Effects of the Soluble Guanylate Cyclase Stimulator Praliciguat in Diabetic Kidney Disease: A Randomized, Placebo-Controlled Clinical Trial

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Supplemental Material and Methods

Eligibility Requirements

Additional eligibility requirements to those described in the text included hemoglobin A1c (HbA1c) ≤12%, seated systolic blood pressure (SBP) of 110 to 160 mmHg by automatic office blood pressure (AOBP) assessment at both the screening and baseline visits, and body mass index of ≥20 and ≤45 kg/m^2. Non-diabetic kidney disease, prior dialysis or kidney transplant, secondary hypertension, malignancy or chronic significant medical comorbidities were exclusionary.

Trial Assessment Visits

Patients had an in-clinic screening visit between 45 and 15 days before randomization and a baseline visit 7 ± 3 days before randomization. Patients had study treatment visits at 1, 4, 8, and 12 weeks and a final in-clinic follow-up visit approximately 4 weeks after treatment.

Assessment Methods

Additional assessment methods to those described in the body of the manuscript are described here.

Kidney Function: UACR measurements were performed on first morning void urine samples collected on the two consecutive mornings before each study visit, and the results averaged. Serum creatinine was also determined at all visits.

Glycemic Control: Fasting plasma glucose and insulin values were collected at the baseline and week 1, 4, 8, and 12 visits, which allowed assessment of the homeostatic model assessment of insulin resistance (HOMA-IR) at each of these timepoints.[39] HbA1c was assessed at the screening, baseline, and week 1, 4, 8, and 12 and follow-up visits.

Hemodynamic Assessments: Continuous ambulatory blood pressure monitoring (ABPM) was performed for a 24-hour period at baseline and week 12, and for a 12-hour daytime period at week 4 and

week 8. Orthostatic (seated to standing) blood pressure by cuff and pulse rate were assessed predose and 1, 3, and 6 hours postdose on the day 1 and week 1 visits, predose at the week 4, 8 and 12 visits, and at the follow-up visit.

Biomarker Assessments: Plasma, serum, and urine samples were obtained for biomarker measurements before dosing on day 1 and at week 12. Biomarkers of endothelial function, inflammation, vascular injury, metabolic function, fibrosis and tubular function (Supplemental Table 1) were assessed by either liquid chromatography with tandem mass spectrometry (LC-MS/MS), enzyme-linked immunosorbent assay (ELISA), or meso scale discovery (MSD) multiplex assays.

Patient-Reported Outcomes: Patients completed the Kidney Disease Quality of Life – Short Form (KDQOL-SF™ v1.3) Health Survey (9 of 12 subscales) and the Patient Global Assessment of Severity (PGIS) questionnaire predose on day 1 and at the week 12 visit. Patients completed the Patient Global Assessment of Change (PGIC) at the week 12 visit and the EuroQol 5-dimension questionnaire (EQ-5D-5L) and SF-12v2 Health Survey Measure at the screening, day 1, week 4, 8, 12, and follow-up visits.

Pharmacokinetics: Blood samples for determination of praliciguat plasma concentrations were collected predose and 1, 3, and 6 hours postdose at the day 1 and week 1 visits, predose at the week 4 and 8 visits, predose and 3 hours postdose at the week 12 visit, and at the follow-up visit. Praliciguat concentrations were measured using a validated LC-MS/MS method as previously described[37].

Laboratory Evaluations: Blood samples for serum chemistry and hematology panels were collected at the screening, baseline, week 4, week 12, and follow-up visits. The chemistry panel included serum electrolytes, liver function tests and serum lipid profile. Hematology evaluation included complete blood counts, leukocyte differential count, platelet count, and serum coagulation panel. Routine urinalysis was

conducted at the screening, baseline, week 12, and follow-up visits. All laboratory assessments were conducted at a central laboratory, the Covance Center for Laboratory Services.

Platelet function: A subset of 26 patients at 7 sites had VerifyNow® aspirin and $P2Y_{12}$ platelet function assays, which assess platelet aggregation induced by arachidonic acid and adenosine diphosphate, respectively. Samples for these assays were collected predose at the day 1 visit, predose and 3 hours postdose at the week 1 visit, and predose at the week 12 visit.

Urine Albumin and Creatinine Measurements

All specimens assessed for urine albumin and creatinine concentrations were performed at a central lab, the Covance Center for Laboratory Services. Urine albumin was assessed by the Siemens BNII Instrument, with an inter-assay precision of 4.4% CV. Urine creatinine was analyzed on Roche Modular and Cobas analyzers using a modified Jaffe reaction, with an inter-assay precision of 1.7-2.2% CV.

