Effects of Selonsertib in Patients with Diabetic Kidney Disease

Glenn M. Chertow,[1] Pablo E. Pergola,[2] Fang Chen,[3] Brian J. Kirby,[3] John S. Sundy,[3] and Uptal D. Patel,[3] for the GS-US-223-1015 Investigators

[1]Division of Nephrology, Department of Medicine, Stanford University School of Medicine, Stanford, California; [2]Renal Associates PA, San Antonio, Texas; and [3]Gilead Sciences, Inc., Foster City, California

ABSTRACT

Background Apoptosis signal-regulating kinase 1 (ASK1) activation in glomerular and tubular cells resulting from oxidative stress may drive kidney disease progression. Findings in animal models identified selonsertib, a selective ASK1 inhibitor, as a potential therapeutic agent.

Methods In a phase 2 trial evaluating selonsertib's safety and efficacy in adults with type 2 diabetes and treatment-refractory moderate-to-advanced diabetic kidney disease, we randomly assigned 333 adults in a 1:1:1:1 allocation to selonsertib (oral daily doses of 2, 6, or 18 mg) or placebo. Primary outcome was change from baseline eGFR at 48 weeks.

Results Selonsertib appeared safe, with no dose-dependent adverse effects over 48 weeks. Although mean eGFR for selonsertib and placebo groups did not differ significantly at 48 weeks, acute effects related to inhibition of creatinine secretion by selonsertib confounded eGFR differences at 48 weeks. Because of this unanticipated effect, we used piecewise linear regression, finding two dose-dependent effects: an acute and more pronounced eGFR decline from 0 to 4 weeks (creatinine secretion effect) and an attenuated eGFR decline between 4 and 48 weeks (therapeutic effect) with higher doses of selonsertib. A post hoc analysis (excluding data for 20 patients from two sites with Good Clinical Practice compliance–related issues) found that between 4 and 48 weeks, rate of eGFR decline was reduced 71% for the 18-mg group relative to placebo (difference 3.11 ± 1.53 ml/min per 1.73 m^2 annualized over 1 year; 95% confidence interval, 0.10–6.13; nominal P=0.043). Effects on urine albumin-to-creatinine ratio did not differ between selonsertib and placebo.

Conclusions Although the trial did not meet its primary endpoint, exploratory post hoc analyses suggest that selonsertib may slow diabetic kidney disease progression.

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Diabetes mellitus is the leading cause of kidney disease, affecting 285 million people worldwide.[1] The incidence of diabetic kidney disease (DKD) continues to increase, paralleling the global prevalence of diabetes.[1,2] ESKD due to DKD is associated with significant mortality and morbidity, with a 5-year survival rate <40%, and multiple complications that impair functional capacity and health-related quality of life.[2] Current primary and secondary interventions for DKD include cardiovascular risk reduction and glycemic and BP control, particularly using inhibitors of the renin-angiotensin-aldosterone system.[3] Although angiotensin-converting enzyme inhibitors (ACEis) and angiotensin receptor blockers (ARBs) are effective at reducing proteinuria when present, and slowing the progression of DKD (defined by the composite end point of death,

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Correspondence: Dr. Glenn M. Chertow, Stanford University School of Medicine, 1070 Arastradero Road, Suite 313, Palo Alto, CA 94034. Email: gchertow@stanford.edu

dialysis, or doubling of serum creatinine) by 15%–20%, a sizeable fraction of patients with DKD progress to ESKD despite institution of these and other therapies, with death as a competing risk.[4–7] Since the approval of ARB therapy for the treatment of DKD approximately 15 years ago, no new therapy has been approved for its management. Additional interventions are needed to target the underlying pathophysiology of DKD, and to reduce the inexorable progression that often complicates its course.

Oxidative stress has been implicated in the pathogenesis of DKD, resulting in disruption of the renal microvasculature, progressive damage to the glomerular capillaries, podocyte and endothelial cell apoptosis, and tubulointerstitial fibrosis.[8–12] Apoptosis signal–regulating kinase 1 (ASK1), a serine/threonine kinase, is a ubiquitously expressed member of the 28 MAP3K family. ASK1 is normally repressed by thioredoxin,[13] an antioxidant protein that dissociates from ASK1 upon being oxidized in settings of oxidative stress, resulting in ASK1 activity. ASK1 is a critical signaling node which, when activated, promotes inflammation, apoptosis, and fibrosis *via* downstream activation of the mitogen-activated protein kinases (MAPKs) p38 and c-Jun N-terminal kinase (c-JNK).[14–16] In DKD, sustained oxidative stress results in activation of ASK1, which then phosphorylates and activates p38 MAPK and c-JNK kinases, promoting inflammation, apoptosis of tubular epithelial cells and podocytes, as well as fibrosis within the tubulointerstitium and glomerulus.[10,17–21] In animal models of DKD, ASK1 inhibition strongly suppresses the activation of ASK1, p38, and JNK in the kidney, reducing progressive kidney injury, inflammation, and fibrosis, which in turn improves kidney function and halts GFR decline.[22,23] On the basis of these findings, it has been hypothesized that ASK1 inhibition may be a promising therapeutic intervention for the management of DKD.

Selonsertib is a highly selective, potent, small-molecule inhibitor of ASK1 being developed as a once-daily oral agent for the management of DKD. In animal models of DKD, selonsertib dose-dependently inhibited ASK1 pathway activation, reduced progressive inflammation and fibrosis in the kidney, and halted the decline of GFR.[23] The aim of this double-blind, placebo-controlled, phase 2 trial was to assess the safety and efficacy of selonsertib in patients with DKD.

