Phosphodiesterase-5 inhibition reduces albuminuria in subjects with overt diabetic nephropathy

SUPPLEMENTAL INFORMATION

Subjects with T2DM and overt nephropathy were selected for study participation if he or she met the following criteria:

Inclusion Criteria:

•Male or female subjects greater than or equal to 18 years. Female subjects must be of non-child bearing potential.

•Clinical diagnosis of type 2 diabetes together with stages 3a, 3b or 4 CKD, based on an eGFR of 25-59 mL/min/1.73m^2.

•Evidence of persistent, overt albuminuria; defined as a UACR greater than or equal to 300 mg/g (greater than or equal to 33.9 mg/mmol) for greater than 3 months.

Exclusion Criteria:

•Subjects with CKD resulting from type 1 diabetes or non-diabetic CKD.

•Subjects with poorly controlled diabetes mellitus, defined as HbA_{1C} >9%.

•Subjects on combination ACE inhibitor/ARB therapy

Assessment of UACR

Analysis of UACR in the PF-00489791 treatment group compared to placebo was conducted within a Bayesian framework; the informative prior for the placebo response (on the natural log

scale) was a normal distribution with a mean of -0.037 and a standard deviation of 0.08. This corresponds to a median Week 12 / baseline UACR ratio of exp (-0.037) = 0.964 (3.6% UACR reduction). As may be seen in Supplemental Figure 1, the resultant posterior distribution of UACR for placebo subjects (on the natural log scale) upon which the primary analysis is based had a normal distribution with mean 0.004 and a standard deviation of 0.056; corresponding to a median Week 12 / baseline UACR ratio of exp (0.004) = 1.004 (0.4% UACR increase) .

Consistent with the albuminuria-lowering effect of PF-00489791, a significant reduction in mean UPCR in the PF-00489791 treatment group compared to placebo was observed from Week 3 through Week 12 (MMRM analysis; -14%, p=0.03 at Week 3; -15%, p=0.03 at Week 6; -21%, p=0.007 at Week 12). The difference between PF-00489791 treatment and placebo group was no longer significant 4 weeks following the end of treatment (Week 16, -14%, p=0.12).

There was no meaningful difference in plasma glycosylated hemoglobin levels observed at Week 0 compared to the baseline for both PF-00489791 treatment and placebo group. However, as seen in supplemental table 1, at the end of the treatment period at Week 12, a mean decrease of 0.28% was observed in the treatment group compared to an increase of 0.12% in the placebo group.

As may be seen in Supplemental Table 2, there were 2 (3%) and 29 (15%) subjects from the placebo and PF-00489791 treatment groups, respectively, that discontinued from the study. In PF-00489791 treatment group, there were no major differences observed in demographic or baseline characteristics between those subjects that remained in the study compared to subjects that discontinued.

As may be seen in Supplemental Table 3, in the PF-00489791 treatment group overall, the most common treatment-related TEAE reported was headache (9 subjects, 4.7%); the most common TEAEs, all causality reported were diarrhea (17 subjects, 8.9%) and dyspepsia, peripheral edema and headache (each respectively in 12 subjects, 6.3%). In the placebo group, the most common all causality and treatment-related TEAE reported was headache (6 subjects, 9.4%; 5 subjects 7.8%, respectively).

Statistical Decision Rules

A 2-part decision criterion for efficacy and futility for this study was used at the end of the study. The pre-defined decision criteria, based on a Bayesian interpretation of the results assuming an informative prior for placebo response and non-informative priors for the treatment difference and variation, were as follows:

Criterion 1. At least 90% sure that PF-00489791 has a reduction (ie, greater than 0% reduction) in albumin:creatinine ratio compared to placebo.

Criterion 2. At least 67% sure that PF-00489791 has a greater than 20% reduction in albumin:creatinine ratio compared to placebo.

The sample size was chosen such that if the true effect of PF-00489791 was a 19% decrease (as compared to placebo) there would be an 80% probability of passing criterion 1.