Statistical Analysis

Mediation analysis to estimate the effect on UACR attributed to direct effect of treatment independent of blood pressure response was performed by fitting a set of generalized linear models with change from baseline in log-transformed UACR as the response variable, change from baseline in blood pressure as the mediation variable, treatment and the interaction between treatment and change from baseline in blood pressure as fixed effects and with baseline log UACR and baseline eGFR stratum as covariates.

Supplemental Results

Exploratory Efficacy Analyses

UACR Responders: In pre-specified responder analyses of the ITT population, numerically greater proportions of patients in the pooled praliciguat treatment groups demonstrated 30% and 40% decreases from baseline in UACR values compared to placebo (Supplemental Figure 2). Odds ratios for praliciguat vs. placebo were 1.51 (90% CI 0.87, 2.63) for a 30% response and 1.38 (0.78, 2.44) for a 40% response.

eGFR: Small reductions in eGFR over the 12-week study period were observed in each praliciguat dose group (Supplemental Figure 3). Mean eGFR changes from baseline over the 12 week treatment period for the 20-mg and 40-mg praliciguat groups were -3.6 (-5.4, -1.8) and -2.0 (-3.9, -0.1) mL/min/1.73 m^2, respectively, compared to -1.4 (-3.1, 0.4) mL/min/1.73 m^2 in the placebo group. At the follow-up visit 4 weeks later, mean eGFR increased 0.9 and 1.0 mL/min/1.73 m^2 from the 12 week end-of-treatment values in the 20mg and 40mg praliciguat groups, respectively, compared to a mean change of -1.5 ml/min/1.73m^2 in the placebo group.

Supplemental Table 1. Results for exploratory efficacy endpoints (change from baseline to Week 12 unless otherwise specified and associated 95% confidence intervals).

Variable	**Placebo (N=54) (95% CI)**	**Praliciguat Pooled (N=102) (95% CI)**	**Between-group Difference (95% CI)**	**Nominal P-value vs. Placebo**
UACR combined week 8 and 12 (%)	-14.8 (-30.0, 3.7)	-27.8 (-37.8, -16.3)	-15.3 (-33.4, 7.7)	0.2
UACR week 12 (%)	-14.8 (-34.2, 10.3)	-30.9 (-43.2, -16.0)	-18.9 (-41.1, 11.5)	0.2
Hemoglobin A1c (%)	-0.1 (-0.3, 0.2)	-0.3 (-0.5, -0.2)	-0.3 (-0.5, 0)	0.06
Cholesterol (mg/dL)	4.3 (-4.7, 13.4)	-5.7 (-12.9, 1.4)	-10.1 (-21.0, 0.8)	0.07
LDL cholesterol (mg/dL)	2.9 (-4.7, 10.6)	-4.6 (-10.5, 1.2)	-7.5 (-16.6, 1.5)	0.1

Note: ITT Population. For UACR, geometric least square means are used; for all other variables, least square mean changes and differences are used.
CI=confidence interval; UACR=urine albumin-to-creatinine ratio.

