Pyridoxamine Dihydrochloride in Diabetic Nephropathy (PIONEER-CSG-17): Lessons Learned from a Pilot Study

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Key Words

Diabetic nephropathy · Clinical trial design · Pyridoxamine

Abstract

Background/Aims: Pyridoxamine dihydrochloride (PyridorinTM) blocks pathogenic oxidative pathways in the progression of diabetic nephropathy. The pyridoxamine pilot study was designed to test entry criteria and outcomes. Subjects had SCr 1.3–3.5 mg/dl, protein-to-creatinine $\geq 1,200$ mg/g and used a surrogate outcome of Δ SCr over 52 weeks. Subjects had to be on a maximally tolerated dose of ACE/ARB for 3 months; stable other antihypertensive doses for 2 months; stable diuretic dose for 2 weeks, and BP $\leq 160/90$ mm Hg; or enter a Pharmacologic-Stabilization Phase (PSP). This pilot failed to detect an effect on Δ SCr in intent-to-treat analysis. **Methods:** We queried the locked clinical trial database for subgroups in which there was a treatment effect. **Results:** Subjects not requiring PSP and those with entry SCr < 2.0 mg/dl had a treatment effect. Subjects entering PSP required more changes in antihypertensive medications and experienced larger Δ SCr over 52 weeks. PSP subjects with BP $> 140/90$ mm Hg had no treatment effect, but those $\leq 140/90$ mm Hg did. **Conclusion:** Time required for acute effects of ACE/ARB to stabilize is unknown, but these data suggest

> 3 months. Thus, subjects in the pivotal trial must be on ACE/ARB for 6 months. Frequent antihypertensive adjustment could engender SCr changes unrelated to CKD progression. Thus, we will require subjects to have BP $\leq 150/90$ mm Hg and on stable antihypertensives for 26 weeks, or $\leq 140/90$ mm Hg and on stable antihypertensives for 13 weeks. Since Δ SCr over 52 weeks is limited as a surrogate outcome, the pivotal trial uses a time-to-event analysis of baseline SCr to at least a 50% increase in SCr or ESRD as the primary outcome. This substantial Δ SCr is protected from noise and is clinically relevant. The pyridoxamine pilot provided critical information to inform the design of PIONEER-CSG-17, which we conducted under the SPA agreement with FDA.

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Introduction

Despite the role of inhibition of the renin-angiotensin aldosterone system (RAAS) in delaying the progression of diabetic nephropathy (DN) [1–3], many patients still progress to end-stage renal disease (ESRD). With the increasing prevalence of diabetes mellitus, the potential rise in incident ESRD due to DN is costly to our healthcare systems and it negatively impacts the quality of life of our

patients with diabetes. Additional therapies beyond RAAS inhibition that can delay the progression of DN are needed to confront the growing epidemic [4, 5].

Pyridoxamine dihydrochloride (Pyridorin™, Nephro-Genex, Inc.) is a small molecule derivative of pyridoxal phosphate (vitamin B₆) with a distinct chemical structure that inhibits the formation of advanced glycation end-products (AGE). Pyridoxamine inhibits a broad range of pathogenic oxidative chemistries that lead to AGE formation, including activity against toxic carbonyls, reactive oxygen species, and the conversion of glycosylated proteins to AGEs [6–18]. AGEs play a significant role in the pathogenesis of DN [19–30].

Early clinical studies of pyridoxamine have been encouraging in terms of its potential for slowing the progression of DN while being well tolerated [31]. A pilot study of pyridoxamine (PYR-210), however, showed no significant treatment effect on progression of DN as measured by the mean change in serum creatinine (SCr) over 52-weeks in the intent-to-treat population. The purpose of the pilot studies is to better identify the optimal target population for therapeutic interventions, test study procedures, evaluate and refine entry criteria, and inform study drug dosing. Typically, pilot studies are underpowered and often use surrogate outcomes. The results are hypothesis generating. Given these limitations, post-hoc analyses of pilot study data can suggest efficacy of therapies in subsets of subjects, which can then be tested formally in rigorous and well-powered trials with more robust endpoints. Applying these principles to the pilot study PYR-210, pyridoxamine showed efficacy in predefined subgroups of subjects based on baseline kidney function, and the timing of establishing stable standard of care prior to randomization [32]. Furthermore, previously conducted studies in other cohorts of subjects with DN can provide invaluable guidance for defining entry criteria for future studies.

Here, we describe the results of these pre-specified and post-hoc analyses and discuss how they informed the design of the pivotal, Phase 3 trial [33] that is evaluating the efficacy and safety of pyridoxamine to delay DN progression due to type 2 diabetes mellitus.

