treatment groups (Figure 2). A total of 164

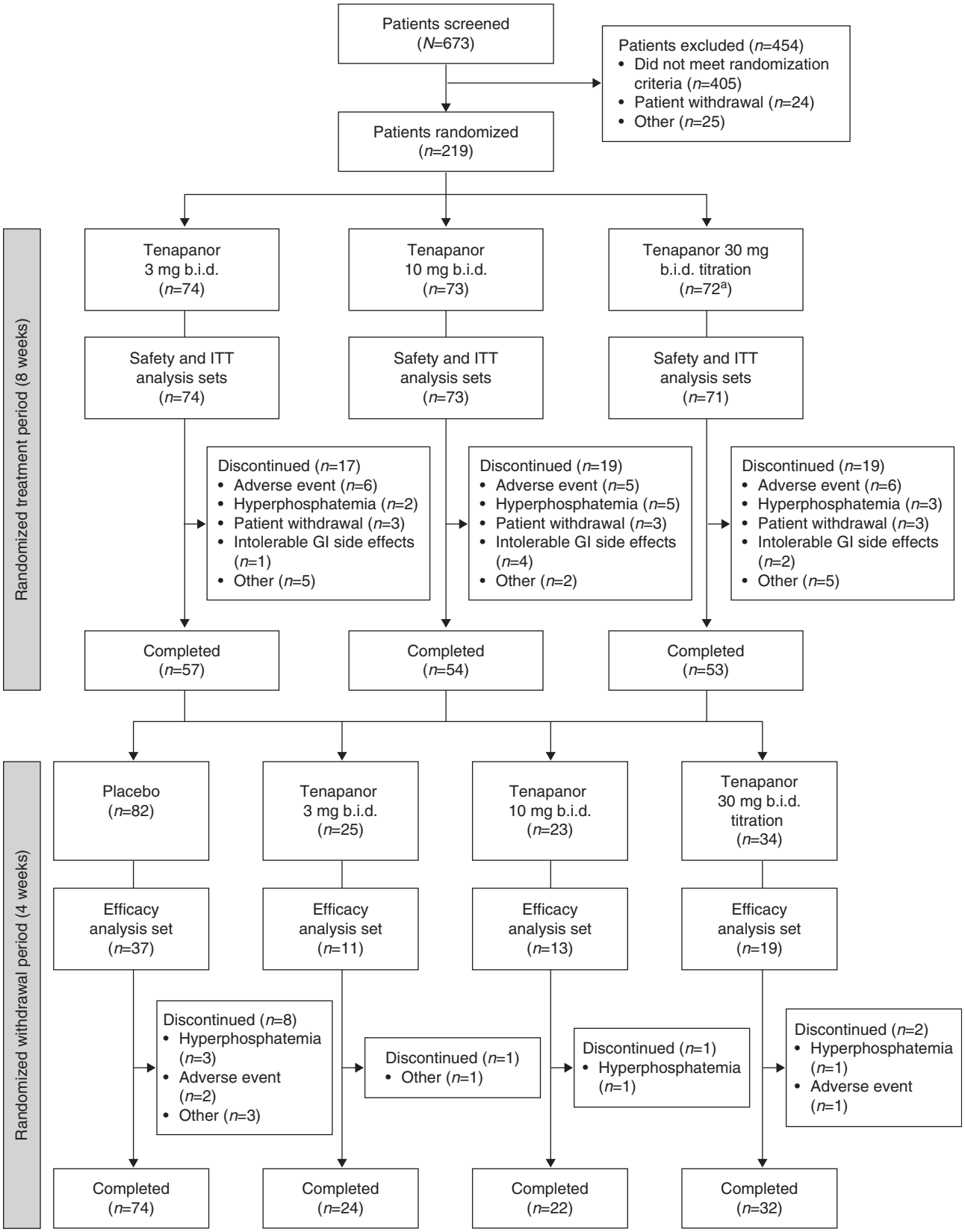


Figure 2. Patient flow. [a]One patient discontinued before receiving a dose of study drug and was excluded from the analyses. *bid*, twice daily; GI, gastrointestinal.

patients (75%) completed the RTP, of whom 152 (93%) completed the 4-week RWP. The proportions of patients not completing the RTP were approximately equal in all three tenapanor dose groups (23%, 26%, and 26% in the 3, 10, and 30 mg [down-titration] twice a day groups, respectively). During the RWP, 10% of patients randomized to placebo and 4%–6% of patients randomized to tenapanor withdrew before completion. The ITT and safety analysis sets for the RTP included 218 patients, because one patient withdrew before receiving study drug, and for the RWP included 164 patients (82 pooled tenapanor, 82 placebo). The secondary (FDA-requested) efficacy analysis set included 80 patients (37 placebo, 43 pooled tenapanor).

Baseline characteristics were similar in all randomized groups (Table 1). Mean adherence to study drug was >92% in all three treatment groups during the RTP and >95% during the RWP. Mean final dose of tenapanor in the 30 mg twice a day down-titration group at the end of the RTP was 24.4 mg twice a day for the safety and ITT analysis sets.

Efficacy

Serum Phosphate

In the RTP, there were significant decreases in serum phosphate in all three tenapanor groups; mean±SD serum phosphate in the ITT set decreased by 1.00±1.73, 1.02±1.66, and 1.19±1.82 mg/dl in patients assigned to tenapanor 3, 10, and 30 mg twice a day down-titration, respectively, from postwashout baseline to week 8 (Figure 3A). There was no clear dose-response relationship during the RTP. The proportion of patients with serum phosphate <5.5 mg/dl at each visit during the RTP was 28.8%–37.7%, 24.6%–41.1%, and 25.0%–40.7% for the tenapanor 3, 10, and 30 mg twice a day down-titration groups, respectively (Supplemental Table 2).

In the RWP, the difference in serum phosphate change between the pooled tenapanor group and the placebo group was significant (mean±SD increase of 0.85±1.68 mg/dl with placebo versus 0.02±1.63 mg/dl with tenapanor; least squares mean difference, −0.72 mg/dl; 95% confidence interval, −1.19 to −0.25 mg/dl; P=0.003; Figure 3A).

Eighty of 164 patients in the RTP were deemed responders (mean±SD serum phosphate reduction, 2.56±1.10 mg/dl) after 8 weeks' treatment. In the RWP, the difference in serum phosphate change between pooled tenapanor and placebo among responders was statistically significant (Figure 3A).

Other Biochemical End Points

Mean changes from baseline to the end of the RTP in mean serum parathyroid hormone concentration were small in magnitude (least squares mean change, +1.0, +7.3, and −24.6 pmol/L in the 3, 10, and 30 mg twice a day down-titration groups, respectively) and none were statistically significant.

Mean FGF23 was reduced from baseline to the end of the RTP in all three treatment groups, with a significant reduction observed in the 3 and 30 mg twice a day down-titration groups (Supplemental Table 3).

Safety and Tolerability

Stool Form and Frequency

Mean bowel movement frequency remained in the normal range for healthy individuals[19] in all groups throughout the study (Figure 4). At the end of the RTP, mean stool frequency