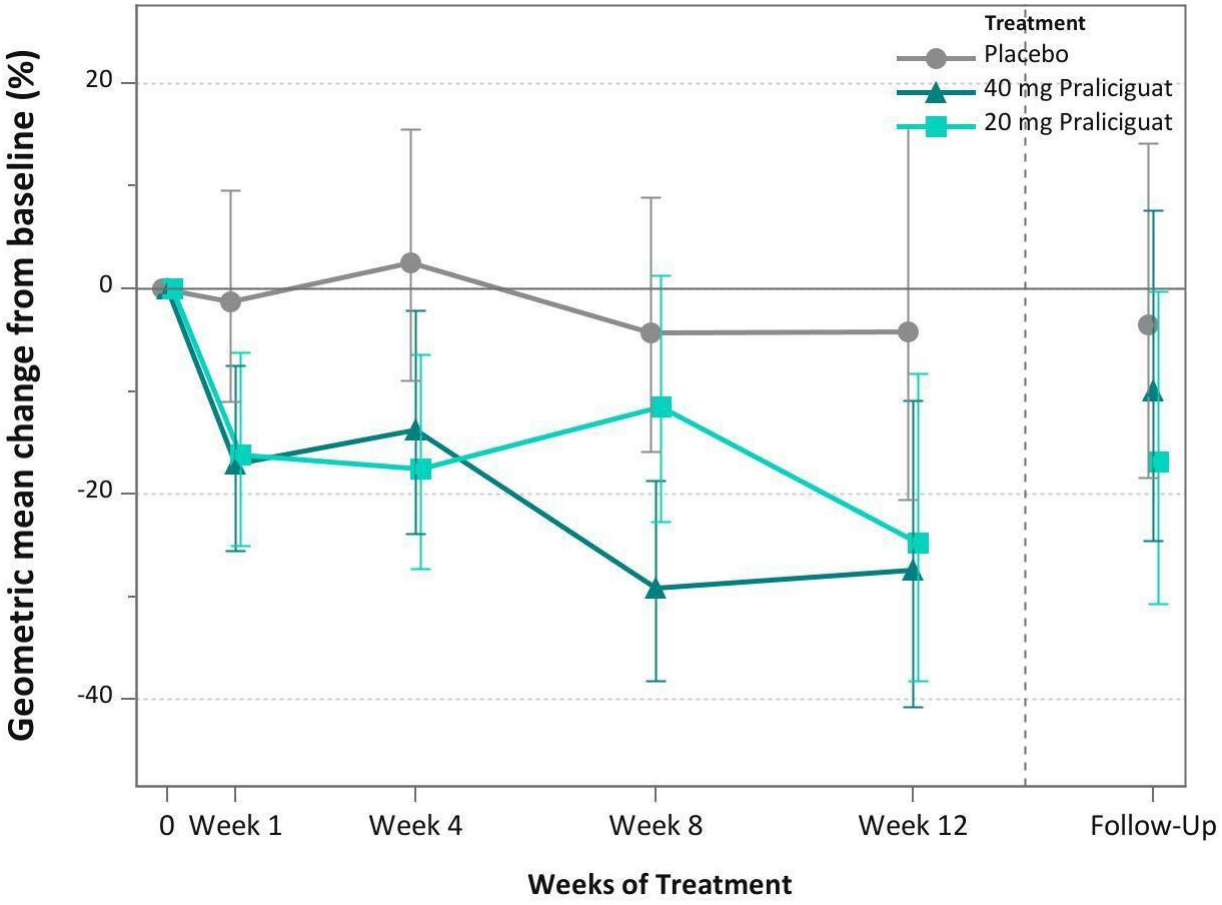
Supplemental Table 2. Summary of biomarker results: change from baseline to week 12 of treatment.

Variable	Placebo (N=54) (90% CI)	Praliciguat Pooled (N=102) (90% CI)	Between-group Difference (90% CI)	Nominal P-value vs. Placebo
L-Arginine (mg/L)	0.814 (-0.057, 1.69)	1.29 (0.602, 1.97)	0.471 (-0.546, 1.49)	0.44
ADMA (µg/L)	-0.268 (-3.59, 3.05)	0.180 (-2.44, 2.80)	0.448 (-3.45, 4.35)	0.85
L-Arginine/ADMA ratio [$x10^3$]	7.45 (0.327, 14.6)	12.5 (6.94, 18.1)	5.07 (-3.24, 13.4)	0.31
SDMA (µg/L)	2.78 (-4.48, 10.0)	5.09 (-0.656, 10.8)	2.31 (-6.16, 10.8)	0.65
FGF23 (RU/mL)	-2.10 (-19.0, 14.8)	2.86 (-9.93, 15.7)	4.96 (-14.8, 24.7)	0.68
TGFβ1 (ng/L)	-301 (-736, 133)	58.0 (-286, 402)	359 (-143, 862)	0.24
MCP-1 (ng/L)	8.87 (-75.2, 92.9)	-5.49 (-70.9, 60.0)	-14.4 (-113, 84.1)	0.81
SAA (pg/mL) [$x10^{-6}$]	9.80 (1.35, 18.25)	-0.85 (-7.49, 5.79)	-10.65 (-20.58, -0.72)	0.08
TNFα (ng/L)	-0.216 (-0.357, -0.074)	-0.251 (-0.359, -0.144)	-0.0357 (-0.198, 0.127)	0.72
TNFR1 (ng/L)	-36.2 (-157, 84.4)	51.4 (-42.1, 145)	87.6 (-49.9, 225)	0.29
β2-microglobulin (mg/L)	-0.0476 (-0.225, 0.129)	-0.0665 (-0.208, 0.0746)	-0.0189 (-0.226, 0.188)	0.88
Cystatin C (mg/L)	-0.0703 (-0.128, -0.0125)	-0.0231 (-0.0696, 0.0234)	-0.0472 (-0.0209, 0.115)	0.25
KIM-1 (µg/L)	-0.715 (-1.03, -0.404)	-0.307 (-0.552, -0.0622)	0.408 (0.0407, 0.776)	0.07
NGAL (µg/L)	-8.55 (-24.2, 7.11)	0.603 (-11.8, 13.1)	9.16 (-9.32, 27.6)	0.41
Endothelin-1 (ng/L)	-0.0667 (-0.212, 0.0785)	0.0863 (-0.0268, 0.200)	0.153 (-0.0159, 0.322)	0.14
ICAM-1 (ng/mL)	-0.0364 (-0.0841, 0.0112)	0.0183 (-0.0194, 0.0559)	0.0547 (-0.0018, 0.111)	0.11
VCAM-1 (ng/mL)	-0.0528 (-0.112, 0.007)	-0.0626 (-0.109, -0.0159)	-0.0098 (-0.0796, 0.0600)	0.82