The Pyridorin™ in Diabetic Nephropathy (PIONEER-CSG-17) trial is a Phase 3, randomized, double blind, placebo-controlled, international multi-center study to evaluate the safety and efficacy of pyridoxamine in subjects with nephropathy due to type 2 diabetes mellitus. The primary outcome is the time to the composite endpoint of ≥50% increase in SCr from baseline, or ESRD. ESRD is defined as the initiation of permanent dialysis, receiving a

kidney transplant, or an SCr ≥6.0 mg/dl confirmed by repeated testing 4–6 weeks later. The study plans to randomize 600 subjects in a 1:1 fashion to pyridoxamine 300 mg BID or placebo. Subjects are adults with advanced DN, defined as SCr ≥1.3 mg/dl for women or ≥1.5 mg/dl for men, a 24-hour urine collection protein-to-creatinine ratio (PCR) ≥1,200 mg/g, SCr <3.0 mg/dl (both sexes), and an estimated glomerular filtration rate (eGFR) ≥20 ml/min/1.73 m², using the 4-variable Modification of Diet in Renal Disease (MDRD) equation. These entry criteria were modified from our pilot trial PYR-210 and were derived from prior DN studies conducted by the Collaborative study Group, including the Irbesartan in Diabetic Nephropathy Trial (IDNT) [3] and Sulodexide Overt Diabetic Nephropathy trial [5]. The expected median follow-up is 30 months, with the first subject enrolled in June 2014. The trial is expected to have results in March 2018, with an interim analysis in 2016. The sample size (n = 600) was computed to provide approximately a 90% power to distinguish a 28% treatment effect of pyridoxamine 300 mg BID from placebo for the primary endpoint after a total of 247 events have accrued. The study will be conducted at approximately 100 sites globally. The trial will be conducted under a Special Protocol Assessment (SPA) with the U.S. Food and Drug Administration (FDA).

Methods

We queried the locked clinical trial database of the pyridoxamine pilot trial PYR-210 (NCT00734253). The design and principal results of this trial have been published previously [32]. It was a double blind, randomized, placebo-controlled trial that assigned 317 subjects with advanced type 2 DN to placebo, pyridoxamine 150 mg twice daily, or pyridoxamine 300 mg twice daily for 52 weeks. Subjects were adults with SCr 1.3–3.3 mg/dl (women) or 1.5–3.5 mg/dl (men) and a 24-hour urine PCR ≥1,200 mg/g, while receiving the maximally tolerated dose of an angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB) for ≥3 months before the qualifying visit. Randomization was stratified on the basis of entry SCr (SCr ≤2.0 mg/dl, SCr >2.0 mg/dl). Blood pressure (BP) medications were required to be stable for ≥2 months and diuretic medications were required to be stable for ≥2 weeks before the qualifying visit. Additionally, BP had to be ≤160/90 mm Hg at the qualifying visit. An optional 3-month Pharmacologic-Stabilization Period (PSP) was allowed for subjects starting ACE-I/ARB, or whose medication durations were not at the protocol-required length at the qualifying visit. Subjects whose BP did not meet entry criteria at the qualifying visit also entered into the PSP. The primary outcome of the trial was the difference in the mean change in SCr concentration from baseline to 52 weeks in the pyridoxamine vs. placebo groups as a whole.

We performed predefined efficacy analyses according to baseline SCr (SCr ≤2.0 mg/dl, SCr >2.0 mg/dl) and requirement for

Table 1. Baseline characteristics of the subjects in the pyridoxamine pilot trial

Variable	Placebo (n = 106)	PYR150 (n = 105)	PYR300 (n = 106)
Age, years	64.4±9.0	63.8±9.1	63.6±10.4
Men, n (%)	78 (74%)	78 (74%)	80 (76%)
Caucasian race, n (%)	90 (85%)	85 (81%)	83 (78%)
DM duration, years	18.8±9.0	16.9±8.1	17.1±8.4
Kidney disease duration, years	5.2±4.0	5.6±5.1	5.2±4.3
BMI, kg/m ²	34.1±6.4	32.5±5.5	34.3±7.3
Systolic BP, mm Hg	138±19	138±15	138±13
Diastolic BP, mm Hg	74±9	74±10	73±9
SCr, mg/dl	2.20±0.56	2.22±0.55	2.17±0.57
Urine PCR, mg/g	2,934±1,805	3,015±1,984	2,971±2,007

PYR150 = Pyridoxamine 150 mg BID group; PYR300 = pyridoxamine 300 mg BID group; DM = diabetes mellitus; BMI = body mass index; BP = blood pressure; SCr = serum creatinine; PCR = protein-to-creatinine ratio.

entry into the PSP upon study enrollment (PSP required, PSP not required). We also performed exploratory post-hoc analyses on subjects based on need for entry into the PSP (PSP required, PSP not required) examining BP change, the number of BP medication changes during the treatment period, and Δ SCr over 52 weeks. These post-hoc analyses were conducted on the combined treatment arms.

