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PODO: Trial Design: Phase 2 Study of PF-06730512 in Focal Segmental Glomerulosclerosis

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Introduction: Focal segmental glomerulosclerosis (FSGS) is characterized by proteinuria and a histologic pattern of glomerular lesions of diverse etiology that share features including glomerular scarring and podocyte foot process effacement. Roundabout guidance receptor 2 (ROBO2)/slit guidance ligand 2 (SLIT2) signaling destabilizes the slit diaphragm and reduces podocyte adhesion to the glomerular basement membrane (GBM). Preclinical studies suggest that inhibition of glomerular ROBO2/SLIT2 signaling can stabilize podocyte adhesion and reduce proteinuria. This clinical trial evaluates the preliminary efficacy and safety of ROBO2/SLIT2 inhibition with the ROBO2 fusion protein PF-06730512 in patients with FSGS.

Methods: The Study to Evaluate PF-06730512 in Adults With FSGS (PODO; ClinicalTrials.gov identifier NCT03448692), an open-label, phase 2a, multicenter trial in adults with FSGS, will enroll patients into 2 cohorts ($n = 22$ per cohort) to receive either high- or low-dose PF-06730512 (intravenous) every 2 weeks for 12 weeks. Key inclusion criteria include a confirmed biopsy diagnosis of FSGS, an estimated glomerular filtration rate (eGFR) $\geq$45 ml/min/1.73 m^2 based on the Chronic Kidney Disease Epidemiology Collaboration formula (30–45 with a recent biopsy), and urinary protein-to-creatinine ratio (UPCR) $>$1.5 g/g. Key exclusion criteria include collapsing FSGS, serious/active infection, $\geq$50% tubulointerstitial fibrosis on biopsy, and organ transplantation. The primary endpoint is change from baseline to week 13 in UPCR; secondary endpoints include safety, changes in eGFR, and PF-06730512 serum concentration.

Results: This ongoing trial will report the efficacy, safety, pharmacokinetics, and biomarker results of PF-06730512 for patients with FSGS.

Conclusion: Findings from this proof-of-concept study may support further development and evaluation of PF-06730512 to treat FSGS and warrant assessment in phase 3 clinical trials.

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FSGS is a common pattern of glomerular injury that often causes proteinuric chronic kidney disease.[1,2] FSGS is characterized by a variety of sclerosing lesions of the glomerular tuft and perturbation of the filtration barrier.[1,2] The filtration barrier itself comprises the fenestrated glomerular endothelial cells, the GBM, and visceral epithelial cells, or podocytes.[3] The interdigitating foot processes of adjacent podocytes are attached to the GBM and are separated by filtration slits bridged by nephrin-containing slit diaphragms that form the final barrier to macromolecular permeation.[3] Many cases of FSGS are classified as primary and are caused by an as yet incompletely understood abnormalities of the podocytes, referred to as "podocytopathies," whereas others may be secondary to hemodynamic or other stresses that affect the podocytes or to one of several genetic mutations of podocyte genes. Regardless of the etiology, however, all forms of FSGS share the common features of variable degrees of podocyte foot process effacement, disruption of the slit diaphragms, and podocyte detachment.[2]

Focal and segmental glomerulosclerosis in all of its forms is often progressive when proteinuria, a hallmark of podocyte injury, cannot be controlled, and is one of the common glomerular etiologies leading to end-stage kidney disease, which has significant health and