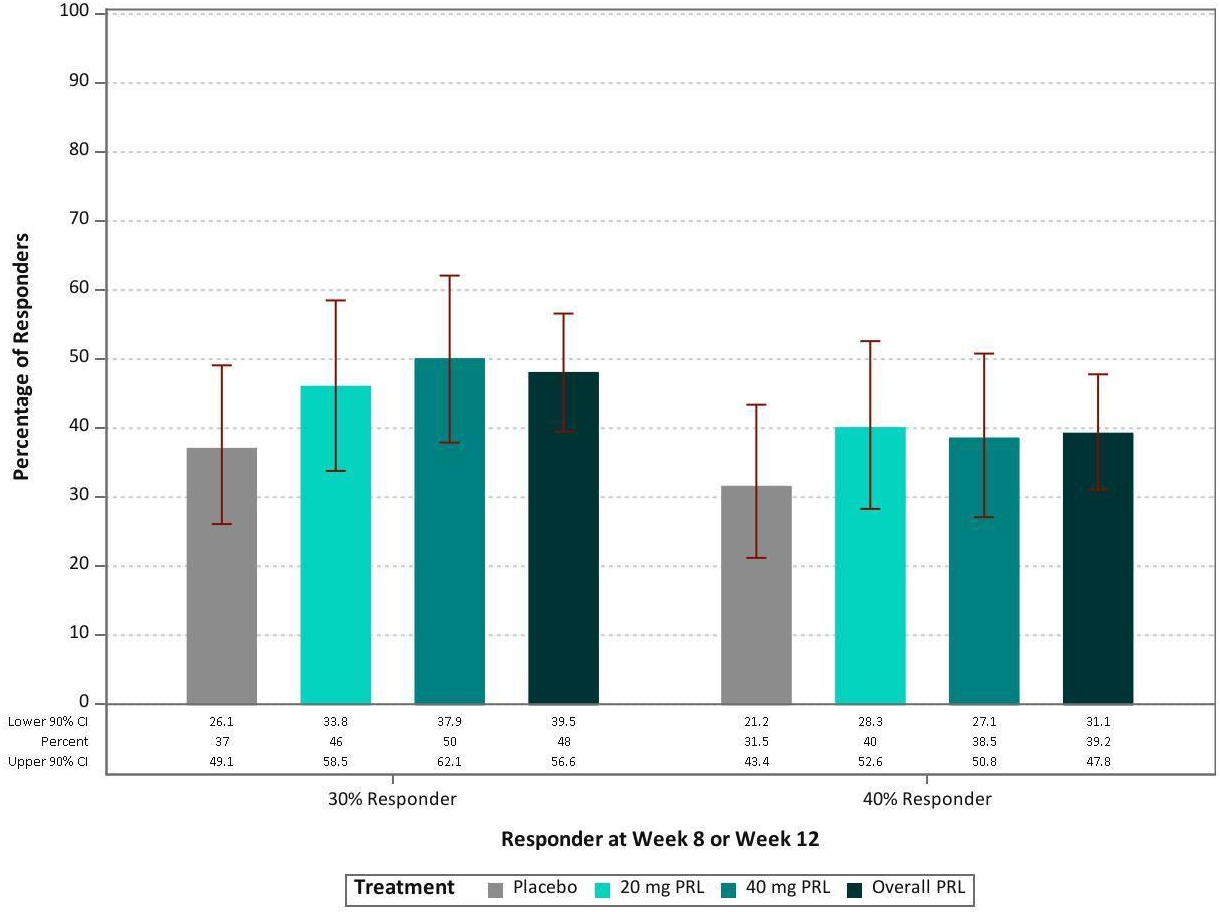
Note: ITT population. ANCOVA least square mean change from baseline to week 12 (90% CI)