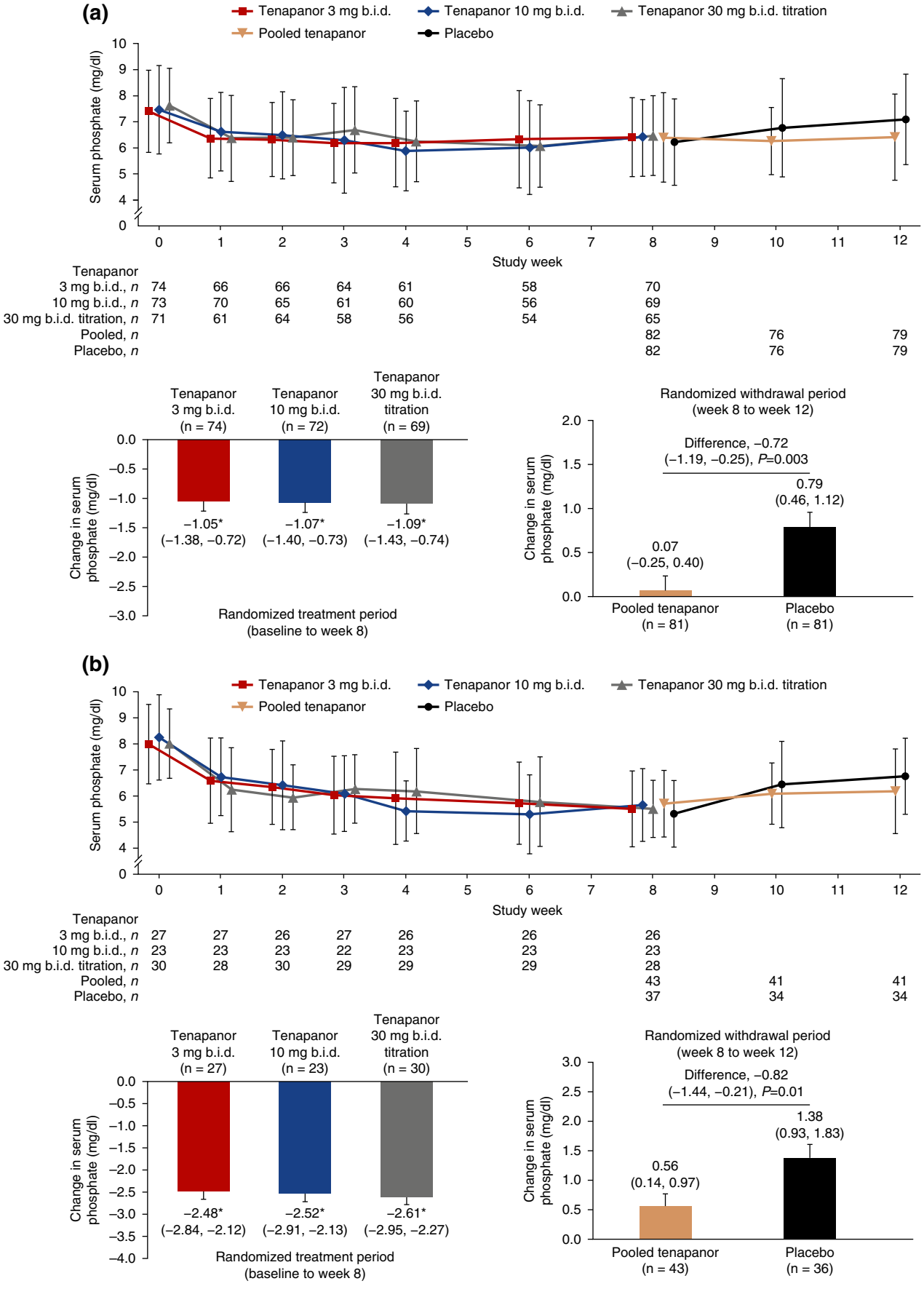
Table 1. Baseline characteristics for patients entering the RTP and RWP

Characteristic	RTP			RWP	
	Tenapanor				
	3 mg Twice Daily, *n*=74	10 mg Twice Daily, *n*=73	30 mg Twice Daily Titration, *n*=71	Placebo, *n*=82	Pooled Tenapanor, *n*=82
Age, yr	55.7±11.5	57.4±10.8	54.2±10.9	55.8±11.8	55.2±10.4
Men, *n* (%)	46 (62.2)	34 (46.6)	48 (67.6)	44 (53.7)	52 (63.4)
Race, *n* (%)					
White	30 (40.5)	25 (34.2)	30 (42.3)	26 (31.7)	29 (35.4)
Black	40 (54.1)	45 (61.6)	40 (56.3)	51 (62.2)	51 (62.2)
Asian	2 (2.7)	0 (0.0)	0 (0.0)	2 (2.4)	0 (0.0)
Native American or Alaskan native	1 (1.4)	2 (2.7)	1 (1.4)	2 (2.4)	2 (2.4)
Other	1 (1.4)	1 (1.4)	0 (0.0)	1 (1.2)	0 (0.0)
Ethnicity, *n* (%)					
Hispanic or Latino	13 (17.6)	8 (11.0)	18 (25.4)	12 (14.6)	16 (19.5)
Baseline BMI, kg/m^2	32.5±8.5	33.6±8.5	33.4±8.1	33.0±7.9	34.3±8.2
Duration since first hemodialysis, mo	58.1±63.1	62.0±53.1	57.1±57.1	55.7±52.2	57.9±51.7
Kt/V value[a]	NA	1.62±0.38	1.61±0.28	1.63±0.32	1.61±0.34
Serum phosphate, mg/dl[b]	7.40±1.57	7.46±1.69	7.62±1.43	NA	NA
PTH value before study entry, pg/ml	471±268	393±237	433±213	405±206	443±241

Data are mean±SD unless otherwise stated. BMI, body mass index; Kt/V, a marker of dialysis adequacy, where K is dialyzer clearance of urea, t is dialysis time, and V is volume distribution of urea (approximately equal to the participant's total body water); NA, not applicable/available; PTH, parathyroid hormone.

[a]Data for 3 mg twice a day group not included due to a recording error.

[b]On day 1, *i.e.*, postwashout of phosphate binders.


(a)
Tenapanor 3 mg b.i.d.
Tenapanor 10 mg b.i.d.
Tenapanor 30 mg b.i.d. titration
Pooled tenapanor
Placebo
Serum phosphate (mg/dl)
Study week
Tenapanor
3 mg b.i.d., n 74 66 66 64 61 58 70
10 mg b.i.d., n 73 70 65 61 60 56 69
30 mg b.i.d. titration, n 71 61 64 58 56 54 65
Pooled, n 82 76 79
Placebo, n 82 76 79
Tenapanor 3 mg b.i.d. (n = 74)
Tenapanor 10 mg b.i.d. (n = 72)
Tenapanor 30 mg b.i.d. titration (n = 69)
Change in serum phosphate (mg/dl)
−1.05* (−1.38, −0.72)
−1.07* (−1.40, −0.73)
−1.09* (−1.43, −0.74)
Randomized treatment period (baseline to week 8)
Randomized withdrawal period (week 8 to week 12)
Difference, −0.72 (−1.19, −0.25), P=0.003
0.07 (−0.25, 0.40)
0.79 (0.46, 1.12)
Pooled tenapanor (n = 81)
Placebo (n = 81)
(b)
Tenapanor 3 mg b.i.d.
Tenapanor 10 mg b.i.d.
Tenapanor 30 mg b.i.d. titration
Pooled tenapanor
Placebo
Serum phosphate (mg/dl)
Study week
Tenapanor
3 mg b.i.d., n 27 27 26 27 26 26 26
10 mg b.i.d., n 23 23 23 22 23 23 23
30 mg b.i.d. titration, n 30 28 30 29 29 29 28
Pooled, n 43 41 41
Placebo, n 37 34 34
Tenapanor 3 mg b.i.d. (n = 27)
Tenapanor 10 mg b.i.d. (n = 23)
Tenapanor 30 mg b.i.d. titration (n = 30)
Change in serum phosphate (mg/dl)
−2.48* (−2.84, −2.12)
−2.52* (−2.91, −2.13)
−2.61* (−2.95, −2.27)
Randomized treatment period (baseline to week 8)
Randomized withdrawal period (week 8 to week 12)
Difference, −0.82 (−1.44, −0.21), P=0.01
0.56 (0.14, 0.97)
1.38 (0.93, 1.83)
Pooled tenapanor (n = 43)
Placebo (n = 36)

increased by 2.8/wk (equivalent to 0.4/d or one incremental movement every 2.5 days) from baseline. During the RWP, the mean bowel movement frequency was 0.8−2.7 movements per week higher in patients receiving tenapanor versus those receiving placebo. The mean Bristol Stool Form Scale score increased by 0.8 from baseline during the RTP and was 0.4–0.9 points higher in tenapanor- versus placebo-treated patients during the RWP.

Adverse Events and Other Assessments

The most common adverse events were gastrointestinal in nature and were largely confined to diarrhea, as defined by any change in stool form or frequency (Tables 2 and 3, Supplemental Tables 4 and 5). During the RTP, diarrhea prompted drug discontinuation in 18 patients (8.3%). No patients discontinued treatment owing to diarrhea during the RWP. Very few serious adverse events occurred during the RTP and no patients receiving tenapanor had a serious adverse event during the RWP. One patient receiving tenapanor 3 mg twice a day died from sudden cardiac death, a finding assessed as unrelated to study treatment. Hyperphosphatemia was reported in 12 patients during the RTP. No clinically meaningful changes from baseline were observed in other laboratory parameters (Supplemental Table 6), including serum bicarbonate, or in vital signs, 12-lead electrocardiograms, or physical examination findings.