We evaluated the treatment effect in subgroups by the differences in Δ SCr over 52 weeks between pyridoxamine and placebo. Change in SCr concentration was determined using an analysis of covariance model (ANCOVA), with treatment group as a factor and baseline SCr concentration as a covariate. The Dunnett test was used to compare the pyridoxamine treatment groups with placebo, with maintenance of the significance level at $\alpha = 0.05$. Analyses were performed with SAS versions 9.3 and 9.4 (Cary, N.C., USA).

Additionally, we analyzed various permutations of entry criteria on the irbesartan arm of the locked clinical trial database of the Irbesartan Diabetic Nephropathy Trial (IDNT) conducted by the Collaborative Study Group. IDNT was a prospective, randomized, controlled trial in subjects with advanced DN (comparable to subjects being studied in PIONEER-CSG-17), which tested the effect of irbesartan to delay the progression of DN [3]. This modeling allowed us to compute the event rate and sample size for the PIONEER-CSG-17 trial for a given set of entry criteria to assess feasibility.

Results

Table 1 shows the baseline characteristics of the subjects in the PYR-210 pilot trial. As expected based on the entry criteria, the mean SCr was approximately 2.2 mg/

Table 2. Results of subgroup analyses for an assessment of treatment effect on Δ SCr over 52 weeks between pyridoxamine and placebo

Subgroup	Placebo	PYR150	PYR300
All subjects			
N	103	99	105
SCr, baseline, mg/dl	2.20±0.56	2.22±0.55	2.17±0.57
Δ SCr at 52 weeks	0.36±0.38	0.42±0.38	0.36±0.37
SCr ≤2 mg/dl			
N	48	39	44
SCr, baseline, mg/dl	1.73±0.18	1.68±0.18	1.62±0.22
Δ SCr at 52 weeks	0.28±0.55	0.13±0.32	0.14±0.22
SCr >2 mg/dl			
N	55	60	61
SCr, baseline, mg/dl	2.62±0.44	2.57±0.41	2.56±0.41
Δ SCr at 52 weeks	0.43±0.71	0.63±0.84	0.54±0.69
PSP not required			
N	69	69	69
SCr, baseline, mg/dl	2.14±0.50	2.18±0.55	2.09±0.55
Δ SCr at 52 weeks	0.38±0.71	0.30±0.59	0.21±0.39
PSP required			
N	34	30	36
SCr, baseline, mg/dl	2.34±0.67	2.33±0.56	2.32±0.59
Δ SCr at 52 weeks	0.31±0.68	0.73±0.90	0.62±0.75
bSCr <3 and PSP not required			
N	63	60	64
SCr, baseline, mg/dl	2.04±0.40	2.03±0.40	2.01±0.49
Δ SCr at 52 weeks	0.42±0.70	0.23±0.45*	0.18±0.34*

PYR150 = Pyridoxamine 150 mg BID group; PYR300 = pyridoxamine 300 mg BID group; SCr = serum creatinine; PSP = pharmacostabilization period; bSCr = baseline serum creatinine. * $p < 0.05$ versus placebo.

dl, with mean proteinuria in the near-nephrotic range, and mean BP approximately 138/74 mm Hg for all subjects. The average number of BP medications and diuretics were the same across all treatment arms. Additionally, the mean Δ SCr at 52 weeks (pyridoxamine 0.39 ± 0.39 mg/dl vs. placebo 0.36 ± 0.38 mg/dl) was successfully predicted by the sample size calculations based on data derived from IDNT.

Table 2 displays the treatment effect of pyridoxamine in defined subgroups. A trend towards a treatment effect is seen in subjects with entry baseline SCr ≤2.0 mg/dl. The mean Δ SCr in the placebo group was 0.28 ± 0.55 mg/dl, while it was 0.13 ± 0.32 mg/dl ($p = 0.10$) for pyridoxamine 150 mg BID (PYR150) group, and 0.14 ± 0.22 mg/dl ($p = 0.19$) for pyridoxamine 300 mg BID (PYR300) group. In contrast, those subjects with entry baseline SCr >2.0 mg/dl showed no trend toward a treatment effect.