CI=confidence interval; ADMA=asymmetric dimethylarginine; SDMA=symmetric dimethylarginine; FGF23=Fibroblast Growth Factor 23; TGFβ1=transforming growth factor beta 1; MCP-1=monocyte chemotactic protein 1; SAA=serum amyloid A; TNFα=tumor necrosis factor alpha; TNFR1=tumor necrosis factor receptor 1; KIM-1=kidney injury molecule-1; NGAL=neutrophil gelatinase-associated lipocalin; ICAM-1=intercellular adhesion molecule 1; VCAM-1=vascular cell adhesion molecule 1.

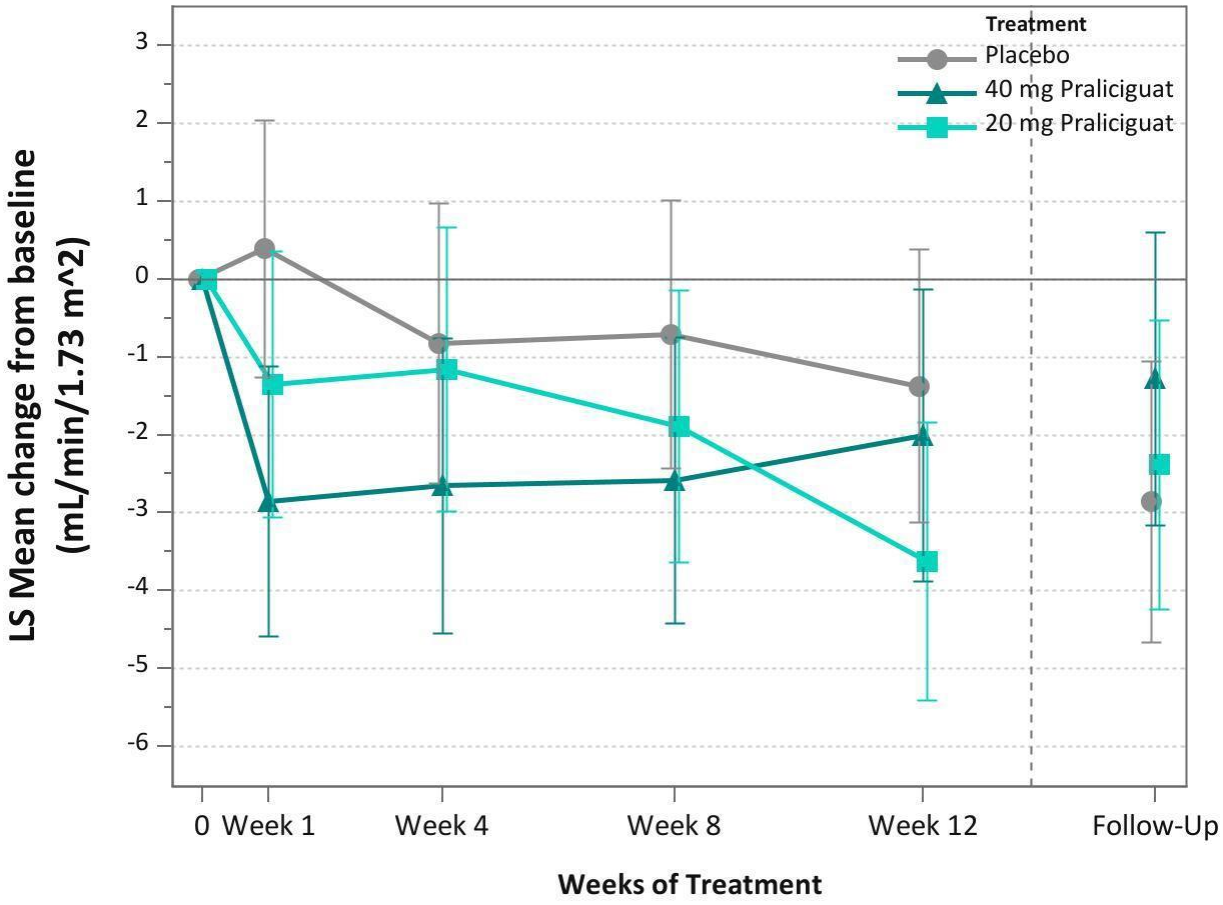
Supplemental Figure 1. Least square mean (90% CI) change from baseline in UACR over 12 weeks for ITT population excluding one site with inconsistent data.