Significance Statement

Findings in animal models of diabetic kidney disease identified selonsertib, a selective inhibitor of apoptosis signal–regulating kinase 1 (ASK1), as a potential therapeutic agent. In a randomized, dose-ranging, placebo-controlled phase 2 trial evaluating selonsertib's safety and efficacy in patients with moderate-to-advanced diabetic kidney disease, the authors found that selonsertib appeared safe, with no dose-dependent adverse effects over 48 weeks, including for the 18-mg daily dose thought to maximally inhibit ASK1. Although the trial did not meet its primary efficacy end point of change in eGFR from baseline to week 48, acute effects related to inhibition of creatinine secretion by selonsertib confounded differences in eGFR. Exploratory post hoc analyses accounting for these effects suggest that selonsertib resulted in a dose-dependent reduction in kidney function decline and merits further study.

METHODS

Study Design and Patients

Seventy-six sites across the United States and four sites across Canada—representing hospitals, outpatient clinics, academic centers, and private research sites—conducted this double-blind, placebo-controlled, dose-finding phase 2 trial (ClinicalTrials.gov identifier NCT02177786) in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines for Good Clinical Practice (GCP). The trial protocol was approved by central and local institutional review boards. All trial participants provided written, informed consent before undergoing any study procedure. The primary end point was the change from baseline in eGFR with selonsertib compared with placebo at 48 weeks. A secondary end point was the effect of selonsertib on albuminuria. Safety assessments included monitoring and collection of adverse events (AEs), clinical laboratory tests, vital sign measurements, physical examinations, and electrocardiograms.

Adults (age 18–75 years) with type 2 diabetes and treatment-refractory moderate-to-advanced DKD (eGFR of $\geq$15 to $<$60 ml/min per 1.73 m^2 at screening) and albuminuria, defined as a urine albumin-to-creatinine ratio (UACR) $\geq$600 mg/g if stage 3a CKD, UACR $\geq$300 mg/g if stage 3b CKD, and UACR $\geq$150 mg/g if stage 4 CKD, receiving standard-of-care therapies (including ACEis or ARBs in the absence of contraindication, at an appropriate and stable dose), were eligible to participate in this study. Additional inclusion and exclusion criteria are provided in Supplemental Table 1. Each patient underwent a 7- to 30-day screening period and a 48-week treatment period, followed by a 30-day follow-up period. The maximum total duration of participation for each patient was approximately 56 weeks.

After screening, patients were randomized in a 1:1:1:1 allocation to receive one of three doses of selonsertib (2, 6, or 18 mg) or matching placebo. Randomization was stratified by DKD strata on the basis of eGFR (stages 3a, 3b, and 4) and UACR. Patients self-administered randomized study drug (one tablet orally once daily) for 48 weeks. Study drug adherence was calculated on the basis of tablet counts dispensed and returned.

We assessed efficacy by surrogate markers of kidney function (eGFR and albuminuria) at each study visit, and calculated eGFR using the abbreviated (four-variable) Modification of Diet in Renal Disease (MDRD) Study formula.

Statistical Analyses

A sample size of 75 patients per treatment group provided 80% power to detect a 50% improvement in eGFR decline from baseline to week 48 relative to placebo for at least one

selonsertib dose. We assumed an eGFR decline of 5 ml/min per year in the placebo group and a common SD of 5 ml/min per year with a 15% dropout rate. The primary analysis in the full analysis set tested the null hypothesis that there was no difference in change in eGFR from baseline to week 48 between selonsertib (2, 6, or 18 mg) and placebo. We conducted inference testing at the two-sided 0.05 significance level in the following sequence: (*1*) high (18 mg), (*2*) medium (6 mg), and (*3*) low (2 mg) dose. Once a nonsignificant test result was reached, no statistical significance could be declared in the remainder of the sequence. For the prespecified analysis of the primary end point, we used a mixed model for repeated measures to handle missing values. Baseline eGFR, treatment group, visit, and treatment-by-visit interaction were included in the mixed model for repeated measures. In consideration of an unanticipated acute increase in serum creatinine (and corresponding drop in eGFR) in the selonsertib groups and issues relating to GCP compliance at two sites (described below), we performed an exploratory post hoc analysis of eGFR decline using a piecewise random slope model with a turning point at week 4. The random slope model included the following terms: baseline eGFR, treatment group, visit, and treatment-by-visit interaction (see Supplemental Table 2 for details). Data anomalies from patients at the two sites were noted across multiple laboratory parameters and vital signs. These issues called into question the reliability of eGFR and other data from the 20 patients enrolled at those sites. Thus, before study completion, we decided to exclude data from these 20 patients in secondary analyses as well as subsequent exploratory post hoc analyses.

Pharmacokinetic/Pharmacodynamic Analyses

Single plasma samples were collected for pharmacokinetic (PK) analyses from all subjects at weeks 1, 4, 12, 24, and 48 of treatment with trough samples (timed to approximately 24 hours after the previous dose of selonsertib) at weeks 4 and 48. Additionally, an optional intensive PK substudy was performed at week 8, 12, 16, and 24 of treatment that involved intensive plasma sampling over a 24-hour period. The PK analysis set included all randomized patients who took ≥1 dose of selonsertib and had ≥1 nonmissing postdose concentration value. Population PK modeling using NONMEM was conducted on the single PK samples as well as the intensive PK substudy samples to estimate area under the plasma concentration–time curve over the dosing interval (AUC_{tau}) for selonsertib and its metabolite.

PK/pharmacodynamic (PD) relationships were evaluated between selonsertib dose or exposure (AUC_{tau}) and ASK1 target engagement (percentage change in phosphorylation status of p38 at weeks 12 and 24 of treatment compared with baseline) as well as the acute decline in eGFR between 0 and 4 weeks and the chronic decline in eGFR between 4 and 48 weeks.