At the end of the study, the posterior probability of a reduction in UACR with PF-00489791 compared to placebo was 0.99 (Criterion 1) and the posterior probability of greater than a 20% reduction in UACR with PF-00489791 compared to placebo was 0.24 (Criterion 2).

Supplemental Table 1. Summary of Plasma Glycosylated Hemoglobin (HbA_{1C})

	Placebo	**PF-00489791**
Randomized subjects with data (FAS) *	**61**	**187**
Mean Baseline HbA_{1C} (SD)	7.13 (1.02)	7.39 (1.14)
Mean change from Baseline at Week 12 (SD)	0.12 (0.86)	-0.28 (0.98)

* All subjects with data available were included in the analysis; at week 12, data from 56 placebo subjects and 157 subjects treated with PF- 00489791 were included in the calculation for mean change from Baseline.
SD=Standard deviations
Subjects were to have an HbA_{1c} < 9% at baseline defined as predose reading at Week 0.

In the per-protocol population, which included only those subjects that completed the study without major protocol deviations, the mean change from Baseline at Week 12 was 0.16 (n=44) for Placebo and -0.30 (n=133) for PF-00489791 treated subjects.

Supplemental Table 2. Demographic and Baseline Characteristics (All Randomized and Discontinued Subjects)[*]

	All Randomized		Discontinued	
Number of Subjects	**Placebo (N=64)**	**PF-00489791 (N=192)**	**Placebo (N=2)**	**PF-00489791 (N=28)[+]**
Female Gender	13 (20%)	48 (25%)	2	9 (32%)
Age (years)	59.8 (11)	62.0 (9)	65	61.1 (8)
Race (n)				
White	27 (42%)	79 (41%)	1	10 (36%)
Black	5 (8%)	13 (7%)	1	2 (7%)
Asian	26 (41%)	83 (43%)		12 (43%)
Other	6 (9%)	17 (9%)		4 (14%)
Weight (kg)	84.3 (22)	83.8 (20))	90.5	86.9 (18)
Body mass index (kg/m^2)	30.3 (7)	29.9 (6)	33.4	30.9 (6)
Height (cm)	166.1 (8)	167.1 (10)	164.5	167.6 (10)
Median T2DM duration since first diagnoses (yrs)	12.2	14.1	22.5	15.7
UACR (mg/mmol)	132.4	127.6	140.3	123.3
eGFR (ml/min/1.73m^2)	38.6 (12)	37.7 (10)	33.7	39.1 (12)
CKD Stage[†](n)				
Stage 2 (eGFR 60 - < 90 ml/min/1.73m^2)	3 (5 %)	2 (1%)		1 (4%)
Stage 3a (eGFR 45-<60 ml/min/1.73m^2)	12 (20%)	48 (26%)		7 (25%)
Stage 3b (eGFR 30-<45 ml/min/1.73m^2)	33 (54%)	94 (50%)	1	14 (50%)
Stage 4 (eGFR 15-<30 ml/min/1.73m^2)	13 (21%)	44 (23%)	1	6 (21%)
Supine SBP (mmHg)	138.2 (9)	135.9 (10)	135.7	134.9 (10)

[*]With the exception of gender and race, shown as number and percent study population, data are presented as Mean (SD) unless otherwise noted. BMI was defined as weight/ (height × 0.01)^2. UACR presented as Geometric Mean.
[†]Some subjects had baseline eGFR outside of the screening criteria due to differences in serum creatinine measured between screening and baseline. Seven subjects had missing eGFR values at baseline (3 subjects on placebo and 4 subjects on PF 00489791).
[+]Subject 10211022 was discontinued from the study treatment due to creatine kinase elevation, but continued to complete the study for data collection, and thus this subject is not included in this table

Supplemental Table 3. Incidence of all TEAEs, all causality and treatment-related