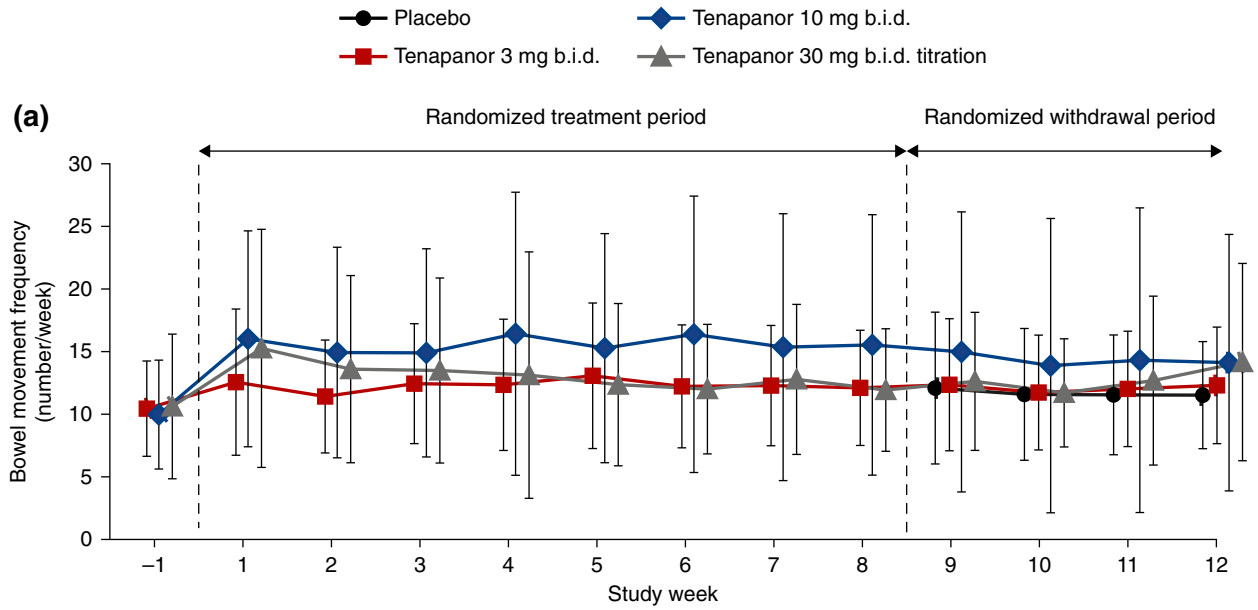
DISCUSSION

In this phase 3 placebo-controlled trial with an RWP, treatment with tenapanor, a minimally absorbed specific inhibitor of NHE3 that neither binds phosphate nor inhibits active phosphate transport, resulted in a significant reduction in serum phosphate among patients receiving maintenance hemodialysis with elevated serum phosphate concentrations. Adverse effects were uncommon; there was an expected modest increase in the frequency of bowel movements (on average one additional movement every 2.5 days) and a detectable, modest softening of the stool by a conventional criterion (the Bristol Stool Form Scale).

Tenapanor is a minimally absorbed compound with detectable levels in serum only rarely being described,[15–17] a potential advantage given the continued concern with inadvertent systemic accumulation of cations from phosphate binders including those containing aluminum, lanthanum, iron, and calcium. Indeed, on the basis of several rigorous calcium balance studies in patients with CKD and from observational data and randomized clinical trials demonstrating higher mortality related to the use of calcium-based phosphate binders,[20–23] the 2017 Kidney Disease Improving Global Outcomes guidelines update recommends limiting calcium exposure from phosphate binders in all patients with CKD.[24] Also relevant is the form and method of administration of tenapanor. One 9-mm-diameter tablet taken twice daily represents a dramatic reduction in pill burden when compared with commonly used doses of phosphate binders (often 9–12 or more tablets or capsules per day). However, the design of this trial precludes any accurate prediction of the proportion of individuals that might be treated successfully with tenapanor monotherapy.

Contrary to conventional belief, serum phosphate concentration is not simply the net result of phosphate ingestion, absorption, and excretion. Elegant work has demonstrated that the nicotinamide phosphoribosyl transferase (Nampt)/(NAD^+) intracellular pathway plays a fundamentally important role in the expression of renal and intestinal phosphate transporters (NaPi-2a, NaPi-2b, and NaPi-2c), and likely plays an additional role in the transcellular shifts from other organs that occur independent of oral phosphate ingestion, and which determine diurnal variation in serum phosphate concentration.[25–27] Even our understanding of what constitutes a "phosphate-restricted diet" is now known to be on the basis of basic misunderstandings about the relative contribution of animal- versus plant- versus additive-based phosphate exposure.[9,28] Our findings here indicate a clear and substantial contribution of paracellular phosphate transport to net phosphate absorption. One conclusion from this body of work is the erroneous and pejorative labeling of patients as "noncompliant" when their serum phosphate fails to conform to clinical expectations despite prescribed phosphate-restricted dietary limits and phosphate binders. The nearly universal finding that phosphate binders provide a maximal serum phosphate reduction of approximately 2.0 mg/dl at their highest dose suggests that novel mechanisms will be required to achieve further improvements in population phosphate control.[29–31] Other interventions that target renal, intestinal,

Figure 3. Tenapanor significantly decreased serum phosphate levels in patients with hyperphosphatemia receiving maintenance hemodialysis. Data presented are for the change in serum phosphate during the RTP and the RWP for (A) the ITT analysis set and (B) the efficacy (responder) analysis set. Line graph data are mean±SD. Bar chart data are LSM change (95% CI) in serum phosphate concentration and error bars show SEM, from an analysis of covariance with treatment and pooled investigator sites as factors and baseline (left) or end of 8-week RTP (right) serum phosphate concentration as a covariate. Data in (B) are shown for the responder population, defined as all patients with a reduction in serum phosphate concentration of at least 1.2 mg/dl during the RTP. The analyses used a patient's last study center visit as the end point visit; there may be apparent discrepancies in patient numbers between figure panels if patients did not visit the study center after the first visit of each period (*i.e.*, had no end point visit for the RTP/RWP). *$P<0.001$ versus baseline. *bid*, twice daily; 95% CI, 95% confidence interval; LSM, least squares mean.



Placebo, n										74	72	71	64
Tenapanor 3 mg b.i.d., *n*	71	68	68	63	61	58	58	54	52	23	25	24	23
Tenapanor 10 mg b.i.d., *n*	68	70	70	68	63	58	57	55	53	22	22	22	21
Tenapanor 30 mg b.i.d. titration, *n*	65	65	61	60	55	53	53	49	49	30	29	28	28

Figure 4. Mean bowel movement frequency increased with tenapanor treatment, but remained within the normal range for healthy individuals. Data presented are for (A) bowel movement frequency and (B) stool consistency during the RTP and the RWP. Data are mean±SD. BSFS scores are weekly averages. *bid*, twice daily; BSFS, Bristol Stool Form Scale.

or cell membrane active phosphate transport (expression or function), or alternatively tight junction protein function, might provide additional novel pathways.

Tenapanor, which increases stool sodium and water content through its effects on NHE3, resulted in softer stool and increased frequency of bowel movements, as we expected. Diarrhea was experienced by approximately 40% of patients receiving tenapanor during the RTP, although by only one patient receiving tenapanor and two patients receiving placebo during the RWP. Interestingly, although we chose to classify any increase in stool

