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Design of a Phase 2 Clinical Trial of an ASK1 Inhibitor, GS-4997, in Patients with Diabetic Kidney Disease

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Key Words
Diabetic Kidney Disease · DKD · Phase 2 · ASK1 · GS-4997 · ACEi · ARB · eGFR · Proteinuria · Albuminuria

Abstract
Background: Most patients with diabetic kidney disease (DKD) experience disease progression despite receiving standard care therapy. Oxidative stress is associated with DKD severity and risk of progression, but currently approved therapies do not directly attenuate the pathologic consequences of oxidative stress. GS-4997 is a once daily, oral molecule that inhibits Apoptosis Signal-regulating Kinase 1 (ASK1), which is a key mediator of the deleterious effects of oxidative stress. ***Methods:*** We describe the rationale and design of a Phase 2 placebo-controlled clinical trial investigating the effects of GS-4997 in patients with T2DM and stage 3/4 DKD receiving standard of care therapy. Approximately, 300 subjects will be randomized in a stratified manner, based on the estimated glomerular filtration rate (eGFR) and urine albumin to creatinine ratio, to one of four arms in this dose-ranging study. The primary endpoint is change in eGFR at 48 weeks, and the key secondary endpoint is change in albuminuria. ***Conclusion:*** Guided by the biology of oxidative stress signaling through ASK1, the biology of DKD pathogenesis, and solid statistical methods, the decisions made for this Phase 2 study regarding delineating study population, efficacy outcomes, treatment period and statistical methods represent innovative attempts to resolve challenges specific to DKD study design.

Introduction

Type 2 diabetes mellitus (T2DM) is the leading cause of chronic kidney disease (CKD) in the world, accounting for nearly half of all incident cases of kidney failure requiring dialysis or transplantation in the United States (US) [1]. Approximately 7.6 million people in the United States have comorbid CKD and T2DM, which is classified as diabetic kidney disease (DKD) and sometimes referred to as diabetic nephropathy [2]. While the pathobiology remains incompletely understood, DKD results from progressive angiopathy and fibrosis in the kidney. Inflammation and apoptosis are other key contributors to DKD pathogenesis [3]. Despite treatment aimed at optimizing glycemic control and reducing systemic hypertension and glomerular hyperfiltration, impaired kidney function often worsens over time. Currently approved therapies do not directly address the pathological consequences of oxidative stress experienced with DKD and progressive loss of kidney function [4–6].

Oxidative stress is deleterious through many mechanisms [7]. An oxidative stress responsive pathway initiated with the activation of Apoptosis Signal-regulating Kinase 1 (ASK1) is emerging as a driver of events relevant to DKD [8–12]. ASK1 is a redox-sensitive mitogen-activated protein kinase kinase kinase (MAPKKK), which functions as a critical signaling node through which oxidative stress promotes deleterious effects. ASK1 activation causes downstream activation of the terminal MAPK

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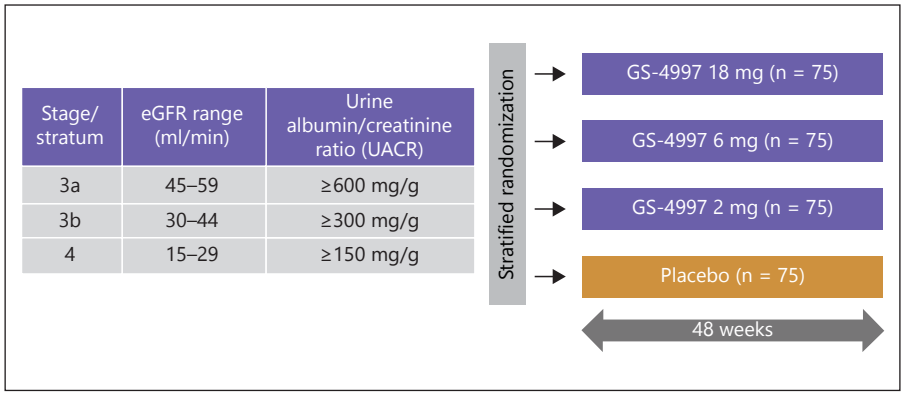

Fig. 1. Study Schema.

kinases p38 and c-Jun N-terminal kinase (JNK) [13–15], which stimulate production of inflammatory cytokines/chemokines, induce expression of matrix remodeling genes (fibrosis), promote apoptotic and necrotic cell death, increase aberrant cell proliferation, and contribute to metabolic perturbations [8–12]. GS-4997 is a highly selective and potent once-daily oral ASK1 inhibitor that competes with ATP in the ASK1 catalytic kinase domain (unpublished data).