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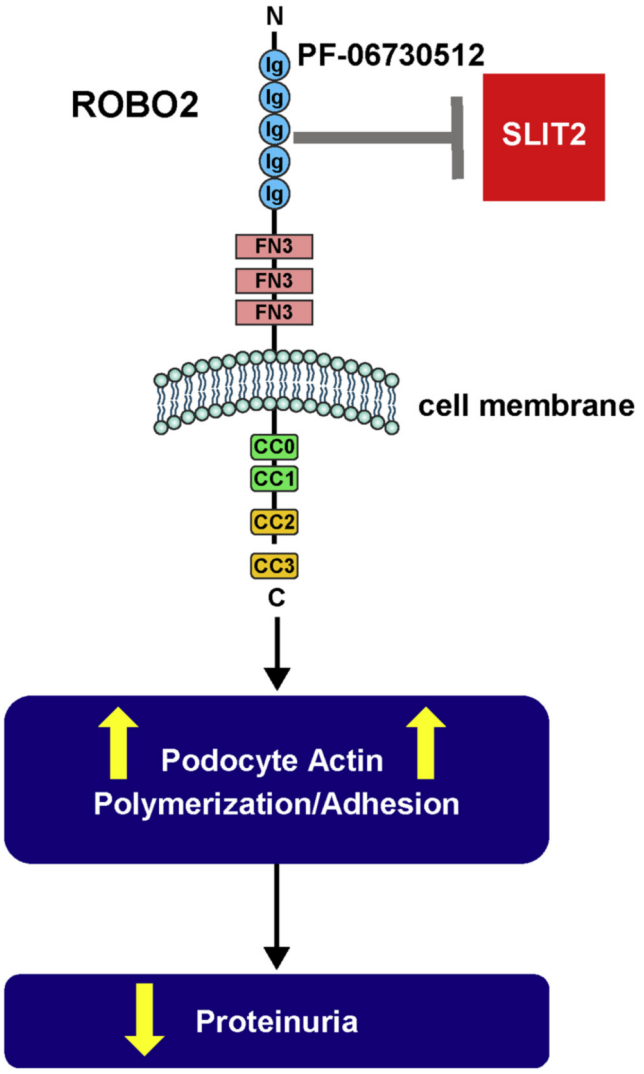

Figure 1. Mechanism of action of PF-06730512. PF-06730512 binds to slit guidance ligand 2 (SLIT2) and prevents its interaction with the roundabout guidance receptor 2 (ROBO2) receptor on podocytes, enhancing podocyte actin polymerization and adhesion, leading to improved structural integrity of podocytes and reduced proteinuria.[7] CC = cytoplasmic conserved region; Ig = immunoglobulin; FN3 = fibronectin type 3.

economic impact.[1,4] Thus, a treatment that targets the final common pathway of injury, namely podocyte foot process effacement and disruption of the slit diaphragms, would have broad therapeutic application.

The roundabout guidance receptor 2 (ROBO2) localizes to the basal surface of the podocytes and colocalizes with the protein nephrin in the glomerulus.[5] ROBO2 is a receptor for slit guidance ligand 2 (SLIT2).[6] ROBO2/SLIT2 signaling has been shown to destabilize the slit diaphragm and reduce podocyte adhesion to the GBM,[7] whereas loss of ROBO2 function has been shown to promote podocyte adhesion, reduce effacement of podocyte foot processes, and reduce proteinuria.[8]

Therefore, targeting improvement in podocyte adhesion, podocyte depletion, and the resulting proteinuria might slow disease progression for patients with FSGS. Data from preclinical studies suggest that one method of stabilizing podocyte adhesion is to inhibit glomerular ROBO2/SLIT2 signaling.[8,9] We have recently generated a therapeutic ROBO2 fusion protein (PF-06730512) that inhibits the ROBO2/SLIT2 signaling pathway. Treatment with PF-06730512 in a preclinical proteinuric animal model reduced proteinuria and improved podocyte foot process ultrastructure.[9] A phase 2a clinical trial to evaluate the preliminary efficacy and safety of ROBO2 fusion protein PF-06730512 in patients with FSGS is currently ongoing (NCT03448692).[9] We describe the study design for this clinical trial herein.

METHODS

Mechanism of Action

PF-06730512 is a recombinant ROBO2 human immunoglobulin G1 crystallized fragment (Fc) fusion protein that contains the first 2 immunoglobulin domains of ROBO2 fused to a human immunoglobulin G1 Fc. Mutations in the Fc region reduce its normal humoral effector functions, such as Fc receptor binding, and complement activation. PF-06730512 therefore acts as a neutralizing ligand trap that binds SLIT2 and prevents its interaction with native ROBO2 on the basal surface of the podocyte, an interaction that would normally serve to destabilize the slit diaphragm. Data from preclinical studies support the hypothesis that by neutralizing endogenous ROBO2/SLIT2 signaling with PF-06730512, podocyte actin polymerization and adhesion should be enhanced, structural integrity of the podocytes should be maintained, and proteinuria should be reduced[9] (Figure 1). PF-06730512 neutralizes SLIT ligand binding to ROBO receptors inhibiting other SLIT/ROBO signaling pathways linked to roles outside of the kidney.[6]