Study Oversight

This trial was approved by the institutional review board or independent ethics committee at all participating sites and conducted in compliance with the Declaration of Helsinki, GCP guidelines, and local regulatory requirements. The trial was designed and conducted according to protocol by the sponsor (Gilead Sciences, Inc., Foster City, CA) in collaboration with the independent principal investigators. The sponsor collected the data, monitored study conduct, and performed the statistical analyses. An independent data monitoring committee reviewed the progress and provided oversight of the trial. The investigators, participating institutions, and sponsor agreed to maintain confidentiality of the data. All authors had access to the data and assume responsibility for the integrity and completeness of the reported data. The trial protocol is available with this article.

RESULTS

Patient Disposition and Baseline Characteristics

The trial ran from June 30, 2014 (first patient screened), until July 25, 2016; patients were primarily from the United States (96%). A total of 893 patients were screened for enrollment; 334 were randomly assigned to receive selonsertib (2, 6, or 18 mg) or placebo (Figure 1). For randomized patients, premature discontinuation of study treatment was recorded for 18.5% (15 of 81), 21.4% (18 of 84), and 28.9% (24 of 83) of patients in the selonsertib 2-, 6-, and 18-mg treatment groups, respectively, and for 22.4% (19 of 85) of patients in the placebo group. The most common reasons for study discontinuation overall (≥2%) were AEs, progression to ESKD, withdrawal of consent, and protocol violations.

Patient demographics and baseline characteristics were well balanced among the groups except for a higher proportion of white patients and a corresponding lower proportion of black patients in the selonsertib 18-mg treatment group (Table 1). The mean (SD) age was 63.0 (8.3) years, and 226 (67.9%) patients were men.

Efficacy

In the prespecified primary analysis, patients receiving selonsertib had similar decreases in the mean (±SEM) eGFR as compared with placebo at 48 weeks (with treatment differences of 0.38±1.21, 0.84±1.22, and −0.87±1.23 ml/min per 1.73 m^2 in the 2-, 6-, and 18-mg groups, respectively; $P>0.4$ for all pair-wise comparisons; Figure 2); thus, the trial failed to meet its primary efficacy end point. Differences in eGFR at 48 weeks were confounded by acute increases in serum creatinine (and corresponding drop in eGFR [Supplemental Figure 1]) in the selonsertib-treated patients that were not anticipated in the original study design.

In an exploratory post hoc analysis isolating the unanticipated acute selonsertib-associated creatinine increase in the high-dose selonsertib treatment arm and excluding the 20 patients from two centers with serious GCP compliance questions, two dose-dependent effects were observed (Figure 3): an acute and larger decline in eGFR from 0 to 4 weeks (creatinine transport effect), and a gradual and smaller chronic decline in

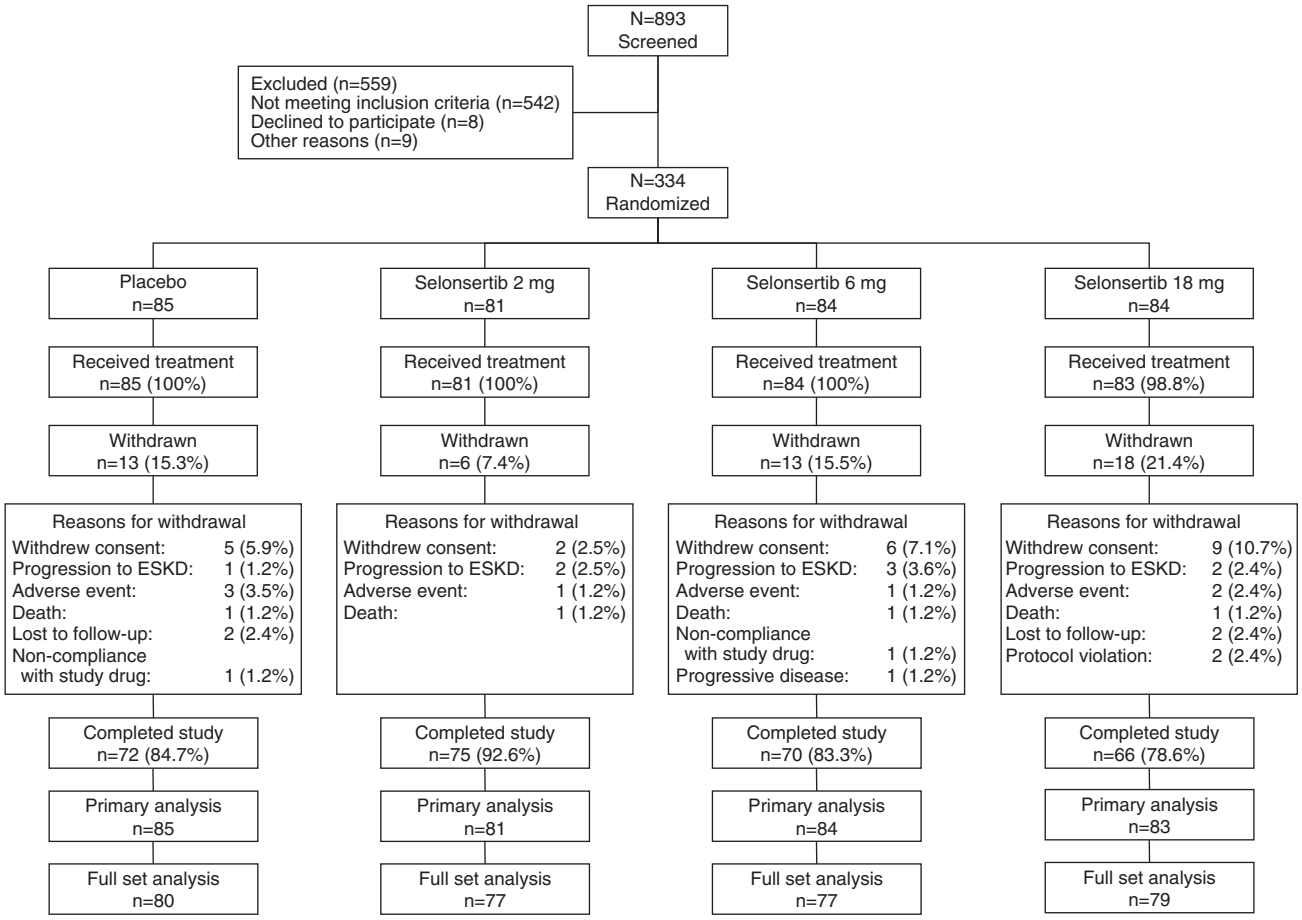

Figure 1. Patient disposition.