Table 2. Summary of AEs

AE category	Tenapanor			Placebo, n=82	Tenapanor		
	3 mg Twice Daily, *n*=74	10 mg Twice Daily, *n*=73	30 mg Twice Daily Titration, *n*=71		3 mg Twice Daily, *n*=25	10 mg Twice Daily, *n*=23	30 mg Twice Daily Titration, *n*=34
RTP							
Any AE	39 (52.7)	51 (69.9)	49 (69.0)				
Treatment-related AE	24 (32.4)	38 (52.1)	33 (46.5)				
AE leading to study discontinuation	8 (10.8)	16 (21.9)	11 (15.5)				
AE leading to death	1 (1.4)	0 (0.0)	0 (0.0)				
SAE	11 (14.9)	5 (6.8)	5 (7.0)				
AEs by system organ class[a]							
Gastrointestinal disorders	24 (32.4)	35 (47.9)	40 (56.3)				
Infections and infestations	11 (14.9)	5 (6.8)	8 (11.3)				
Metabolism and nutrition disorders	4 (5.4)	10 (13.7)	9 (12.7)				
Injury, poisoning, and procedural complications	5 (6.8)	11 (15.1)	5 (7.0)				
General disorders and administration site conditions	7 (9.5)	5 (6.8)	3 (4.2)				
Respiratory, thoracic, and mediastinal disorders	3 (4.1)	3 (4.1)	5 (7.0)				
Skin and subcutaneous tissue disorders	3 (4.1)	4 (5.5)	2 (2.8)				
Cardiac disorders	3 (4.1)	2 (2.7)	3 (4.2)				
Vascular disorders	0 (0.0)	4 (5.5)	3 (4.2)				
Investigations	3 (4.1)	2 (2.7)	1 (1.4)				
Nervous system disorders	4 (5.4)	1 (1.4)	1 (1.4)				
Musculoskeletal and connective tissue disorders	2 (2.7)	1 (1.4)	2 (2.8)				
Blood and lymphatic system disorders	2 (2.7)	2 (2.7)	0 (0.0)				
Renal and urinary disorders	2 (2.7)	1 (1.4)	1 (1.4)				
RWP							
Any AE				21 (25.6)	4 (16.0)	7 (30.4)	12 (35.3)
Treatment-related AE				5 (6.1)	0 (0.0)	1 (4.3)	0 (0.0)
AE leading to study discontinuation				5 (6.1)	0 (0.0)	1 (4.3)	1 (2.9)
AE leading to death				0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
SAE				4 (4.9)	0 (0.0)	0 (0.0)	0 (0.0)
AEs by system organ class[a]							
Metabolism and nutrition disorders				7 (8.5)	0 (0.0)	1 (4.3)	3 (8.8)
Injury, poisoning, and procedural complications				4 (4.9)	2 (8.0)	0 (0.0)	2 (5.9)
Infections and infestations				2 (2.4)	2 (8.0)	1 (4.3)	2 (5.9)
Gastrointestinal disorders				4 (4.9)	0 (0.0)	0 (0.0)	2 (5.9)
Investigations				2 (2.4)	1 (4.0)	0 (0.0)	2 (5.9)
Respiratory, thoracic, and mediastinal disorders				1 (1.2)	1 (4.0)	3 (13.0)	0 (0.0)
General disorders and administration site conditions				1 (1.2)	0 (0.0)	0 (0.0)	2 (5.9)
Skin and subcutaneous tissue disorders				2 (2.4)	0 (0.0)	1 (4.3)	0 (0.0)
Cardiac disorders				2 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)

Data are number of patients experiencing AE (%). AE, adverse event; SAE, serious adverse event.
[a]Data shown for system organ classes for which two or more patients in any group experienced an AE.

Table 3. Gastrointestinal AEs

AE category	Tenapanor			Placebo, *n*=82	Tenapanor		
	3 mg Twice Daily, *n*=74	10 mg Twice Daily, *n*=73	30 mg Twice Daily Titration, *n*=71		3 mg Twice Daily, *n*=25	10 mg Twice Daily, *n*=23	30 mg Twice Daily Titration, *n*=34
RTP							
Gastrointestinal disorders	24 (32.4)	35 (47.9)	40 (56.3)				
Gastrointestinal disorders by preferred term[a]							
Diarrhea	22 (29.7)	30 (41.1)	34 (47.9)				
Mild	9 (12.2)	11 (15.1)	14 (19.7)				
Moderate	12 (16.2)	16 (21.9)	17 (23.9)				
Severe	1 (1.4)	3 (4.1)	3 (4.2)				
Vomiting	2 (2.7)	3 (4.1)	3 (4.2)				
Flatulence	2 (2.7)	3 (4.1)	2 (2.8)				
Abdominal discomfort	1 (1.4)	4 (5.5)	1 (1.4)				
Abdominal distension	0 (0.0)	1 (1.4)	2 (2.8)				
Abdominal pain	0 (0.0)	3 (4.1)	0 (0.0)				
Abdominal pain upper	2 (2.7)	1 (1.4)	0 (0.0)				
Frequent bowel movements	0 (0.0)	3 (4.1)	0 (0.0)				
Nausea	2 (2.7)	1 (1.4)	0 (0.0)				
Defecation urgency	0 (0.0)	2 (2.7)	0 (0.0)				
RWP							
Gastrointestinal disorders				4 (4.9)	0 (0.0)	0 (0.0)	2 (5.9)
Gastrointestinal disorders by preferred term[a]							
Diarrhea				2 (2.4)	0 (0.0)	0 (0.0)	1 (2.9)
Mild				1 (1.2)	0 (0.0)	0 (0.0)	1 (2.9)
Moderate				1 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
Severe				0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Abdominal pain				0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Food poisoning				0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)

Data are number of patients experiencing AE (%). AE, adverse event.
[a]Data shown for AEs that were experienced by >2% of patients in any group.

softness or frequency as "diarrhea," patients themselves only rarely discontinued study drug as a result—only 18 patients after 8 weeks of exposure in the RTP. It is plausible to speculate that the acute effect on stool sodium and water content may become the "new normal," with patients becoming accustomed to the effect. Alternatively, the constipating effect of most phosphate binders may allow for some individuals to prefer more frequent and slightly softer, yet still formed, stool.

We did not observe a dose-response relationship in the RTP of our study. We had previously conducted a dose-ranging study[13] and chose two doses that were deemed efficacious, along with a down-titration arm, recognizing that the effects of tenapanor on stool frequency and consistency were not necessarily dose related. The trial design precluded any ability to titrate the dose of tenapanor to a particular phosphate target or to match tenapanor dose to those requiring the highest amount of phosphate binder at baseline. An ongoing phase 3 trial of tenapanor includes a 26-week, open-label treatment period followed by a 12-week, placebo-controlled RWP, and includes the capacity to titrate tenapanor dose to patient response[32]; this design should be more capable of robustly estimating the dose response over the longer term. The mean 2.56 mg/dl reduction in serum phosphate observed in the responder population suggests the possibility of improving phosphate control relative to historical or current standard of care. Studies using tenapanor in combination with intestinal phosphate binders would also be informative.

This trial has two key limitations. First, the protocol was modified after the trial was launched, at the request of the FDA. Thus, the primary outcome was changed from the original (change in serum phosphate relative to baseline at completion of the RTP) to the revised (change in serum phosphate relative to end of RTP at completion of the RWP) version. The FDA also requested a secondary analysis examining the change in serum phosphate during the RWP among "responders," defined as patients with a reduction in serum phosphate during the RTP of at least 1.2 mg/dl (the efficacy analysis set). For transparency, we present all three results, although we consider the analysis in responders as secondary; because only responders were included, any conclusions drawn from this analysis have limited generalizability. Second, the primary efficacy end point—the mean change in serum phosphate—is a surrogate. Ideally, a long-term, randomized, placebo-controlled trial would be conducted examining the effects of tenapanor on mortality, cardiovascular events, and fractures. However, many experts are concerned about the risks of untreated hyperphosphatemia for periods longer than 4–6 weeks. Alternatively, a trial could be conducted comparing the effects of tenapanor with those of phosphate binders, or a placebo-controlled trial of tenapanor in patients who remain hyperphosphatemic on fixed doses of phosphate binders. Blinding of such trials would be difficult, given the disparate effects of tenapanor and phosphate binders on the gastrointestinal tract.

Our trial has several strengths as well. The population was diverse in terms of age, sex, race/ethnicity, and primary cause of kidney disease. On the basis of experience from an earlier phase 2 trial, we carefully evaluated the effects of tenapanor on stool form and the frequency of bowel movements, and patients who started on the highest dose of tenapanor (30 mg twice a day) could be titrated down if there were untoward gastrointestinal effects. Adherence to tenapanor dosing was excellent.

In conclusion, we demonstrate the relative safety and efficacy of tenapanor in lowering serum phosphate through the inhibition of paracellular phosphate transport. Adverse effects were limited to those expected by its mechanism of action, which increases stool sodium and water content.

ACKNOWLEDGMENTS

This study was funded by Ardelyx. Medical writing support was provided by Richard Claes, of Oxford PharmaGenesis, UK, funded by Ardelyx.

Individual deidentified participant data and additional study-related documents will not be available.

G.A.B., conception, design, and conduct of the study; interpretation of data; drafting and revision of the manuscript for important intellectual content; and approval of the final version for submission. D.P.R., conception, design, and conduct of the study; analysis and interpretation of data; revision of the manuscript for important intellectual content; and approval of the final version for submission. A.Y., design and conduct of statistical analyses and interpretation of data, revision of the manuscript for important intellectual content, and approval of the final version for submission. G.M.C., conception and design of study, interpretation of data, drafting and revision of the manuscript for important intellectual content, and approval of the final version for submission.

DISCLOSURES

G.A.B. has received consulting fees from and has ownership interest in Ardelyx. He was affiliated with Denver Nephrology at the time this study was conducted and has since become an employee of Reata Pharmaceuticals. D.P.R. and A.Y. are employees of and have ownership interest in Ardelyx. G.M.C. is a consultant to and has equity ownership interest in Ardelyx.