Methods

Design and conduct of this study are in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines for Good Clinical Practice. The study protocol was approved by central and local IRBs in North America. This study is registered on clinicaltrials.gov with identifier NCT02177786.

Study Conduct

This will be a double blind, placebo-controlled study as depicted in figure 1. Three dose levels of GS-4997 will be tested vs. placebo. The treatment period is 48 weeks with a safety visit at 52 weeks. An external data monitoring committee (DMC) will monitor unblinded safety data.

The mean of two sets of screening values for estimated glomerular filtration rate (eGFR) and urine albumin excretion (urine albumin to creatinine ratio, or UACR) will be used for stratification and randomization purposes. eGFR will be calculated by the 4-variable Modification of Diet in Renal Disease (MDRD) Study formula.

Given that the study population is at risk for medical complications and has an established burden of comorbidities, the study incorporates 4 weekly visits after initiation of therapy in order to frequently assess for any potential adverse events. The study visit frequency decreases thereafter, with visits scheduled at weeks 8, 12, 16, 24, 36, 47, 48, and 52. The primary endpoint will be assessed at 48 weeks of therapy, but the additional eGFR/UACR measurements at week 47 will mitigate to some degree the effects of intrasubject variability in these measurements.

Study Population

Approximately, 75 patients will be randomized into each arm for a total sample size of approximately 300. Subjects must have T2DM, stage 3/4 CKD (eGFR 15 to 59 ml/min/1.73 m^2), albuminuria, and be on a stable dose of angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) for at least 3 months. Co-administration of ACEi with ARB is not allowed on study. The use of a direct renin inhibitor (aliskiren) is allowed, but not concurrently with ACEi or ARB. There are different qualifying UACR thresholds by CKD stage (UACR >600 mg/g for stage 3a, UACR >300 mg/g for stage 3b, UACR >150 mg/g for stage 4). Randomization will be stratified by these three tiers of DKD stage matched with UACR. Other inclusion and exclusion criteria are highlighted in table 1.

Endpoints

The key objectives of this study are to evaluate the safety and efficacy of GS-4997 in DKD in a Phase 2 setting. Safety endpoints include changes in vital signs, laboratory values, 12-lead ECG, and adverse events. The study population is at a great risk for developing or worsening heart failure, and therefore a clinical marker of fluid status and heart failure severity, NT-pro-BNP, will be explored as a safety parameter. The primary efficacy endpoint is change in eGFR at 48 weeks of therapy. The secondary endpoint will be change in albuminuria. Cystatin-c, another surrogate of glomerular filtration that is not subject to the confounding effects of creatinine generation (a correlate of muscle mass), is being measured, recognizing that cystatin-c is an imperfect surrogate of kidney function.

Exploratory endpoints include examination of the pharmacokinetics of GS-4997 and its metabolites, and biomarkers. Selected biomarkers will interrogate CKD and acute kidney injury, inflammation, fibrosis, metabolism, oxidative stress, and the ASK1 pathway. Metabolic parameters will include HbA1c, fasting insulin, and fasting glucose. Physical examination parameters such as weight and waist circumference at each visit will add to an understanding of metabolic effects.

Statistical Considerations

The sample size of 75 subjects per arm, given potential subject annual dropout rate of 15% and standard deviation of 5 ml/min/1.73 m^2, yielded 80% power to detect an absolute treatment difference of 2.5 ml/min/1.73 m^2 for at least one GS-4997 dose