Number (%) of Subjects with Treatment-Emergent Adverse Events by: System Organ Class and MedDRA (v16.0) preferred term	Placebo (n=64) N (%) (All Causality)	PF-00489791 20 mg (n=192) N (%) (All Causality)	Placebo (n=64) N (%) (Treatment Related)	PF-00489791 20 mg (n=192) N (%) (Treatment Related)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	1 (1.6)	10 (5.2)		
Anaemia	1 (1.6)	7 (3.6)		
Haemorrhagic anaemia	0	1 (0.5)		
Iron deficiency anaemia	0	2 (1.0		
Neutrophilia	0	1 (0.5)		
CARDIAC DISORDERS	2 (3.1)	7 (3.6)		
Acute myocardial infarction	1 (1.6)			
Cardiac failure	1 (1.6)	2 (1.0)		
Cardiac failure congestive	0	2 (1.0)		
Coronary artery disease	1 (1.6)	2 (1.0)		
Mitral valve incompetence	0	1 (0.5)		
Palpitations	0	2 (1.0)		
EAR AND LABYRINTH DISORDERS	0	1 (0.5)		
Ear haemorrhage	0	1 (0.5)		
ENDOCRINE DISORDERS	0	1 (0.5)		
Autoimmune thyroiditis	0	1 (0.5)		
EYE DISORDERS	0	8 (4.2)	0	3 (1.6)
Eye irritation			0	1 (0.5)
Conjunctivitis	0	1 (0.5)		
Diabetic retinopathy	0	1 (0.5)		
Lacrimation increased			0	1 (0.5)
Visual acuity reduced			0	1 (0.5)
Visual impairment				
GASTROINTESTINAL DISORDERS	8 (12.5)	46 (24.0)	1 (1.6)	20 (10.4)
Constipation	2 (3.1)	2 (1.0)	0	1 (0.5)
Diarrhoea	2 (3.1)	17 (8.9)	0	7 (3.6)
Dyspepsia	1 (1.6)	12 (6.3)	1 (1.6)	7 (3.6)
Gastritis	0	3 (1.6)	0	1 (0.5)
Gastrooesophageal reflux disease	0	4 (2.1)	0	3 (1.6)
Nausea	2 (3.1)	4 (2.1)	0	3 (1.6)
Vomiting	2 (3.1)	7 (3.6)	0	3 (1.6)
Abdominal discomfort	1 (1.6)	0		
Abdominal distension	0	1 (0.5)		
Colitis	1 (1.6)	0		
Dry mouth	1 (1.6)	0		
Flatulence	0	1 (0.5)		

Number (%) of Subjects with Treatment-Emergent Adverse Events by: System Organ Class and MedDRA (v16.0) preferred term	Placebo (n=64) N (%) (All Causality)	PF-00489791 20 mg (n=192) N (%) (All Causality)	Placebo (n=64) N (%) (Treatment Related)	PF-00489791 20 mg (n=192) N (%) (Treatment Related)
Frequent bowel movements	0	1 (0.5)		
Gastritis	0	3 (1.6)		
Gastritis erosive	0	1 (0.5)		
Gastroduodenitis	0	1 (0.5)		
Gastrointestinal haemorrhage	1 (1.6)	1 (0.5)		
Irritable bowel syndrome	0	1 (0.5)		
Large intestine polyp	0	1 (0.5)		
Melaena	0	1 (0.5)		
Nausea	2 (3.1)	4 (2.1)		
Oesophageal ulcer	0	1 (0.5)		
Oesophagitis	0	1 (0.5)		
Pancreatitis	0	1 (0.5)		
Vomiting	2 (3.1)	7 (3.6)		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	8 (12.5)	26 (13.5)	1 (1.6)	8 (4.2)
Chest discomfort	0	2 (1.0)	0	2 (1.0)
Chest pain	0	2 (1.0)	0	2 (1.0)
Feeling hot	0	1 (0.5)	0	1 (0.5)
Oedema	1 (1.6)	3 (1.6)	0	1 (0.5)
Oedema peripheral	6 (9.4)	12 (6.3)	1 (1.6)	1 (0.5)
Pyrexia	0	4 (2.1)	0	1 (0.5)
Chills	0	1 (0.5)		
Face oedema	0	1 (0.5)		
Fatigue	1 (1.6)	2 (1.0)		
IMMUNE SYSTEM DISORDERS	0	2 (1.0)		
Drug hypersensitivity	0	1 (0.5)		
Hypersensitivity	0	1 (0.5)		
INFECTIONS AND INFESTATIONS	8 (12.5)	28 (14.6)	0	2 (1.0)
Abscess limb	0	1 (0.5)		
Bronchitis	2 (3.1)	1 (0.5)		
Cellulitis	1 (1.6)	1 (0.5)		
Eye infection bacterial	0	1 (0.5)		
Gastroenteritis	0	1 (0.5)	0	1 (0.5)
Influenza	0	3 (1.6)		
Laryngitis	1 (1.6)	0		
Localised infection	0	1 (0.5)		
Lower respiratory tract infection	0	1 (0.5)		
Gastroenteritis			0	1 (0.5)
Nasopharyngitis	1 (1.6)	9 (4.7)	0	1 (0.5)
Mastitis fungal	0	1 (0.5)		