eGFR between 4 and 48 weeks (therapeutic effect). Patients receiving 18 mg selonsertib had an acute dose- and exposure-dependent decrease in eGFR of –3.33 ml/min per 1.73 m^2. This acute decrease in eGFR (which plateaued by weeks 3–4 of treatment) was larger (approximately 10% of the baseline value at week 4) than for the 2- and 6-mg selonsertib dose groups or placebo (Supplemental Figure 1). Between 4 and 48 weeks, the rate of decline for the 18-mg group was nominally significantly lower than that of the placebo group (difference of 3.11 ml/min per 1.73 m^2 annualized over 1 year, or 71% reduction in eGFR decline rate from placebo; 95% confidence interval, 0.10 to 6.13; P=0.043; Figure 4). Changes from baseline in eGFR using the CKD Epidemiology Collaboration cystatin C 2012 equation also showed no statistically significant differences from placebo for any selonsertib treatment group. Supplemental Figure 2 shows the cystatin C–based eGFR change from baseline over time for selonsertib 18 mg versus placebo, and suggests that there may be a difference in the decline in kidney function between selonsertib 18 mg versus placebo, because the cystatin C alternative method to estimate GFR is not affected by the inhibition of creatinine secretion.

Changes from baseline in UACR are presented in Figure 5; there was no statistically significant difference from placebo for any selonsertib treatment group in the proportion of patients achieving at least a 30% reduction from baseline in albuminuria (as measured by UACR) at week 48. Supplemental Figure 3 shows an absence of strong effects on two urine biomarkers (endothelial growth factor and kidney injury molecule–1) both with and without correction for urinary creatinine because of the inhibition of tubular secretion of creatinine by selonsertib.

PK/PD Analyses

Selonsertib steady-state exposures were as expected and comparable to those observed in healthy volunteers on a dose-normalized basis. Selonsertib metabolite exposures were moderately lower in patients with DKD as compared with healthy volunteers on a dose-normalized basis. The percent phospho-p38 (%P-p38), a PD marker of ASK1 inhibition by selonsertib, declined in a dose-dependent manner (Supplemental Figure 4). Additionally, the relation between P-p38 inhibition and chronic eGFR slope by dose group showed a trend toward improved efficacy with increasing dose of selonsertib, with minimal overlap between the selonsertib 18-mg group and the placebo group (Supplemental Figure 5).

Table 1. Baseline demographics and characteristics (full analysis set)

Characteristic	Placebo (*n*=85)	Selonsertib 2 mg (*n*=81)	Selonsertib 6 mg (*n*=84)	Selonsertib 18 mg (*n*=83)	Total (*N*=333)
Mean age (SD), yr	62 (7.9)	63 (8.1)	62 (8.1)	63 (9.0)	63 (8.3)
Men, *n* (%)	53 (62.4)	53 (65.4)	64 (76.2)	56 (67.5)	226 (67.9)
Race, *n* (%)					
Asian	3 (3.5)	3 (3.7)	4 (4.8)	2 (2.4)	12 (3.6)
Black	19 (22.4)	20 (24.7)	23 (27.4)	11 (13.3)	73 (21.9)
White	61 (71.8)	53 (65.4)	57 (67.9)	68 (81.9)	239 (71.8)
Other	2 (2.4)	5 (6.1)	0	2 (2.4)	9 (2.7)
Hispanic or Latino ethnicity, *n* (%)	30 (35.3)	25 (30.9)	31 (36.9)	33 (39.8)	119 (35.7)
Mean body mass index (SD), kg/m^2	34.7 (6.78)	34.6 (8.19)[a]	35.6 (8.54)[b]	35.4 (8.06)	35.1 (7.89)[c]
Prior medications of interest, *n* (%)					
ACEi	31 (36.5)	33 (40.7)	31 (36.9)	34 (41.0)	129 (38.7)
ARB	44 (51.8)	36 (44.4)	42 (50.0)	39 (47.0)	161 (48.3)
Medical history					
Mean serum creatinine (SD), mg/dl	2.3 (0.73)	2.3 (0.72)	2.3 (0.74)	2.2 (0.68)	2.3 (0.71)
Mean eGFR (SD), ml/min per 1.73 m^2	31.4 (11.87)	31.6 (10.92)	31.9 (11.58)	31.1 (10.44)	31.5 (11.18)
Diabetes, *n* (%)	68 (80.0)	64 (79.0)	74 (88.1)	67 (80.7)	273 (82.0)
Diabetes: neuropathy	43 (50.6)	35 (43.2)	47 (56.0)	42 (50.6)	167 (50.2)
Diabetes: retinopathy	27 (31.8)	30 (37.0)	30 (35.7)	24 (28.9)	111 (33.3)
Diabetes: gastroparesis	0	2 (2.5)	2 (2.4)	0	4 (1.2)
Congestive heart failure, *n* (%)[d]	12 (14.1)	12 (14.8)	8 (9.5)	14 (16.9)	46 (13.8)
Coronary artery bypass graft, *n* (%)[e]	6 (7.1)	8 (9.9)	6 (7.1)	13 (15.7)	33 (9.9)
Coronary artery disease, *n* (%)	24 (28.2)	27 (33.3)	20 (23.8)	34 (41.0)	105 (31.5)
Dyslipidemia, *n* (%)	68 (80.0)	60 (74.1)	64 (76.2)	66 (79.5)	258 (77.5)

[a]*n*=79.
[b]*n*=83.
[c]*N*=330.
[d]All classes were used for each subject if applicable.
[e]The most recent event was collected for each subject.