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2018080832/-/DCSupplemental.

Key Protocol Amendments
Study Outcome Measures
Study Assessments
Study drug exposure and adherence
Serum intact FGF23 assay
Serum intact PTH assay
Stool frequency and consistency
Adverse event (AE) recording
Statistical Analyses

Sample size calculation

Other

Supplemental Table 1. Tenapanor dosing regimens.

Supplemental Table 2. Proportion of patients with serum phosphate below 5.5 mg/dL during the randomized treatment period (RTP) (intention-to-treat analysis set).

Supplemental Table 3. Change in serum FGF23 from baseline to the end of the randomized treatment period (RTP) (intention-to-treat analysis set).

Supplemental Table 4. AEs occurring in at least 2% of patients in any treatment group.

Supplemental Table 5. Treatment-related AEs occurring in at least 2% of patients in any treatment group.

Supplemental Table 6. Serum chemistry and hematology values.

REFERENCES

1. Block GA, Hulbert-Shearon TE, Levin NW, Port FK: Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: A national study. *Am J Kidney Dis* 31: 607–617, 1998
2. Chue CD, Edwards NC, Moody WE, Steeds RP, Townend JN, Ferro CJ: Serum phosphate is associated with left ventricular mass in patients with chronic kidney disease: A cardiac magnetic resonance study. *Heart* 98: 219–224, 2012
3. Eddington H, Hoefield R, Sinha S, Chrysochou C, Lane B, Foley RN, et al.: Serum phosphate and mortality in patients with chronic kidney disease. *Clin J Am Soc Nephrol* 5: 2251–2257, 2010
4. McGovern AP, de Lusignan S, van Vlymen J, Liyanage H, Tomson CR, Gallagher H, et al.: Serum phosphate as a risk factor for cardiovascular events in people with and without chronic kidney disease: A large community based cohort study. *PLoS One* 8: e74996, 2013
5. Tonelli M, Sacks F, Pfeffer M, Gao Z, Curhan G; Cholesterol And Recurrent Events Trial Investigators: Relation between serum phosphate level and cardiovascular event rate in people with coronary disease. *Circulation* 112: 2627–2633, 2005
6. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group: KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl* (113): S1–S130, 2009
7. Chertow GM, Levin NW, Beck GJ, Depner TA, Eggers PW, Gassman JJ, et al.: FHN Trial Group: In-center hemodialysis six times per week versus three times per week. *N Engl J Med* 363: 2287–2300, 2010
8. Galassi A, Cupisti A, Santoro A, Cozzolino M: Phosphate balance in ESRD: Diet, dialysis and binders against the low evident masked pool. *J Nephrol* 28: 415–429, 2015
9. Moe SM, Zidehsarai MP, Chambers MA, Jackman LA, Radcliffe JS, Trevino LL, et al.: Vegetarian compared with meat dietary protein source and phosphorus homeostasis in chronic kidney disease. *Clin J Am Soc Nephrol* 6: 257–264, 2011
10. Portale AA, Halloran BP, Morris RC Jr.: Dietary intake of phosphorus modulates the circadian rhythm in serum concentration of phosphorus. Implications for the renal production of 1,25-dihydroxyvitamin D. *J Clin Invest* 80: 1147–1154, 1987
11. Trivedi H, Szabo A, Zhao S, Cantor T, Raff H: Circadian variation of mineral and bone parameters in end-stage renal disease. *J Nephrol* 28: 351–359, 2015
12. King AJ, Siegel M, He Y, Nie B, Wang J, Koo-McCoy S, et al.: Inhibition of sodium/hydrogen exchanger 3 in the gastrointestinal tract by tenapanor reduces paracellular phosphate permeability. *Sci Transl Med* 10: pii eaam6474, 2018. doi: 10.1126/scitranslmed.aam6474
13. Block GA, Rosenbaum DP, Leonsson-Zachrisson M, Åstrand M, Johansson S, Knutsson M, et al.: Effect of tenapanor on serum phosphate in patients receiving hemodialysis. *J Am Soc Nephrol* 28: 1933–1942, 2017
14. Johansson S, Leonsson-Zachrisson M, Knutsson M, Spencer AG, Labonté ED, Deshpande D, et al.: Preclinical and healthy volunteer studies of potential drug–drug interactions between tenapanor and phosphate binders. *Clin Pharmacol Drug Dev* 6: 448–456, 2017
15. Johansson S, Rosenbaum DP, Knutsson M, Leonsson-Zachrisson M: A phase 1 study of the safety, tolerability, pharmacodynamics, and pharmacokinetics of tenapanor in healthy Japanese volunteers. *Clin Exp Nephrol* 21: 407–416, 2017
16. Spencer AG, Labonte ED, Rosenbaum DP, Plato CF, Carreras CW, Leadbetter MR, et al.: Intestinal inhibition of the Na^+/H^+ exchanger 3 prevents cardiorenal damage in rats and inhibits Na^+ uptake in humans. *Sci Transl Med* 6: 227ra36, 2014
17. Rosenbaum DP, Yan A, Jacobs JW: Pharmacodynamics, safety, and tolerability of the NHE3 inhibitor tenapanor: Two trials in healthy volunteers. *Clin Drug Investig* 38: 341–351, 2018
18. Lewis SJ, Heaton KW: Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol* 32: 920–924, 1997
19. Walter SA, Kjellström L, Nyhlin H, Talley NJ, Agréus L: Assessment of normal bowel habits in the general adult population: The popcol study. *Scand J Gastroenterol* 45: 556–566, 2010
20. Jamal SA, Vandermeer B, Raggi P, Mendelssohn DC, Chatterley T, Dorgan M, et al.: Effect of calcium-based versus non-calcium-based phosphate binders on mortality in patients with chronic kidney disease: An updated systematic review and meta-analysis. *Lancet* 382: 1268–1277, 2013
21. Hill KM, Martin BR, Wastney ME, McCabe GP, Moe SM, Weaver CM, et al.: Oral calcium carbonate affects calcium but not phosphorus balance in stage 3-4 chronic kidney disease. *Kidney Int* 83: 959–966, 2013
22. Spiegel DM, Brady K: Calcium balance in normal individuals and in patients with chronic kidney disease on low- and high-calcium diets. *Kidney Int* 81: 1116–1122, 2012
23. Patel L, Bernard LM, Elder GJ: Sevelamer versus calcium-based binders for treatment of hyperphosphatemia in CKD: A meta-analysis of randomized controlled trials. *Clin J Am Soc Nephrol* 11: 232–244, 2016
24. Ketteler M, Block GA, Evenepoel P, Fukagawa M, Herzog CA, McCann L, et al.: Executive summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) guideline update: What's changed and why it matters. *Kidney Int* 92: 26–36, 2017
25. Miyagawa A, Tatsumi S, Takahama W, Fujii O, Nagamoto K, Kinoshita E, et al.: The sodium phosphate cotransporter family and nicotinamide phosphoribosyltransferase contribute to the daily oscillation of plasma inorganic phosphate concentration. *Kidney Int* 93: 1073–1085, 2018
26. Zheng J, Glezerman IG, Sadot E, McNeil A, Zarama C, Gonen M, et al.: Hypophosphatemia after hepatectomy or pancreatectomy: Role of the nicotinamide phosphoribosyltransferase. *J Am Coll Surg* 225: 488–497 e482, 2017
27. Nomura K, Tatsumi S, Miyagawa A, Shiozaki Y, Sasaki S, Kaneko I, et al.: Hepatectomy-related hypophosphatemia: A novel phosphaturic factor in the liver-kidney axis. *J Am Soc Nephrol* 25: 761–772, 2014
28. Karp HJ, Kemi VE, Lamberg-Allardt CJ, Kärkkäinen MU: Mono- and polyphosphates have similar effects on calcium and phosphorus metabolism in healthy young women. *Eur J Nutr* 52: 991–996, 2013
29. Renagel (sevelamer hydrochloride) tablet for oral use. Prescribing information (revised 2016). Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/021179s032lbl.pdf. Accessed May 23, 2018
30. Fosrenol (lanthanum carbonate) chewable tablets/oral powder for oral use. Prescribing information (revised 2016). Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/021468s020,204734s001lbl.pdf. Accessed May 23, 2018
31. Auryxia (ferric citrate) for oral use. Prescribing information (revised 2015). Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/205874s004lbl.pdf. Accessed May 23, 2018
32. A phase 3 study of tenapanor to treat hyperphosphatemia in ESRD patients on dialysis. ClinicalTrials.gov identifier: NCT03427125. 2018. Available at: https://clinicaltrials.gov/ct2/show/NCT03427125. Accessed March 15, 2018

SUPPLEMENTAL MATERIAL

Efficacy and Safety of Tenapanor in Patients Receiving Maintenance Hemodialysis with Hyperphosphatemia: A Randomized Phase 3 Trial

Geoffrey A. Block,* David P. Rosenbaum,† Andrew Yan,† and Glenn M. Chertow‡

*Denver Nephrology, Denver, Colorado; †Ardelyx Inc., Fremont, California; ‡Division of Nephrology, Stanford University School of Medicine, Stanford, California

Correspondence: Dr. Geoffrey A. Block, Denver Nephrology Research Division, 130 Rampart Way Suite 175, Denver CO 80230.