Table 1. Key inclusion and exclusion criteria

Inclusion criteria
Prior diagnosis of DKD
Male or female between 30 and 75 years of age, inclusive
Anyone between 18 and 30 years of age may be screened if the diagnosis of type 2 diabetes mellitus is confirmed with a C-peptide level
Type 2 diabetes mellitus diagnosis for at least 6 months
Screening eGFR ≥15 ml/min/1.73 m^2 to <60 ml/min/1.73 m^2
eGFR screening value will be calculated by MDRD formula and will be the average of 2 values obtained at least 5 days apart
If the 2 screening eGFR values are more than 25% different, the subject will be considered screen failure, but may re-screen
UACR (morning void) see left panel of figure 1
Receiving ACEi or ARB at a minimum dose (protocol defined) deemed appropriate for the subject by the investigator and be at a stable dose for the last 3 months
Subjects not on a qualifying dose ACEi/ARB (including no ACEi or ARB) may be screened if there is documented intolerance to ACEi and ARB
SBP 110 to 160 mm Hg and DBP 50 to 90 mm Hg
Exclusion criteria
Type 1 diabetes mellitus
HbA1c >9.5%
Non-diabetic kidney disease
UACR > 5,000 mg/g on any measurement during screening
End-stage renal disease (ESRD; peritoneal dialysis, hemodialysis, or status post-renal transplantation) or anticipated to occur within the treatment period
Anticipated progression to ESRD (need for dialysis or listing for renal transplantation) within the study treatment period
Sickle cell anemia or trait
QT interval corrected for heart rate using the Fredericia formula (QTcF) >450 ms
Unstable cardiovascular disease as defined by:
Unstable angina within 3 months prior to screening
Myocardial infarction, coronary artery bypass graft surgery or coronary angioplasty within 3 months prior to screening
Transient ischemic attack or cerebrovascular accident within 3 months prior to screening
Obstructive valvular heart disease or hypertrophic cardiomyopathy
Class IIIb or IV congestive heart failure
Immunosuppressive drugs including systemic corticosteroids, tacrolimus, sirolimus, cyclosporine, azathioprine, mycophenolate mofetil, and methotrexate (intra-articular, topical, nasal, or inhaled steroids are allowed)
Diagnostic or interventional procedure that requires intravenous contrast agent during, or within 30 days prior to screening and during study treatment period

group with a two-sided alpha of 0.05. In this study, a mixed-effect model repeated measures (MMRM) analysis will be used to account for missing data. Sensitivity analyses will be conducted to assess the robustness of the results across different analysis approaches for missing data.

Discussion

ASK1 inhibitors such as GS-4997 are a novel target class among agents being developed for DKD. In animal models of kidney disease, ASK1 inhibition resulted in reductions in pathway markers such as phospho-p38, but also improvements in kidney histopathologic scores, and in functional readouts such as direct measurements of GFR, serum creatinine, and proteinuria [16]. In models of metabolic syndrome, reductions in body weight, fasting insulin, fasting glucose, HbA1c, and OGTT were also observed [17]. These observations in animal models, along with human disease observations of elevations in on-target proteins such as phospho-p38 combined with the fact that oxidative stress is correlated with disease severity and progression [4–6, 10] led us to initiate a clinical investigation into the potential efficacy and tolerability of GS-4997 in persons with T2DM and stage 3/4 CKD.

Oxidative stress has been a target of DKD therapy. Development of bardoloxone methyl for DKD was recently stopped in the Western hemisphere due to cardiovascular safety concerns. ASK1 inhibition by GS-4997 is notably different in mechanism from bardoxolone methyl. GS-4997 shuts down cell signaling in a subset of cells involved in pathogenesis where oxidative stress is elevated, whereas bardoxolone methyl activates mRNA transcription in every cell exposed to drug. GS-4997 should selectively target affected cells in which the oxidative burden is high.

The study population is intended to capture the unmet need of progressive disease despite treatment with standard of care. Employing proteinuria as a surrogate of disease progression (requiring varying thresholds of albuminuria by stage of eGFR) enriches the study sample with patients more likely to experience progressive loss of kidney function during the study period. We chose different albuminuria cutoffs in an effort to identify patients with stage 3 CKD at higher risks of progression. While there is a direct correlation between the extent of proteinuria and decline in eGFR [18], non-proteinuric DKD is becoming more prevalent [2]. Proteinuria is currently the best surrogate for disease progression, and there is hope for developing superior predictive markers as the field advances.

The process of selecting efficacy outcomes and treatment period strove to balance the competing needs of generating a robust result in a modest time frame while testing the most clinically relevant outcomes. Changes in eGFR and albuminuria are evaluable over a shorter treatment period and may lead the way in demonstrating a

Table 2. Interventional and observational studies reporting rates of eGFR decline in DKD

Author	Population	n	Timing of assessment	Decline in GFR	Annualized change in GFR
House et al. [20]	Diabetics, mean GFR 51 ml/min, median proteinuria 900 mg/day	111	12 months	–5.4±10.5 ml/min	–5.4 ml/min
Mann et al. [21]	T2DM, mean GFR 33 ml/min, median UACR 1,500 mg/g	236	6 months	–2.5±6.9 ml/min	–5 ml/min
Tuttle et al. [22]	T2DM, mean GFR 69 ml/min, mean UACR 800 mg/g	62	12 months	–4.8±1.8 ml/min	–4.8 ml/min
Evans et al. [23]	T2DM, mean serum creatinine 1.69 mg/dl, median proteinuria 2.9 g/day	569	39 months	–14.8 ml/min	–4.6 ml/min
Altemtam et al. [24]	T2DM, mean serum creatinine 2.12 mg/dl, median proteinuria 0.91 g/day	270	retrospective	N/A	Progressors –3.57 ml/min Non-progressors –1.31 ml/min

clinical benefit that enables more definitive investigation of GS-4997 in DKD. Powering a Phase 2 trial for a clinically relevant change in eGFR may prove more informative in planning a Phase 3 trial that may employ a composite of sustained, clinically significant decline in kidney function and 'hard' endpoints such as death or progression to dialysis or transplantation.