Safety

The incidence of AEs was similar across all groups (Table 2). There was no dose-related pattern in overall AE incidence among all selonsertib treatment groups. The majority of AEs were mild to moderate in severity. AEs, as determined by the site investigators, with ≥5% occurrence in any treatment group and that occurred in the selonsertib pooled group at ≥2 times the rate in the placebo group included constipation, AKI, back pain, hypoglycemia, cellulitis, hyperglycemia, and gout. In the placebo group, 18.8% (16 patients) experienced a serious AE, compared with 25.8% (64 patients) in the pooled selonsertib group. There was one fatal AE reported (myocardial infarction on the patient's last planned day of receiving study drug).

AEs leading to study drug discontinuation occurred in 9.4% (eight patients) in the placebo group, compared with 12.1% (30 patients) in the pooled selonsertib group (8.6% [seven patients], 13.1% [11 patients], and 14.5% [12 patients] for the selonsertib 2-, 6-, and 18-mg groups, respectively). ESKD was the most common AE leading to discontinuation of study drug in each treatment group (Figure 1).

Three AEs of special interest were prospectively identified: ARF, cardiac failure, and fluid overload. The occurrence of AEs potentially indicative of ARF was more frequent in all selonsertib treatment groups (10.7%–16.0%) than in the placebo group (8.2%); nevertheless, the occurrence of laboratory abnormalities of severity grade ≥3 for serum creatinine was numerically highest in the placebo group. Occurrences of AEs potentially indicative of cardiac failure and fluid overload were similar among the selonsertib treatment and placebo groups. No clinically relevant changes in electrocardiogram results were observed.

Analyses of fasting glucose and HbA1c indicated no significant difference from placebo for any selonsertib treatment group. Mean changes from baseline in BP, body mass index, and BUN in all treatment groups at all timepoints were small in magnitude, with little variation among the treatment groups.

DISCUSSION

This double-blind, placebo-controlled, dose-finding phase 2 trial evaluated the safety and efficacy of selonsertib, a potent

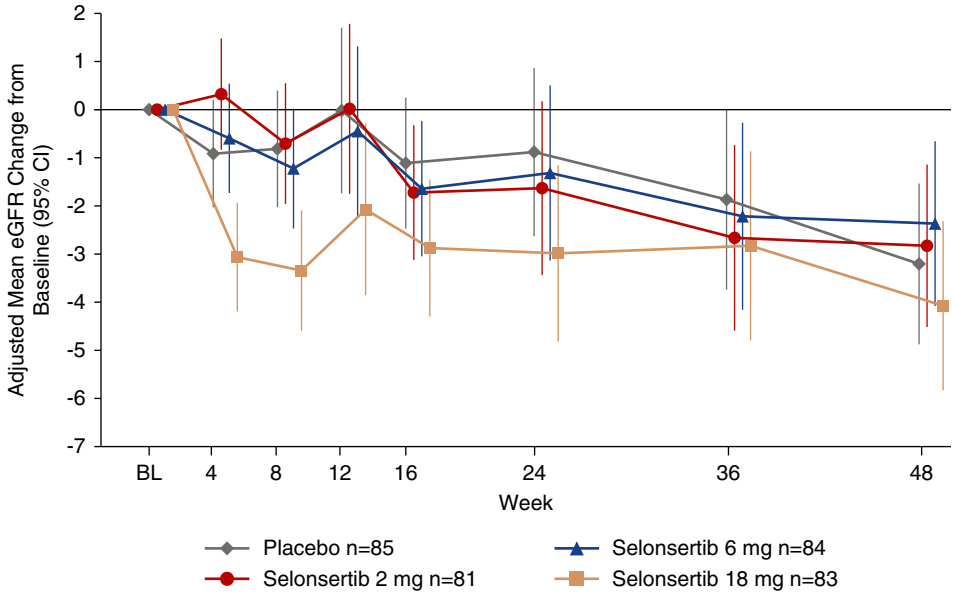

Figure 2. No significant differences versus placebo in eGFR in the primary analysis. Adjusted mean (95% CI) of eGFR (MDRD) change from baseline by visit (full analysis set, *n*=333). 95% CI, 95% confidence interval; BL, baseline.

and selective ASK1 inhibitor, in patients with treatment-refractory moderate-to-advanced DKD. The trial did not meet its predefined primary efficacy end point; treatment with selonsertib did not demonstrate a statistically significant difference from placebo in the mean eGFR after 48 weeks of therapy. However, differences in eGFR from baseline to 48 weeks were confounded by unanticipated acute effects of selonsertib on serum creatinine (decrease approximately 10% of the baseline value at week 4 with the 18-mg dose). Regulatory guidance at the time of study design/conduct[24] indicated low potential for selonsertib to inhibit the renal creatinine transporters; subsequent *in vitro* experiments and clinical studies in healthy volunteers indicate that the acute effect of selonsertib on eGFR is a result of inhibition of multidrug and toxin extrusion protein (MATE) 1 and 2K.[25] Review of all clinical data, after results of the data for this study became available,