Phone: +1 303-364-4775; Fax: +1 303-364-4776; email: gablock@dnresearch.org

Table of Contents

Key Protocol Amendments

The original protocol was dated November 24, 2015.

- The primary objective was to show the effect of tenapanor on the change in serum phosphate levels from baseline to the end of 8-weeks of treatment in hyperphosphatemic ESRD-HD subjects.
- The primary efficacy variable was serum phosphate measured as change from baseline to the last week of the 8-week randomized treatment period (RTP).

Protocol Edition No. 2 was dated March 3, 2016. The key changes are summarized below.

- The primary efficacy variable was modified to include “the difference in the change in serum phosphate from the end of the 8-week RTP to the end of the randomized withdrawal period (RWP) between treatment and placebo” (for the 4-week RWP), in addition to “serum phosphate measured as change from baseline to the last week of the 8-week RTP” (for the 8-week RTP).
- The efficacy analysis set was defined as follows: “All subjects who are randomized into the RWP and have at least one serum phosphate assessment will be members of this analysis set. The efficacy analysis set will be the primary analysis set for efficacy analysis of the 4-week RWP”.

Protocol Edition No. 3 was dated May 27, 2016. The key changes are summarized below.

- The primary objective was changed from “to show the effect of tenapanor on the change in serum phosphate levels from baseline to the end of 8-weeks of treatment in hyperphosphatemic ESRD-HD subjects” to “to compare the effect of tenapanor versus placebo by comparing the difference in the change in serum phosphate from the end of

the 8-week RTP to the end of the 4-week RWP or the end point visit for this period, between the pooled tenapanor treatments and placebo".

- The first secondary objective was changed from "to compare the effect of tenapanor versus placebo in phosphate-lowering treatment by comparing serum phosphate levels between groups from the end of the 8-week RTP to the end of the 4-week RWP" to "to show the effect of tenapanor on the change in serum phosphate levels from baseline to the end of 8 weeks of treatment".
- The number of sites was changed from 25 to 35 to 35 to 45.
- The sample size was changed from 150 male and female participants to 200 male and female participants, and the power calculation was updated accordingly.
- The primary efficacy variable was changed from "For the 8-week treatment period, the primary efficacy variables will be serum phosphate measured as change from baseline to the last week of the 8-week RTP. For the 4-week placebo-controlled RWP, the primary efficacy variable will be the difference in the change in serum phosphate from the end of the 8-week RTP to the end of the RWP between treatment and placebo" to "The primary efficacy variable will be the change in serum phosphate from the end of the 8-week RTP to the end of the 4-week RWP or the end point visit for this period. The primary efficacy analysis will be based on the difference between the pooled tenapanor treatment and placebo treatment groups".
- The efficacy analysis set was changed from "All subjects who are randomized into the RWP and have at least one serum phosphate assessment" to "All subjects who meet the study entry inclusion and exclusion criteria, complete the 8-week treatment period, and subjects who achieve at least a 1.2 mg/dL reduction in serum phosphate from baseline to the end of the 8-week RTP".

Study Outcome Measures

The primary objective of this study was to compare the effect of tenapanor versus placebo on serum phosphate by comparing the difference in the change in serum phosphate from the end of the 8-week randomized treatment period (RTP) to the end of the 4-week randomized withdrawal period (RWP), or the end point visit for this period, between the pooled tenapanor treatments and placebo.

The secondary objectives of this study were:

- to show the effect of tenapanor on the change in serum phosphate levels from baseline to the end of 8 weeks of treatment
- to compare the effect of different tenapanor dosing regimens on the number of participants reaching serum phosphate goal levels defined as <5.5 mg/dL during 8 weeks of treatment
- to evaluate the safety and tolerability of tenapanor as assessed by adverse event recording, stool form and frequency, vital signs, 12-lead electrocardiogram, physical examination, and clinical laboratory tests.

The exploratory objectives of this study included:

- to compare the effect of tenapanor on serum parathyroid hormone (PTH) levels during 8 weeks of treatment
- to compare the effect of tenapanor on intact serum fibroblast growth factor 23 (FGF23) levels during 8 weeks of treatment.

Study Assessments

Study drug exposure and adherence

Days of exposure to study drug were summarized with descriptive statistics by study period and treatment group for each of the analysis sets. Summary statistics were also presented for adherence to study drug in each treatment period by treatment group for each of the analysis sets. The percentage adherence to study drug was calculated as the total number of tablets dispensed minus the total number of tablets returned divided by two times the number of days during the treatment period, then multiplied by 100.

Serum intact FGF23 assay

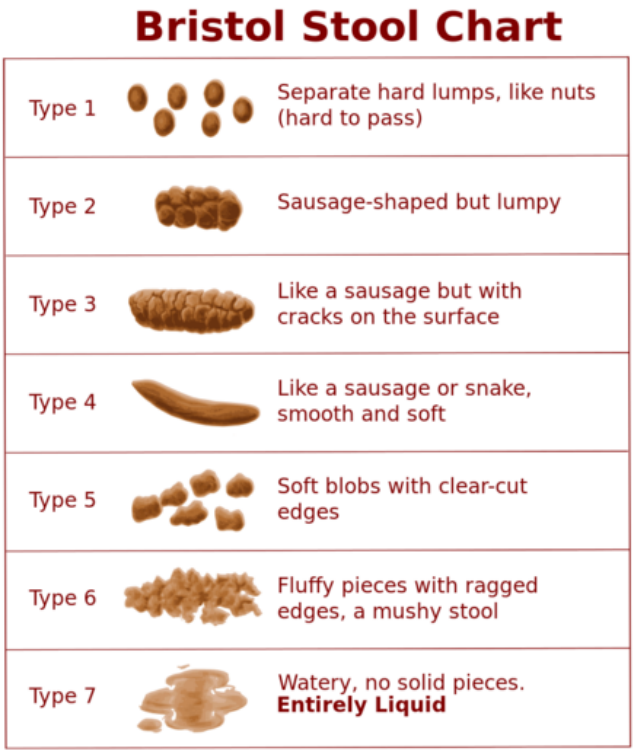
Intact FGF23 in serum was assessed using the Kainos Laboratories (Tokyo, Japan) FGF23 ELISA kit. This is a two-site enzyme-linked immunosorbent assay, with two specific murine monoclonal antibodies that bind to full-length FGF23. One antibody is immobilized onto a microtiter plate well for capture, and the other antibody is conjugated to horseradish peroxidase for detection. A sandwich complex is formed after the addition of the horseradish peroxidase-labelled antibody. Tetramethylbenzidine substrate is added to the wells and then measured on a Tecan Sunrise microplate reader at 450 nm. The enzymatic activity of the complex bound to the well is directly proportional to the amount of FGF23 in the sample.

Serum intact PTH assay

Intact PTH in serum was assessed using the Roche Diagnostics (Indianapolis, Indiana) Elecsys assay. The assay employs a sandwich test principle, in which a biotinylated monoclonal antibody reacts with the N-terminal fragment (1–37) of PTH and a monoclonal antibody labeled with a ruthenium complex reacts with the C-terminal fragment (38–84) of PTH.

Stool frequency and consistency

Participants called into a phone diary every day between 17:00 and 23:59 (local time) from the screening visit through to the last visit at the end of the study. They answered questions about stool form for each bowel movement, according to the Bristol Stool Form Scale (Lewis SJ, Heaton KW. *Scand J Gastroenterol* 32: 920–924, 1997) shown below and the number of bowel movements they have each day. Any increase in bowel movement frequency or loosening of the stool, regardless of the magnitude of the effect, was classified as an adverse event of 'diarrhea'.