Table 3. Results of subgroup analyses among those subjects who required the pharmaco-stabilization period (PSP) to achieve standard of care, versus those subjects who were already on standard of care at screening (PSP not required). In this analysis, all three treatment arms are combined in each group

Subgroup	N	bSCr, mg/dl	Baseline BP, mm Hg, mean	Ending BP, mm Hg, mean	ΔBP at 52 weeks, mm Hg, mean	# BP medication changes, mean	ΔSCr at 52 weeks, mg/dl
PSP required	100	2.3	146/75	138/72	-8/-3	1.61	0.55
PSP not required	207	2.2	138/74	137/74	-1/0	1.09	0.30

PSP = Pharmaco-stabilization period; bSCr = baseline serum creatinine; SCr = serum creatinine.

Additionally, those subjects not requiring the PSP demonstrated a trend toward a treatment effect. The mean ΔSCr in the placebo group was 0.38 ± 0.71 mg/dl, while it was 0.30 ± 0.19 mg/dl for the PYR150 group and 0.21 ± 0.39 mg/dl for the PYR300 group. In contrast, those subjects requiring a PSP prior to enrollment showed no trend toward a treatment effect.

In subjects with a baseline SCr <3.0 mg/dl and on stable standard of care at screening, in whom a PSP was not required, the mean ΔSCr in the placebo group was 0.42 ± 0.70 mg/dl, while it was 0.23 ± 0.45 mg/dl ($p = 0.04$) for the PYR150 group and 0.18 ± 0.34 mg/dl ($p = 0.009$) for the PYR300 group (table 2).

Table 3 displays the results of the post-hoc analysis of the three combined treatment arms for subjects with and without the need for PSP. Subjects who required a PSP had a higher mean baseline BP than subjects who did not require PSP. However, at the end of the trial, BP was similar in both groups. The PSP subjects had a larger reduction in BP and more BP medication changes over the course of the study to achieve comparable end-of-study BP versus those subjects on stable therapy at screening. Despite similar baseline SCr, those subjects who required PSP also demonstrated a larger mean ΔSCr over the course of the trial versus subjects on stable standard of care at screening.

Table 4 shows the ΔSCr at 52 weeks in subjects requiring PSP who also required 3 or more blood pressure medication changes over the course of the trial. These subjects had ΔSCr higher than all other subjects.

Discussion

The analyses presented here offer insights into patient populations who may benefit from treatment with pyridoxamine to delay the progression of DN due to type 2

Table 4. Results of subgroup analysis for those subjects who required both the pharmaco-stabilization period (PSP) and 3 or more post-randomization medication changes to achieve the goal BP

Subgroup	N	bSCr, mg/dl	ΔSCr at 52 weeks, mg/dl
PSP/3BP	25	2.5	0.94
All other subjects	282	2.2	0.33

PSP = Pharmaco-stabilization period; PSP/3BP = the group of subjects who required the PSP and required 3 or more medication changes to achieve goal BP.

diabetes. They demonstrate how pilot trial data can better inform the design of larger and more costly pivotal trials. Despite the limitations of PYR-210 as a pilot study, including the use of a surrogate outcome, interesting observations have emerged from the subgroup analyses that have informed our Phase 3 study design.

The PYR-210 pilot study demonstrated a trend toward treatment effect among subjects with a baseline SCr ≤2.0 mg/dl in contrast to those enrolled with SCr >2.0 mg/dl. Subjects who entered with SCr ≤2.0 mg/dl had less advanced DN. The larger changes in SCr averaged over 52 weeks in subjects with higher entry SCr, which we observed in the pilot study suggested the possibility of ascertainment bias associated with the use of this particular surrogate endpoint (mean ΔSCr) as a measure of decline in GFR in this subset. Figure 1 shows the well-known exponential relationship between SCr and GFR, whereby equivalent changes in GFR are associated with numerically greater changes in SCr in subjects with higher baseline SCr than those with SCr <2.0 mg/dl. Furthermore, ΔSCr in patients with more advanced DN is a non-specific endpoint because of the numerous variables known to

Fig. 1. Given the exponential relationship between SCr and GFR, equivalent changes in GFR are associated with numerically greater changes in SCr in subjects with higher baseline SCr than those with lower baseline SCr. This relationship contributes to the potential confounding of the primary outcome in the PYR-210 pilot trial.

transiently affect the SCr measurement in this cohort: minor hemodynamic changes due to BP variability, overdiuresis, predilection to bouts of acute kidney injury, for example. We hypothesized that our inability to detect a treatment effect in subjects with SCr >2.0 mg/dl may have been obscured by changes in SCr due to causes other than the progression of DN and did not necessarily suggest a limited benefit of pyridoxamine to early stages of DN. In addition, since preliminary animal data [34] suggests a putative effect of pyridoxamine on regression of glomerular scarring, exclusion of subjects with moderately advanced DN could reduce the chances of finding a treatment effect in the pivotal trial. Balancing all this information, rather than restricting entry criteria for the pivotal trial to subjects with SCr <2.0 mg/dl, we made modifications to exclude subjects with the most far-advanced DN (eGFR <20 ml/min/1.73 m² or SCr >3.0 mg/dl) in whom a 50% rise in SCr represented a less clinically meaningful endpoint.