GS-4997 has anti-inflammatory and anti-fibrotic properties, and does not appear to have any clinically evident hemodynamic effects. We therefore do not expect acute changes in eGFR, akin to those that accompany ACEi or ARB therapy. The choice of a one-year treatment period was guided by the efficacy endpoints. Recognizing that a treatment difference may become more apparent over longer treatment periods, and given the natural history of DKD, the treatment period of one year represents the minimum period of time that clinically relevant magnitudes (30–50% attenuation) may be observed in the rate of decline in eGFR. The natural history of eGFR decline in this population is approximately 5 ml/min/1.73 m^2, as determined by precedent interventional and observational studies (table 2). A relative reduction of 50% translates to an absolute reduction of 2.5 ml/min/1.73 m^2. Based on these studies, where the range of standard deviation (STDEV) in eGFR was 3–8 ml/min/1.73 m^2, the assumed STDEV for this study is 5 ml/min/1.73 m^2. The anticipated effect size and the STDEV estimate are key determinants of sample size. The potential limitations in these assumptions are that the study group may be healthier than expected and may not progress at the expected rate, or that there may be more heterogeneity than anticipated.

There was a consideration of composite primary endpoint, which also included events such as cardiovascular events or progression to ESRD. While these are crucial to follow for definitive long-term efficacy in DKD, the modest sample size and the relatively short treatment period preclude detecting a valid signal regarding these clinical events. There was also consideration of proteinuria as the primary endpoint. Other agents have shown disparate effects on proteinuria and kidney function, raising concern about the validity of proteinuria as a valid surrogate endpoint in CKD clinical trials, particularly those addressing non-hemodynamic mechanisms of injury [19].

Another potential limitation for the study surrounds missing data. When chronically ill patients are enrolled in a clinical trial, non-study, drug-related adverse events, either due to worsening primary disease or comorbidity, may warrant therapy that would confound evaluation of study drug treatment effects. In these cases, it will be necessary to stop treatment with blinded GS-4997. However, subjects will be encouraged to remain on study (off study drug) to better understand the natural history of disease in this population exposed to GS-4997 (or placebo) for any period of time. Despite such measures, some dropout is inevitable, and the resulting missing data may bias the efficacy evaluation if these missing data are not appropriately handled. Use of MMRM analysis, which considers the entire longitudinal profile of patients, would produce results that are more robust to potential biases from missing data than an analysis that uses only cross-sectional data (e.g., last observation carried forward [LOCF]).

This study is now underway; the study investigators, study team, and advisory panel look forward to reporting results of this study to the diabetes and nephrology communities.

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Disclosure Statement

J.H.L., J.J.Z., S.L.L. are employees of Gilead Sciences, Inc. G.M.C. has a consulting agreement with Gilead Sciences, Inc. that does not include manuscript preparation.

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References

1 Centers for Disease Control and Prevention: National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011. US Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA 2011, pp 1–12.

2 de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J: Temporal trends in the prevalence of diabetic kidney disease in the United States. JAMA 2011;305:2532–2539.

3 El Nahas AM, Muchaneta-Kubara EC, Essawy M, Soylemezoglu O: Renal fibrosis: insights into pathogenesis and treatment. Int J Biochem Cell Biol 1997;29:55–62.

4 Karamouzis I, Sarafidis PA, Karamouzis M, Iliadis S, Haidich AB, Sioulis A, Triantos A, Vavatsi-Christaki N, Grekas DM: Increase in oxidative stress but not in antioxidant capacity with advancing stages of chronic kidney disease. Am J Nephrol 2008;28:397–404.

5 Dounousi E, Papavasiliou E, Makedou A, Ioannou K, Katopodis KP, Tselepis A, Siamopoulos KC, Tsakiris D: Oxidative stress is progressively enhanced with advancing stages of CKD. Am J Kidney Dis 2006;48:752–760.

6 Hinokio Y, Suzuki S, Hirai M, Suzuki C, Suzuki M, Toyota T: Urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine as a predictor of the development of diabetic nephropathy. Diabetologia 2002;45:877–882.