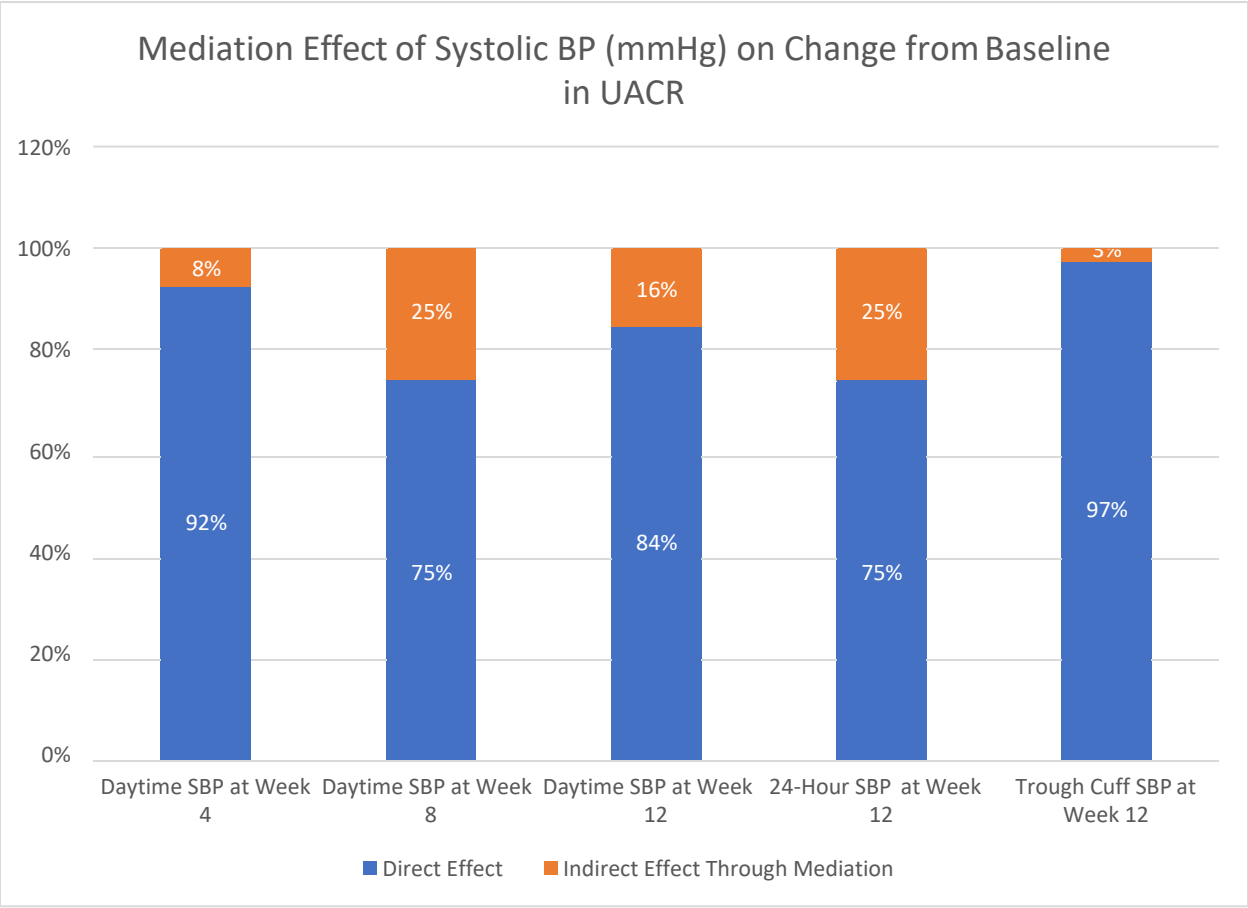
Supplemental Figure 2. Proportion of 30% and 40% UACR responders at Week 8 or 12.



Supplemental Figure 3. Least square mean (90% CI) change from baseline in eGFR.



Supplemental Figure 4. Results of mediation analysis.



Supplemental Figure 5: Praliciguat trough concentrations in plasma: C_{trough} (ng/mL) median (90% CI) over 12 Weeks (PK Population)