It is considered standard of care to treat patients with proteinuric DN with maximally tolerated doses of ACE-I/ARB [1, 3, 5]. In addition, achieving a goal BP in patients with DN often requires the use of diuretics [35]. However, the introduction of, or an increase in RAAS inhibition, is usually associated with an increase in SCr due to alterations in glomerular capillary hemodynamics. Indeed, these changes in SCr have been reported to identify the very subset of DN patients most likely to experience a slowing in the rate of decline of GFR associated with

RAAS inhibition [36]. Because the time course and trajectory of early changes in SCr due to RAAS inhibition is uncertain, the evaluation of data from the pilot study provided important information to inform the design of the pivotal trial. The pilot study required stable doses of RAAS inhibitors for 3 months prior to entry, stable doses of other antihypertensives for 2 months, and 2 weeks of stable doses of diuretics. Protocols involving stabilization periods are standard in cardiovascular clinical trials, in which a PSP can be used to establish background standard therapy [37].

Data from the pilot study guided the determination of the entry criteria for the pivotal trial. Subjects in the pilot trial who required entry into the PSP for adjustments in ACE-I/ARB had larger Δ SCr over the 52 weeks than those subjects who did not enter the PSP. Subjects who entered the PSP to achieve target BP required more BP medications to get to goal, compared to those who did not enter the PSP. These changes in medications and BP can alter SCr, independent of disease progression, as described above, and again introduce the treatment effect ascertainment error. In addition, this suggests that some subjects are inherently less stable and require more time and interventions to achieve target BP. Hence, we concluded that a time period longer than 3 months may be required for SCr stabilization following the establishment of standard of care.

We utilized this finding to design our pivotal trial, such that subjects in PIONEER-CSG-17 must be on stable, maximally tolerated doses of ACE-I/ARB for 6 months or longer. We also expect that investigators enroll subjects whose dose of ACE-I/ARB is not anticipated to change over the course of the trial. Similarly, subjects entering PIONEER-CSG-17 must be on stable doses of BP medication, including diuretics for 13–26 weeks. Specifically, subjects must have a BP $\leq$ 150/90 mm Hg and on stable doses of antihypertensive medication including diuretics for 26 weeks, or $\leq$ 140/90 mm Hg and on stable antihypertensive medication including diuretics for 13 weeks. We have also made provisions in the design of the study for the management of acute changes in SCr (acute kidney injury) and for hyperkalemia. These modifications of the pilot protocol will allow for improved opportunity for the pivotal trial to provide unambiguous detection of a treatment effect of pyridoxamine with minimization of confounding by hemodynamic influences on SCr.

PYR-210 not only provided insight into the factors influencing reproducibility of the SCr measurement, but the entry criteria in the pilot trial accurately estimated the Δ SCr over 52 weeks. This suggests that the entry criteria

will ensure that enough subjects will experience the necessary change in the kidney function over the course of the now longer follow-up period to discern a treatment effect.

Finally, in the pivotal trial, the primary outcome is a time-to-event analysis of the composite of a 50% increase from baseline in SCr or ESRD. This time-to-event design avoids confounding introduced by averaging all subjects' SCr over the course of the trial. Specifically, this avoids the inclusion of slow progressors, who have no opportunity for a treatment effect, and whose data dilute the treatment effect on the fast progressors. Also, this modified endpoint is protected from the noise introduced by events that produce transient small changes in SCr. The requirement of a 50% increase in SCr or ESRD is a clinically relevant event, and an endpoint, which has been endorsed by the FDA-NKF Endpoints Conference (Washington, D.C., USA, December 2012). In the pilot, 307 of the 317 subjects completed the 52-week study, and an excellent

safety profile was observed both at 150 and 300 mg doses. We selected the 300 mg/day dose for the pivotal trial.

Thus, the pyridoxamine PYR-210 pilot trial provided critical information to guide the design of the pivotal trial, PIONEER-CSG-17. Pivotal trials that require significant financial and patient resources are best designed from information gleaned from smaller pilot studies that increase our ability to design, conduct, and analyze a pivotal trial that generates an unambiguous result.

Disclosure Statements

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