Study Design

PODO is an ongoing, phase 2a, open-label, multicenter, adaptive study to evaluate the efficacy, safety, tolerability, and pharmacokinetics of PF-06730512 after multiple intravenous (IV) administrations in adult patients with FSGS (ClinicalTrials.gov identifier: NCT03448692). Patients will be enrolled into 2 cohorts (Figure 2). Cohort 1 will receive the higher IV dose of PF-06730512 every 2 weeks (Q2W) from week 1 through week 12. Cohort 2 will receive the lower IV dose of PF-06730512 (Q2W) from week 1 through week 12. Both cohorts will undergo the same schedule of events. Up to 3 interim analyses are currently planned for the study. The first is planned to be conducted after ≥50% of the planned patients in cohort 1 complete the primary efficacy assessment; the second after

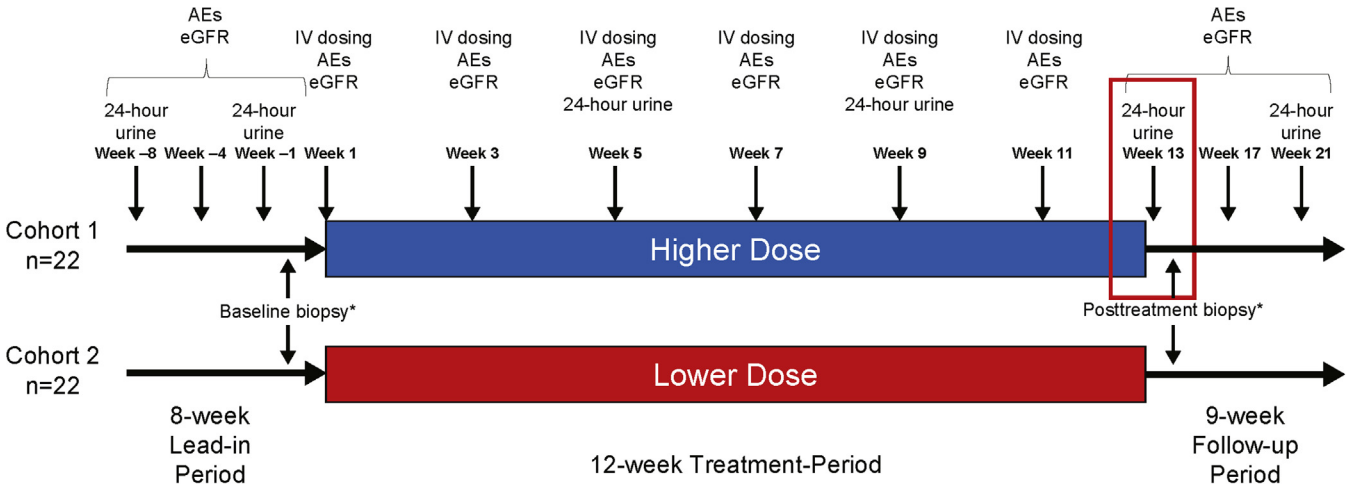

Figure 2. Study to Evaluate PF-06730512 in Adults With FSGS (PODO) study design. Patients will be enrolled sequentially into 1 of 2 cohorts; cohort 1 will be enrolled first. For both cohorts, an initial 8-week lead-in period will be followed by a 12-week investigational period. During this period, they will receive intravenous study treatment at 2-week intervals starting with week 1. A 9-week follow-up period will follow the investigational period. The red box denotes the primary endpoint (change from baseline to week 13 in urinary protein-to-creatinine ratio). The asterisk (*) indicates biopsy specimens obtained to measure the change in podocyte ultrastructure from baseline to week 13 (<14 days) for the biopsy substudy. AE = adverse event; eGFR = estimated glomerular filtration rate; IV = intravenous.

all patients from cohort 1 complete the primary efficacy assessment or discontinue from the study; and the third after ≥50% of the planned patients from cohort 2 complete the primary efficacy assessment. At each interim analysis, the study may be stopped for futility. This trial is being conducted in accordance with the Declaration of Helsinki, and all patients are providing written informed consent before enrollment.