Adverse event (AE) recording

Treatment-emergent AEs are presented. AEs were considered to be treatment-emergent during the RTP if the start date of the event was on or after the day of first dose of study drug through the completion of the RTP. Any AE considered drug-related regardless of the start date of the event, or any event that was present at baseline but worsened in severity or was subsequently

considered drug-related by the investigator, was also considered to be a treatment-emergent AE. AEs were considered treatment-emergent during the RWP if the start date of the event was on or after the day of first dose of study drug in the RWP through the final visit of the study. Any AE considered drug-related regardless of the start date of the event, or any event that was present at screening/washout/baseline and/or the 8-week RTP but worsened in severity (compared with both screening/washout/baseline and the 8-week RTP if applicable) in the RWP or was subsequently considered drug-related by the investigator, was also considered to be a treatment-emergent AE. If a participant had more than one occurrence of the same treatment-emergent AE, he/she was counted only once within the system organ class and preferred term.

Statistical Analyses

Sample size calculation

A sample size of 39 participants in the pooled tenapanor treatment and placebo groups would have 90% power to detect a difference in the change in mean serum phosphate from the end of the 8-week RTP to the end of the 4-week RWP with at least a 75% effect size; this effect size was based on a minimum 1.5 mg/dL difference between placebo and pooled tenapanor treatment with a standard deviation no greater than 2.0 mg/dl. A target enrollment of 200 participants allowed for a 20% dropout rate and a 50% responder rate (≥1.2 mg/dL serum phosphate reduction from baseline to end of RTP).

Other

The efficacy analyses utilized a patient's last study center visit as the endpoint visit. All statistical analyses were conducted using SAS (version 9.1.3 or higher; SAS institute, Inc, Cary, North Carolina).

Supplemental Table 1. Tenapanor dosing regimens

Tenapanor Regimen	Morning	Evening	Total Daily Dose
0 mg (RWP only)	0 + 0 mg	0 + 0 mg	0 mg
3 mg b.i.d.	3 + 0 mg	3 + 0 mg	6 mg
10 mg b.i.d.	10 + 0 mg	10 + 0 mg	20 mg
15 mg b.i.d.	15 + 0 mg	15 + 0 mg	30 mg
20 mg b.i.d.	10 + 10 mg	10 + 10 mg	40 mg
30 mg b.i.d.	30 + 0 mg	30 + 0 mg	60 mg

b.i.d., twice daily; RWP, randomized withdrawal period.

Supplemental Table 2. Proportion of patients with serum phosphate below 5.5 mg/dL during the randomized treatment period (RTP) (intention-to-treat analysis set)

	Tenapanor		
	3 mg b.i.d., *n* = 74	**10 mg b.i.d., *n* = 73**	**30 mg b.i.d. titration, *n* = 71**
Week 1			
Proportion	20/66	19/70	16/61
Percentage (%)	30.3	27.1	26.2
95% CI (%)	(19.6, 42.9)	(17.2, 39.1)	(15.8, 39.1)
Week 2			
Proportion	19/66	16/65	16/64
Percentage (%)	28.8	24.6	25.0
95% CI (%)	(18.3, 41.3)	(14.8, 36.9)	(15.0, 37.4)
Week 3			
Proportion	22/64	21/61	15/58
Percentage (%)	34.4	34.4	25.9
95% CI (%)	(22.9, 47.3)	(22.7, 47.7)	(15.3, 39.0)
Week 4			
Proportion	23/61	21/60	15/56
Percentage (%)	37.7	35.0	26.8
95% CI (%)	(25.6, 51.0)	(23.1, 48.4)	(15.8, 40.3)
Week 6			
Proportion	20/58	23/56	22/54
Percentage (%)	34.5	41.1	40.7
95% CI (%)	(22.5, 48.1)	(28.1, 55.0)	(27.6, 55.0)
Week 8			
Proportion	24/70	22/69	18/65
Percentage (%)	34.3	31.9	27.7
95% CI (%)	(23.3, 46.6)	(21.2, 44.2)	(17.3, 40.2)
End of RTP			
Proportion	24/74	23/72	20/69
Percentage (%)	32.4	31.9	29.0
95% CI (%)	(22.0, 44.3)	(21.4, 44.0)	(18.7, 41.2)

b.i.d., twice daily; CI, confidence interval; RTP, randomized treatment period.

Supplemental Table 3. Change in serum FGF23 from baseline to the end of the randomized treatment period (RTP) (intention-to-treat analysis set)

	Tenapanor		
	3 mg b.i.d., *n* = 74	**10 mg b.i.d., *n* = 73**	**30 mg b.i.d. titration, *n* = 71**
Baseline			
n	59	57	54
Mean ± SD	8137 ± 13 178	10 467 ± 22 682	10 994 ± 11 498
Geo. mean ± geo. CV	3455 ± 253	4112 ± 261	6089 ± 182
End of RTP			
n	57	57	54
Mean ± SD	6586 ± 11 245	9244 ± 13 883	8161 ± 8199
Geo. mean ± geo. CV	2489 ± 300	3682 ± 283	4558 ± 183
Change from baseline to end of RTP			
n	57	57	54
Mean ± SD	−102 ± 3890	−1223 ± 13 554	−2833 ± 8187
Ratio of geo. means (95% CI)	0.768 (0.656, 0.899)	0.887 (0.759, 1.037)	0.767 (0.652, 0.902)

FGF23 data are pg/mL.

Geo. means, geo. CVs, and 95% CIs are from an ANCOVA model with treatment and pooled investigator site as fixed factors, and baseline FGF23 (log-transformed) as a covariate.

ANCOVA, analysis of covariance; b.i.d., twice daily; CI, confidence interval; FGF23, fibroblast growth factor 23; geo. CV, geometric coefficient of variation (%); geo. mean, geometric mean; RTP, randomized treatment period.

Supplemental Table 4. AEs occurring in at least 2% of patients in any treatment group

Randomized treatment period			
	Tenapanor		
	3 mg b.i.d., *n* = 74	**10 mg b.i.d., *n* = 73**	**30 mg b.i.d. titration, *n* = 71**
Participants with any AE	39 (52.7)	51 (69.9)	49 (69.0)
Gastrointestinal disorders	24 (32.4)	35 (47.9)	40 (56.3)
Diarrhea	22 (29.7)	30 (41.1)	34 (47.9)
Vomiting	2 (2.7)	3 (4.1)	3 (4.2)
Flatulence	2 (2.7)	3 (4.1)	2 (2.8)
Abdominal discomfort	1 (1.4)	4 (5.5)	1 (1.4)
Abdominal distension	0 (0.0)	1 (1.4)	2 (2.8)
Abdominal pain	0 (0.0)	3 (4.1)	0 (0.0)
Abdominal pain upper	2 (2.7)	1 (1.4)	0 (0.0)
Frequent bowel movements	0 (0.0)	3 (4.1)	0 (0.0)
Nausea	2 (2.7)	1 (1.4)	0 (0.0)
Defecation urgency	0 (0.0)	2 (2.7)	0 (0.0)
Infections and infestations	11 (14.9)	5 (6.8)	8 (11.3)
Cellulitis	3 (4.1)	2 (2.7)	1 (1.4)
Nasopharyngitis	1 (1.4)	1 (1.4)	2 (2.8)
Pneumonia	2 (2.7)	1 (1.4)	0 (0.0)
Upper respiratory tract infection	1 (1.4)	0 (0.0)	2 (2.8)
Metabolism and nutrition disorders	4 (5.4)	10 (13.7)	9 (12.7)
Hyperphosphatemia	3 (4.1)	5 (6.8)	4 (5.6)
Fluid overload	1 (1.4)	1 (1.4)	2 (2.8)
Hypocalcemia	0 (0.0)	1 (1.4)	2 (2.8)
Injury, poisoning, and procedural complications	5 (6.8)	11 (15.1)	5 (7.0)
Arteriovenous fistula site complication	0 (0.0)	2 (2.7)	2 (2.8)
Vascular graft complication	0 (0.0)	3 (4.1)	1 (1.4)
Wound	1 (1.4)	2 (2.7)	0 (0.0)

Arteriovenous fistula thrombosis	2 (2.7)	0 (0.0)	0 (0.0)
General disorders and administration site conditions	7 (9.5)	5 (6.8)	3 (4.2)
Non-cardiac chest pain	2 (2.7)	0 (0.0)	0 (0.0)
Skin and subcutaneous tissue disorders	3 (4.1)	4 (5.5)	2 (2.8)
Pruritus	1 (1.4)	2 (2.7)	1 (1.4)
Cardiac disorders	3 (4.2)	2 (2.7)	3 (4.2)
Tachycardia	2 (2.7)	1 (1.4)	0 (0.0)