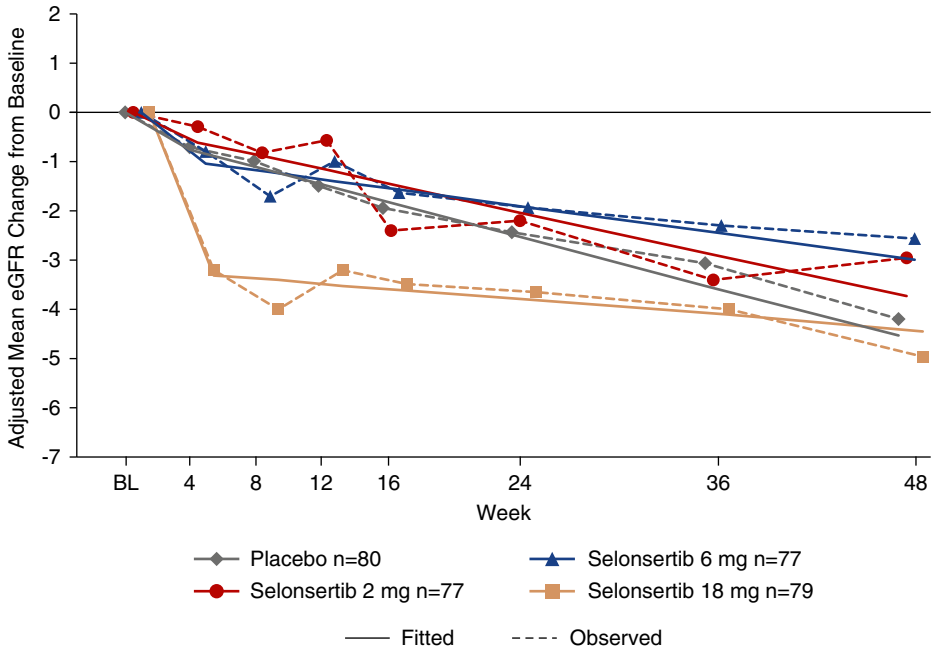

Figure 3. Selonsertib shows dose-dependent effects on eGFR. eGFR (MDRD) decline rate using a piecewise linear random slope model (full analysis set excluding data collected from two sites with GCP compliance findings; *n*=313). BL, baseline.

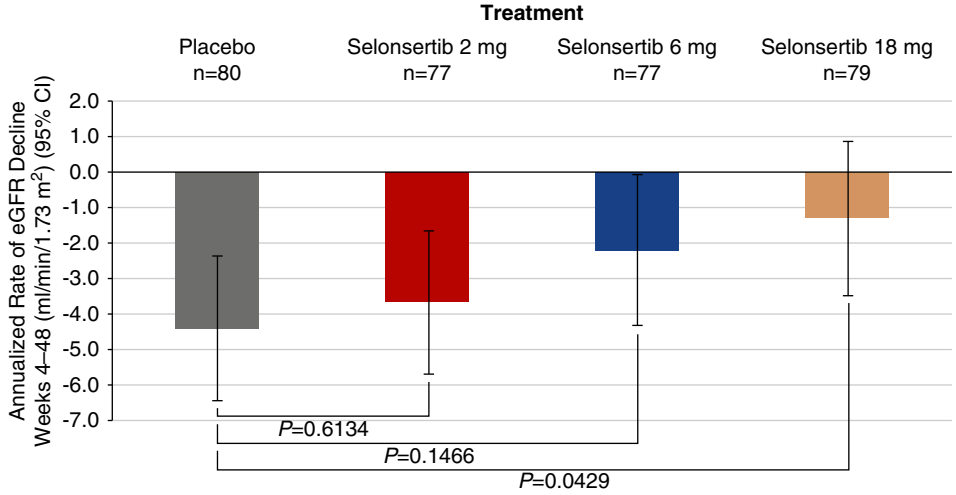

Figure 4. In a post hoc analysis, the 18 mg selonsertib dose slowed eGFR decline between 4 and 48 weeks. eGFR (MDRD) decline rate from week 4 to 48 (95% CI) (full analysis set excluding data collected from two sites with GCP compliance findings; *n*=313). 95% CI, 95% confidence interval.

demonstrates consistency in the findings between phase 1 studies of healthy volunteers (Gilead Sciences, Inc., GS-US-223-3973 and GS-US-223-0102, unpublished data), as well as a phase 2 study of patients with nonalcoholic steatohepatitis.[26] In our study, treatment with selonsertib appeared to slow the decline of kidney function in exploratory post hoc analyses, which accounts for these effects.

In the original trial design, change in eGFR, calculated using the MDRD formula, was selected as the primary efficacy end point, because eGFR is considered an appropriate surrogate marker for CKD. However, what was not adequately considered and what has been demonstrated is that selonsertib acutely affects serum creatinine concentrations and, by extension, eGFR *via* the effects on MATE1 and MATE2K that have been recognized in Food and Drug Administration guidance released after the design of this study. An acute effect on GFR, whereby the early change in GFR is different in direction or magnitude from the later change in GFR, has been observed with other interventions—including ACEis and ARBs—and can complicate the interpretation of study results.[27] Specifically, in contrast to ACEis and ARBs, the acute effect on eGFR with selonsertib is related to inhibition of renal transporters and not related to a change in glomerular hemodynamics that could be correlated with reducing the subsequent decline in kidney function.[28]

The findings in this study and the continued discussion regarding the role of change in eGFR as a surrogate end point for kidney failure in clinical trials[29–31] highlight the challenges inherent in the design and implementation of studies evaluating interventions for DKD. These findings also underscore the need for additional validated surrogate markers for kidney disease progression to facilitate drug discovery and development of DKD therapeutics. To establish efficacy, a clinical trial must demonstrate that the therapy influences a clinically meaningful end point. However, because DKD is a slowly progressive disease, clinically meaningful end points such as ESKD or eGFR reduction may take years to develop, requiring longer-duration trials in large numbers of patients to demonstrate benefit, both of which drive up trial costs. Indeed, the 1-year study duration of this trial may have been too short to allow demonstration of a treatment effect.[32] However, currently, no novel biomarkers are routinely used in clinical practice or as prognostic end points in clinical trials.[33] It may be that slope comparisons will prove to be more statistically powerful than proportions of or times to dichotomized changes, especially when measurements are not dense and event times are not assessed precisely, as recent research and workshop discussions are recognizing.[34,35] This may, in turn, allow trials to be sufficiently powered without the need for quite as high patient numbers or extended study durations.