Key Inclusion and Exclusion Criteria

This study will enroll male and female patients ≥18 years of age with a diagnosis of FSGS confirmed by a biopsy procedure performed within the past 5 years. Additional key inclusion and exclusion criteria are listed in Table 1. Of note, type II diabetes mellitus does not exclude the patient from the trial if a kidney biopsy specimen obtained within the preceding 12 months shows no evidence of diabetic nephropathy. The following abnormalities in clinical laboratory tests at screening, as assessed by the study-specific laboratory and confirmed by a repeat test if deemed necessary, will also be exclusionary: aspartate aminotransferase or alanine aminotransferase level exceeding 1.5 times the upper limit of normal and total bilirubin level >1.5 times the upper limit of normal, although patients with a history of Gilbert syndrome may have direct bilirubin measured and would be eligible for this study provided the direct bilirubin level is less than or equal to the upper limit of normal.

Primary and Secondary Endpoints

The primary endpoint for the study is the percent change from baseline (i.e., before receiving study drug) to week 13 in urinary protein-to-creatinine ratio (UPCR) as assessed by 24-hour urine collection by a central laboratory. Secondary endpoints are safety (including adverse events [AEs], safety laboratory tests, body weight, blood pressure, pulse rate, oral temperature and electrocardiograms [ECGs]); percentage change from baseline in UPCR to weeks 3, 5, and 9; partial (i.e., >40% reduction in UPCR from baseline to week 13 to a level between 0.3–1.5 g/g at week 13) or complete (i.e., UPCR ≤0.3 g/g at week 13) remission of proteinuria; percentage change from baseline to weeks 3, 5, 9, and 13 in eGFR based on the Chronic Kidney Disease Epidemiology Collaboration formula; PF-06730512 serum concentration; and incidence of the development of an antidrug antibody and neutralizing antibody. Tertiary endpoints include change from baseline in serum albumin at weeks 3, 5, 9, and 13; percentage change from baseline in urinary albumin-to-creatinine ratio at weeks 3, 5, 9, and 13; and clinical outcome assessments to measure the effect on health-related quality of life. Pharmacokinetic samples will be collected at weeks 1, 3, 5, 7, 9, 11, 12, 13, 15, 17, and 21, and concentrations will be summarized descriptively by nominal pharmacokinetic sampling time and dose. Banked biospecimens and additional urine samples will also be collected.

Statistical Analyses

A sample size of 18 completers for each dose cohort was chosen based on the primary endpoint (percent change from baseline to week 13 in 24-hour UPCR), with an estimated 80% power to detect a mean reduction from baseline UPCR to week 13 of 50%, using a 1-sided *t* test at

Table 1. Key inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• eGFR of ≥45 ml/min/1.73 m^2 o If eGFR is 30–45 ml/min/1.73 m^2, a recent biopsy specimen (<12 months before screening) must demonstrate <50% tubulo-interstitial fibrosis	• Diagnosis of collapsing FSGS • ≥50% tubulointerstitial fibrosis at biopsy • Serious/active infection • Evidence or history of a clinically significant comorbid condition[a]
• UPCR >1.5 g protein/g creatinine at screening	• Previous heroin use
• Discontinuation of ongoing corticosteroid treatment or taper to a stable prednisone (or equivalent) dose ≤7.5 mg per day (or 15 mg every other day) ≥1 week before the lead-in period	• Organ transplantation • BMI >45 kg/m^2 • Treatment with rituximab <6 months before start of lead-in period
• Treatment with 1–3 classes of immunosuppressants, either alone or in combination, or contraindication to any class of immunosuppressant (which may include steroids) or intolerance to any class of immunosuppressant (may include steroids) per investigator judgment	• Screening sitting BP ≥155 mm Hg (systolic) or ≥95 mm Hg (diastolic), following ≥5 minutes of sitting rest • Pregnancy or breastfeeding • Positive urinary drug test at screening (except positivity for THC)
• Treatment with CNIs or MMF (but not both) may be continued upon entry into the lead-in period at the discretion of the investigator. o Treatment with the agent (CNIs or MMF) must have been ongoing for ≥6 months before lead-in, and the dose (or level) of the agent must have been stable for ≥1 month before the lead-in period	