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Onychomycosis	1 (1.6)	0		
Pharyngitis	0	2 (1.0)		
Pneumonia	0	2 (1.0)		
Respiratory tract infection	0	1 (0.5)		
Rhinitis	0	1 (0.5)		
Sinusitis	0	1 (0.5)		
Tooth infection	1 (1.6)	0		
Upper respiratory tract infection	1 (1.6)	6 (3.1)		
Urinary tract infection	1 (1.6)	0		
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	4 (6.3)	5 (2.6)	1 (1.6)	0
Contusion	1 (1.6)	0		
Excoriation	1 (1.6)	1 (0.5)		
Fall	0	1 (0.5)		
Humerus fracture	0	1 (0.5)		
Laceration	0	1 (0.5)		
Ligament sprain	0	1 (0.5)		
Lip injury	1 (1.6)	0	1 (1.6)	0
Procedural hypotension	1 (1.6)	0		
Procedural pain	1 (1.6)	0		
Spinal compression fracture	0	1 (0.5)		
INVESTIGATIONS	11 (17.2)	23 (12.0)	1 (1.6)	5 (2.6)
Amylase increased	1 (1.6)	1 (0.5)	0	1 (0.5)
Blood calcium decreased	0	1 (0.5)		
Blood creatinine increased	1 (1.6)	1 (0.5)		
Blood creatine phosphokinase increased	3 (4.7)	6 (3.1)	1 (1.6)	1 (0.5)
Blood glucose abnormal	1 (1.6)	0		
Blood glucose increased	1 (1.6)	1 (0.5)		
Blood lactate dehydrogenase increased	1 (1.6)	0		
Blood potassium increased	0	3 (1.6)		
Blood pressure abnormal	0	1 (0.5)		
Blood pressure increased	0	1 (0.5)		
Blood urea increased	0	2 (1.0)	0	1 (0.5)
Blood uric acid increased	2 (3.1)	0		
Electrocardiogram ST segment depression	0	1 (0.5)		
Gamma-glutamyltransferase increased	1 (1.6)	0		
Glycosylated haemoglobin increased	0	1 (0.5)		
Haemoglobin decreased	1 (1.6)	0		
International normalised ratio increased	0	1 (0.5)		