Despite a greater understanding of the importance of oxidative stress in the development of glomerulosclerosis and tubulointerstitial fibrosis characteristic of DKD,[12,36–41] therapeutic strategies generally targeting reduction in oxidative stress have not demonstrated clear clinical benefits,[7,10–12] and the approach to oxidative stress reduction has evolved to focus on the specific downstream cellular events. However, extensive preclinical data support the potential therapeutic benefits of inhibiting ASK1, a serine/threonine kinase that mediates cellular responses contributing to the inflammation, fibrosis, and apoptosis characteristic of DKD.[14–16,37,40–42] Consistent with these preclinical findings, this study demonstrated a dose-dependent reduction in %P-p38 at weeks 12 and 24, and a relation between P-p38 inhibition and chronic eGFR slope by dose group, demonstrating minimal overlap between the selonsertib 18-mg and placebo groups.

Interpretation of the exploratory post hoc analysis of eGFR resulting from the unanticipated dose-dependent acute

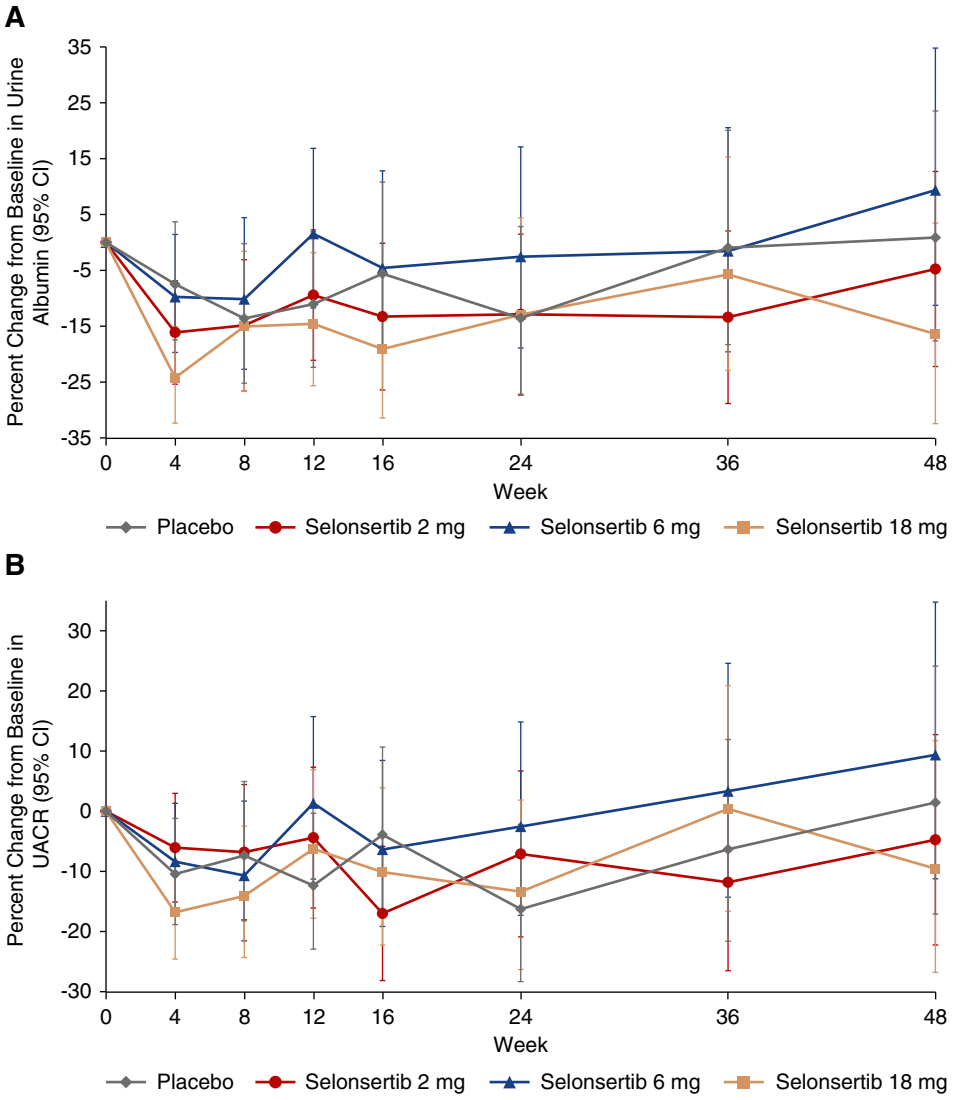

Figure 5. No significant changes from baseline in urine albumin or UACR. Panel A, uncorrected albumin; Panel B, albumin normalized for creatinine (UACR). 95% CI, 95% confidence interval.

reduction in eGFR is limited by several factors: (*1*) the data-driven model selection; (*2*) marginal statistical significance; (*3*) the assumption that dropouts were missing at random so that the mixed model analysis controls for missing data bias; and (*4*) perhaps most importantly, the underlying assumption of the analysis that longitudinal trends in eGFR in patients on high-dose selonsertib after stabilization of the acute creatinine increase, but in the presence of renal creatinine transport inhibition, reflect underlying kidney function in the same manner as the cross-sectional creatinine-GFR relationships in MDRD patients from which the eGFR formula was derived.

Another limitation of this study relates to issues concerning GCP in study conduct identified at two sites, and highlights the importance of appropriate site selection and monitoring of data quality for potential misconduct. There can be trade-offs between more sites to improve the speed of enrollment versus fewer sites to allow better vetting and data quality control. Future studies will need to consider this balance to avoid similar occurrences.