7 Ray PD, Huang BW, Tsuji Y: Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 2012;24:981–990.

8 Nagai H, Noguchi T, Takeda K, Ichijo H: Pathophysiological roles of ASK1-MAP kinase signaling pathways. J Biochem Mol Biol 2007;40:1–6.

9 Soga M, Matsuzawa A, Ichijo H: Oxidative stress-induced diseases via the ASK1 signaling pathway. Int J Cell Biol 2012;2012:439587.

10 Adhikary L, Chow F, Nikolic-Paterson DJ, Stambe C, Dowling J, Atkins RC, Tesch GH: Abnormal p38 mitogen-activated protein kinase signalling in human and experimental diabetic nephropathy. Diabetologia 2004;47:1210–1222.

11 De Borst MH, Prakash J, Melenhorst WB, van den Heuvel MC, Kok RJ, Navis G, van Goor H: Glomerular and tubular induction of the transcription factor c-Jun in human renal disease. J Pathol 2007;213:219–228.

12 Sakai N, Wada T, Furuichi K, Iwata Y, Yoshimoto K, Kitagawa K, Kokubo S, Kobayashi M, Hara A, Yamahana J, Okumura T, Takasawa K, Takeda S, Yoshimura M, Kida H, Yokoyama H: Involvement of extracellular signal-regulated kinase and p38 in human diabetic nephropathy. Am J Kidney Dis 2005;45:54–65.

13 Tobiume K, Matsuzawa A, Takahashi T, Nishitoh H, Morita K, Takeda K, Minowa O, Miyazono K, Noda T, Ichijo H: ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. EMBO Rep 2001;2:222–228.

14 Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H: Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 1998;17:2596–2606.

15 Bunkoczi G, Salah E, Filippakopoulos P, Fedorov O, Muller S, Sobott F, Parker SA, Zhang H, Min W, Turk BE, Knapp S: Structural and functional characterization of the human protein kinase ASK1. Structure 2007;15:1215–1226.

16 Liles JT, Yang H, Fogo AB, Breckenridge DG: A Small Molecule Inhibitor of Apoptosis Signal-regulating Kinase 1 (ASK1) reduces Key Pathological Processes Related to CKD. American Society of Nephrology Annual Conference Abstracts, 2014, Abstract 5028.

17 Karnik S, Charlton M, Popov Y, Nash M, Barry V, Huntzicker E, French D, Li K, Decaris M, Emson C, Turner S, Goodman Z, Breckenridge D, Tumas D: Pharmacological inhibition of apoptosis signal-regulating kinase 1 (ASK1) in a murine model of NASH with pre-existing disease blocks fibrosis, steatosis, and insulin resistance. American Association for the Study of Liver Disease, The Liver Meeting 2014, Abstract 2028810.

18 Babazono T, Nyumura I, Toya K, Hayashi T, Ohta M, Suzuki K, Kiuchi Y, Iwamoto Y: Higher levels of urinary albumin excretion within the normal range predict faster decline in glomerular filtration rate in diabetic patients. Diabetes Care 2009;32:1518–1520.

19 Levey AS, Inker LA, Matsushita K, Greene T, Willis K, Lewis E, de Zeeuw D, Cheung AK, Coresh J: GFR Decline as an Endpoint for Clinical Trials in CKD: A Scientific Workshop Sponsored by the National Kidney Foundation and the US Food and Drug Administration. Am J Kidney Dis 2014, in press.

20 House AA, Eliasziw M, Cattran DC, Churchill DN, Oliver MJ, Fine A, Dresser GK, Spence JD: Effect of B-vitamin therapy on progression of diabetic nephropathy: a randomized controlled trial. JAMA 2010;303:1603–1609.

21 Mann JF, Green D, Jamerson K, Ruilope LM, Kuranoff SJ, Littke T, Viberti G: Avosentan for overt diabetic nephropathy. J Am Soc Nephrol 2010;21:527–535.

22 Tuttle KR, Bakris GL, Toto RD, McGill JB, Hu K, Anderson PW: The effect of ruboxistaurin on nephropathy in type 2 diabetes. Diabetes Care 2005;28:2686–2690.

23 Evans M, Bain SC, Hogan S, Bilous RW: Irbesartan delays progression of nephropathy as measured by estimated glomerular filtration rate: post hoc analysis of the Irbesartan Diabetic Nephropathy Trial. Nephrol Dial Transplant 2012;27:2255–2263.

24 Altemtam N, Russell J, El Nahas M: A study of the natural history of diabetic kidney disease (DKD). Nephrol Dial Transplant 2012;27:1847–1854.