BMI = body mass index; BP = blood pressure; CNI = calcineurin inhibitor; eGFR = estimated glomerular filtration rate; FSGS = focal segmental glomerulosclerosis; MMF = mycophenolate mofetil; THC = tetrahydrocannabinol; UPCR = urinary protein-to-creatinine ratio.
[a]Clinically significant conditions include hematologic, endocrine (including type 1 diabetes mellitus), pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or hepatic disease, like cirrhosis or chronic active liver disease.

a 5% significance level. This approach assumes a conservative standard deviation on the $\log_e$ scale of 1.09, based on previously published reports,[10,11] and a reanalysis of patient-level data from the FSGS clinical trial,[12] obtained through the National Institutes of Health. A conservative dropout rate was estimated to be 15% (based on similar previous studies); therefore, approximately 22 patients are planned to be enrolled in each cohort.

The primary analysis will be based on the full analysis set, defined as all enrolled patients who have received ≥1 dose of study treatment and have ≥1 postbaseline measurement of UPCR. A mixed-effects model of repeated measures will be fitted to the post-dose 24-hour urine collection data on UPCR (weeks 5, 9, and 13). Baseline for the primary endpoint is defined as week −1 UPCR measurement based on 24-hour urine collection; week 13 values will also be based on the 24-hour urine collection. The model will include treatment (if >1 dose), baseline (week −1 UPCR), week (as a factor), baseline by week interaction and the week by treatment (if >1 dose) interaction, with week fitted as a repeated effect and patient as a random effect. An unstructured correlation matrix will be used, and the Kenward-Roger approximation will be used for estimating degrees of freedom for the model parameters.

The secondary endpoints of percentage change from baseline in UPCR at weeks 3, 5, and 9 will come from the primary analysis mixed-effects model of repeated measures model. A similar mixed-effects model of repeated measures model will also be applied separately to the single morning void assessments of UPCR (at weeks 3, 5, 9, and 13). Percentage changes from baseline at weeks 3, 5, 9, and 13 in eGFR will be analyzed using the same model as defined for the primary endpoint. Partial and complete remission of proteinuria at week 13 will be summarized descriptively using frequency and percentage. No formal statistical analyses are planned for the safety analyses. All patients who received ≥1 dose of study medication will be included in the safety analyses, and data will be reported in accordance with the sponsor reporting standards, which include summarizing by dose (if applicable) AEs, safety laboratory abnormalities, body weight, blood pressure, pulse rate, and ECGs.

Biopsy Substudy

In lieu of measurable serum or urine biomarkers that demonstrate target engagement by PF-06730512, changes in podocyte morphology may serve as a marker of PF-06730512 activity. The objective of the biopsy substudy is to explore the pharmacodynamic effect of PF-06730512 on podocyte ultrastructure. The main endpoint of the substudy is change from baseline in mean foot process width to week 13. Patients enrolled in cohorts 1 and 2 in the United States and Canada can consent to undergo renal biopsy procedures. The target enrolment for the biopsy substudy is approximately 5 patients.

The baseline biopsy procedure will be performed at the end of the screening period or during the lead-in period, before administration of the first dose of PF-06730512; the second biopsy procedure will be performed after 12 weeks of treatment with PF-06730512, shortly after week 13. Inclusion/exclusion criteria are the same as for the main study, except for a proteinuria entry level UPCR of ≥3.0 g protein/g creatinine in the 24-hour urine collection at screening. eGFR must be ≥45 ml/min/1.73 m^2 at screening, even if a patient

had a recent biopsy procedure and was considered eligible for the main study with an eGFR of 30 to 45 ml/min/1.73 m^2. Specimens will be analyzed by a central renal histopathology facility.

DISCUSSION

The results of the PODO trial will provide information regarding the safety and efficacy of PF-06730512 inhibition of ROBO2 signaling and the resulting effect on podocyte injury in FSGS. Because the ROBO2/SLIT2 signaling pathway may also be involved in other diseases that cause proteinuria, and given the central role of the podocyte in maintaining the glomerular filtration barrier and the proposed role of podocyte injury in many proteinuric kidney diseases, the antiproteinuric effects identified during this trial may have broader applicability to other kidney diseases considered to be podocytopathies with significant proteinuria. Findings from this proof-of-concept study may support further development and evaluation of PF-06730512 and warrant assessment in phase 3 clinical trials.

DISCLOSURES

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