In conclusion, this phase 2 trial evaluating the safety and efficacy of once-daily selonsertib, a selective ASK1 inhibitor, in patients with moderate-to-advanced DKD, did not demonstrate significant dose-related toxicity. A statistically significant difference from placebo in the mean eGFR from baseline to 48 weeks after initiation of therapy, the predefined study end point, was not achieved. Although this observed difference was not statistically significant in the primary analysis, exploratory post hoc analyses accounting for the acute effects of selonsertib on serum creatinine (examining differences in eGFR slope from week 4 to 48) suggest that selonsertib treatment resulted in a dose-dependent reduction in the decline of kidney function. Further exploration of whether selonsertib may reduce progression to kidney failure in patients with DKD with a

Table 2. AEs of grade 3 or greater severity reported in at least two subjects in any treatment group (safety analysis set, *n*=333)

Adverse Event	Placebo (*n*=85)	Selonsertib 2 mg (*n*=81)	Selonsertib 6 mg (*n*=84)	Selonsertib 18 mg (*n*=83)
Any AE of grade 3 or greater severity, *n* (%)	15 (17.6)	21 (25.9)	20 (23.8)	21 (25.3)
AKI	2 (2.4)	1 (1.2)	3 (3.6)	1 (1.2)
Cardiac failure, congestive	2 (2.4)	1 (1.2)	1 (1.2)	3 (3.6)
Cellulitis	1 (1.2)	1 (1.2)	2 (2.4)	2 (2.4)
Pneumonia	0	1 (1.2)	1 (1.2)	2 (2.4)
Dyspnea	1 (1.2)	0	2 (2.4)	1 (1.2)
ESRD	2 (2.4)	0	2 (2.4)	0
Acute myocardial infarction	0	1 (1.2)	2 (2.4)	0
Hyperkalemia	0	0	1 (1.2)	2 (2.4)
Hypertension	1 (1.2)	2 (2.5)	0	0
Chronic obstructive pulmonary disease	0	2 (2.5)	0	0
Renal failure	0	2 (2.5)	0	0
CKD	2 (2.4)	0	0	0

AEs were mapped according to MedDRA Version 19. Treatment-emergent AEs are those that started on or after first study drug dose date up to and including 30 d after permanent discontinuation, or that led to premature study drug discontinuation. Severity grades (1=mild, 2=moderate, 3=severe, 4=life-threatening, 5=death) were from modified CTCAE Version 4.0. Multiple AEs were counted once per subject for each preferred term. CTCAE, Common Terminology Criteria for Adverse Events; MedDRA, Medical Dictionary for Regulatory Activities.

longer duration of therapy and without undue safety or tolerability issues is warranted.

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Dr. Chertow reports personal fees from Gilead Sciences, Inc., during the conduct of the study; personal fees from Akebia, personal fees from AMAG, personal fees from Amgen, personal fees and other from Ardelyx, personal fees from AstraZeneca, personal fees from Baxter, other from Cricket Health, other from Durect, other from DxNow, other from Outset, personal fees from Reata, personal fees from Sanifit, personal fees from Bayer, personal fees from ReCor, and personal fees from Vertex, outside the submitted work. Dr. Pergola reports personal fees from Gilead Sciences, Inc., personal fees from Akebia, personal fees from AstraZeneca, personal fees from Keryx, personal fees from Reata, personal fees from ExThera, personal fees from Vifor, personal fees from AbbVie, other from Renal Associates, and PA (employer), during the conduct of the study. Dr. Chen reports employment from Gilead Sciences, Inc., during the conduct of the study. Dr. Kirby reports employment from Gilead Sciences, Inc., during the conduct of the study. Dr. Sundy reports employment from Gilead Sciences, Inc., during the conduct of the study. Dr. Patel reports employment from Gilead Sciences, Inc., beginning in 2016, and served as a consultant to Gilead Sciences, Inc., between 2013–2016. Individual participant data will not be available.

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2018121231/-/DCSupplemental.

Supplemental Table 1. Inclusion and exclusion criteria.

Supplemental Table 2. Statistical methods.

Supplemental Figure 1. Acute selonsertib dose- and exposure-dependent change in eGFR between weeks 0 and 4 of treatment (post hoc efficacy analysis set). SEL, selonsertib; AUC_{tau}, area under the plasma concentration time curve over the dosing interval; 95% CI, 95% confidence interval.

Supplemental Figure 2. Adjusted mean (95% CI) of eGFR (cystatin C–based CKD-EPI) change from baseline by visit: post hoc analysis (full analysis set with available samples for cystatin C from selonsertib 18-mg and placebo arms, excluding data collected from two sites with GCP compliance findings; n=148). 95% CI, 95% confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; GCP, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines for Good Clinical Practice; SE, standard error; BL, baseline; MMRM, mixed model for repeated measures.

Supplemental Figure 3. Percent change in urine EGF (A, uncorrected; B, normalized for creatinine) and KIM-1 (C, uncorrected; D, normalized for creatinine) from baseline by visit. EGF, endothelial growth factor; KIM-1, kidney injury molecule–1; 95% CI, 95% confidence interval.

Supplemental Figure 4. Relationship between percent change in P-p38 from baseline and selonsertib dose and exposure (AUC_{tau}). P-p38, phospho-p38; AUC_{tau}, area under the plasma concentration–time curve over the dosing interval; SEL, selonsertib; 95% CI, 95% confidence interval; %P-p38, percent P-p38; PBO, placebo.

Supplemental Figure 5. Relationship between percent change in P-p38 from baseline and annualized rate of eGFR decline from week 4 to 48 by dose group. 95% CI, 95% confidence interval; PBO, placebo.

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