Randomized withdrawal period				
	Placebo, *n* = 82	**Tenapanor**		
		3 mg b.i.d., *n* = 25	**10 mg b.i.d., *n* = 23**	**30 mg b.i.d. titration, *n* = 34**
Participants with any AE	21 (25.6)	4 (16.0)	7 (30.4)	12 (35.3)
Metabolism and nutrition disorders	7 (8.5)	0 (0.0)	1 (4.3)	3 (8.8)
Hyperphosphatemia	3 (3.7)	0 (0.0)	1 (4.3)	0 (0.0)
Hyperkalemia	0 (0.0)	0 (0.0)	1 (4.3)	2 (5.9)
Fluid overload	2 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)
Hypermagnesemia	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Injury, poisoning, and procedural complications	4 (4.9)	2 (8.0)	0 (0.0)	2 (5.9)
Arteriovenous fistula site complication	1 (1.2)	1 (4.0)	0 (0.0)	0 (0.0)
Contusion	1 (1.2)	0 (0.0)	0 (0.0)	1 (2.9)
Arteriovenous fistula site hemorrhage	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
Vascular graft thrombosis	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Infections and infestations	2 (2.4)	2 (8.0)	1 (4.3)	2 (5.9)
Sinusitis	0 (0.0)	1 (4.0)	0 (0.0)	1 (2.9)
Fungal skin infection	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Gastrointestinal viral infection	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)

Upper respiratory tract infection	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
Gastrointestinal disorders	4 (4.9)	0 (0.0)	0 (0.0)	2 (5.9)
Diarrhea	2 (2.4)	0 (0.0)	0 (0.0)	1 (2.9)
Abdominal pain	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Food poisoning	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Investigations	2 (2.4)	1 (4.0)	0 (0.0)	2 (5.9)
Anticoagulation drug level above therapeutic	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Blood urea increased	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)
Venous pressure increased	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
Respiratory, thoracic, and mediastinal disorders	1 (1.2)	1 (4.0)	3 (13.0)	0 (0.0)
Asthma	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)
Rales	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)
Rhinorrhea	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)
Throat irritation	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)

Data are number of patients experiencing AE (%) by system organ class and preferred term.

AE, adverse event; b.i.d., twice daily.

Supplemental Table 5. Treatment-related AEs occurring in at least 2% of patients in any treatment group

Randomized treatment period				
		Tenapanor		
	3 mg b.i.d., *n* = 74	**10 mg b.i.d., *n* = 73**	**30 mg b.i.d. titration, *n* = 71**	
Participants with any treatment-related AE	24 (32.4)	38 (52.1)	33 (46.5)	
Gastrointestinal disorders	21 (28.4)	34 (46.6)	31 (43.7)	
Diarrhea	19 (25.7)	30 (41.1)	28 (39.4)	
Flatulence	1 (1.4)	3 (4.1)	2 (2.8)	
Abdominal discomfort	0 (0.0)	3 (4.1)	1 (1.4)	
Abdominal distension	0 (0.0)	1 (1.4)	2 (2.8)	
Abdominal pain	0 (0.0)	3 (4.1)	0 (0.0)	
Frequent bowel movements	0 (0.0)	3 (4.1)	0 (0.0)	
Abdominal pain upper	2 (2.7)	0 (0.0)	0 (0.0)	
Defecation urgency	0 (0.0)	2 (2.7)	0 (0.0)	
Metabolism and nutrition disorders	1 (1.4)	6 (8.2)	2 (2.8)	
Hyperphosphatemia	1 (1.4)	4 (5.5)	1 (1.4)	
Randomized withdrawal period				
	Placebo, *n* = 82	**Tenapanor**		
		3 mg b.i.d., *n* = 25	**10 mg b.i.d., *n* = 23**	**30 mg b.i.d. titration, *n* = 34**
Participants with any treatment-related AE	5 (6.1)	0 (0.0)	1 (4.3)	0 (0.0)
Metabolism and nutrition disorders	3 (3.7)	0 (0.0)	1 (4.3)	0 (0.0)
Hyperphosphatemia	2 (2.4)	0 (0.0)	1 (4.3)	0 (0.0)

Data are number of patients experiencing AE (%) by system organ class and preferred term.

AE, adverse event; b.i.d., twice daily.

Supplemental Table 6. Serum chemistry and hematology values

Randomized Treatment Period			
		Tenapanor	
	3 mg b.i.d., *n* = 74	**10 mg b.i.d., *n* = 73**	**30 mg b.i.d. titration, *n* = 71**
Albumin, g/dl			
Baseline	3.90 ± 0.33	3.89 ± 0.27	3.94 ± 0.28
End of period	3.92 ± 0.34	3.84 ± 0.25	3.90 ± 0.27
Bicarbonate, mmol/L			
Baseline	24.5 ± 3.2	24.2 ± 3.0	23.9 ± 2.6
End of period	23.9 ± 3.2	24.0 ± 3.5	23.7 ± 2.8
Calcium, mg/dl			
Baseline	8.68 ± 0.90	8.69 ± 0.72	8.59 ± 0.77
End of period	8.77 ± 0.73	8.71 ± 0.74	8.57 ± 0.93
Chloride, mmol/L			
Baseline	96.6 ± 3.3	96.9 ± 3.5	96.8 ± 3.4
End of period	97.0 ± 3.3	96.9 ± 3.2	97.3 ± 3.5
Glucose, mg/dl			
Baseline	156.4 ± 80.3	154.2 ± 65.2	157.4 ± 109.7
End of period	150.2 ± 78.7	165.4 ± 70.1	153.2 ± 71.0
Hemoglobin, g/dl			
Baseline	11.11 ± 1.45	10.75 ± 1.37	10.77 ± 1.32
End of period	11.16 ± 1.60	10.96 ± 1.22	11.15 ± 1.26
Potassium, mmol/L			
Baseline	4.62 ± 0.65	4.72 ± 0.61	4.74 ± 0.69
End of period	4.72 ± 0.66	4.65 ± 0.67	4.82 ± 0.83
Sodium, mmol/L			
Baseline	136.1 ± 2.6	136.3 ± 2.8	136.6 ± 3.2
End of period	136.1 ± 2.3	135.8 ± 3.0	136.1 ± 2.5

Randomized Withdrawal Period				
	Placebo, *n* = 82	**Tenapanor**		
		3 mg b.i.d., *n* = 25	**10 mg b.i.d., *n* = 23**	**30 mg b.i.d. titration, *n* = 34**
Albumin, g/dl				
Baseline	3.91 ± 0.34	3.87 ± 0.32	3.92 ± 0.28	3.97 ± 0.22
End of period	3.88 ± 0.29	3.97 ± 0.34	3.89 ± 0.23	3.97 ± 0.28

Bicarbonate, mmol/L				
Baseline	24.5 ± 2.7	24.0 ± 3.2	23.6 ± 3.2	24.0 ± 3.0
End of period	24.0 ± 2.7	23.1 ± 2.2	23.3 ± 2.6	23.4 ± 3.0
Calcium, mg/dl				
Baseline	8.67 ± 0.78	8.68 ± 0.94	8.68 ± 0.81	8.47 ± 0.80
End of period	8.63 ± 0.73	8.65 ± 0.74	8.80 ± 0.64	8.74 ± 0.86
Chloride, mmol/L				
Baseline	97.2 ± 3.4	96.1 ± 3.2	97.2 ± 3.2	96.5 ± 3.1
End of period	97.3 ± 3.4	95.7 ± 3.9	97.8 ± 3.7	97.2 ± 3.5
Glucose, mg/dl				
Baseline	145.4 ± 58.4	189.2 ± 105.1	145.7 ± 60.9	159.8 ± 82.1
End of period	152.4 ± 84.7	164.3 ± 83.9	160.0 ± 89.2	155.5 ± 74.0
Hemoglobin, g/dl				
Baseline	11.00 ± 1.40	11.33 ± 1.59	10.56 ± 1.44	10.72 ± 1.35
End of period	10.96 ± 1.19	11.77 ± 1.90	10.73 ± 1.34	11.09 ± 1.57
Potassium, mmol/L				
Baseline	4.60 ± 0.56	4.66 ± 0.59	4.80 ± 0.67	4.85 ± 0.81
End of period	4.59 ± 0.68	4.54 ± 0.48	4.80 ± 0.69	4.90 ± 0.81
Sodium, mmol/L				
Baseline	136.4 ± 2.6	136.0 ± 2.6	136.6 ± 2.6	136.6 ± 3.1
End of period	136.3 ± 3.3	135.6 ± 2.5	136.3 ± 3.1	136.1 ± 2.8

Data are mean ± SD. Baseline is the pre-dose value on day 1.

b.i.d., twice daily.