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Lipase increased	0	2 (1.0)	0	1 (0.5)
Liver function test normal	0	1 (0.5)	0	1 (0.5)
Weight decreased	0	1 (0.5)		
Weight increased	0	1 (0.5)		
METABOLISM AND NUTRITION DISORDERS	5 (7.8)	13 (6.8)		
Decreased appetite	0	1 (0.5)		
Gout	0	2 (1.0)		
Hyperamylasaemia	1 (1.6)	0		
Hyperglycaemia	1 (1.6)	4 (2.1)		
Hyperkalaemia	1 (1.6)	2 (1.0)		
Hyperuricaemia	1 (1.6)	3 (1.6)		
Hypoglycaemia	2 (3.1)	1 (0.5)		
Hyponatraemia	1 (1.6)	0		
Vitamin D deficiency	0	1 (0.5)		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	7 (10.9)	19 (9.9)	1 (1.6)	10 (5.2)
Arthralgia	1 (1.6)	3 (1.6)	0	2 (1.0)
Arthritis	0	1 (0.5)		
Back pain	2 (3.1)	5 (2.6)	0	3 (1.6)
Gouty arthritis	1 (1.6)	0		
Joint swelling	0	2 (1.0)	0	1 (0.5)
Muscle spasms	2 (3.1)	1 (0.5)	1 (1.6)	1 (0.5)
Musculoskeletal pain	1 (1.6)	0		
Myalgia	0	2 (1.0)	0	1 (0.5)
Neck pain	0	1 (0.5)	0	1 (0.5)
Osteoarthritis	0	1 (0.5)		
Osteopenia	1 (1.6)	0		
Pain in extremity	0	3 (1.6)	0	1 (0.5)
Spinal osteoarthritis	0	1 (0.5)		
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)	1 (1.6)	0		
Monoclonal gammopathy	1 (1.6)	0		
NERVOUS SYSTEM DISORDERS	9 (14.1)	26 (13.5)	6 (9.4)	16 (8.3)
Balance disorder	0	1 (0.5)	0	1 (0.5)
Cerebrovascular accident	0	2 (1.0)	0	1 (0.5)
Diabetic neuropathy	0	1 (0.5)		
Dizziness	1 (1.6)	7 (3.6)	0	5 (2.6)
Drooling	0	1 (0.5)		
Dysarthria	0	1 (0.5)		

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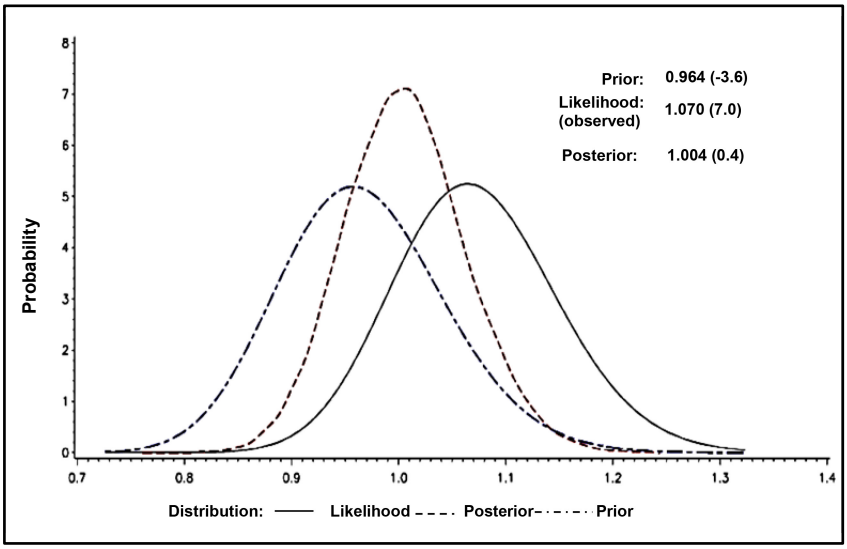
Headache	6 (9.4)	12 (6.3)	5 (7.8)	9 (4.7)
Hemiparesis	0	1 (0.5)		
Memory impairment	1 (1.6)	0	1 (1.6)	0
Paraesthesia	0	1 (0.5)		
Restless legs syndrome	0	1 (0.5)		
Somnolence	0	2 (1.0)		
Tremor	1 (1.6)	1 (0.5)	0	1 (0.5)
PSYCHIATRIC DISORDERS	1 (1.6)	0	1 (1.6)	0
Nervousness	1 (1.6)	0	1 (1.6)	0
RENAL AND URINARY DISORDERS	5 (7.8)	6 (3.1)	1 (1.6)	1 (0.5)
Nocturia	1 (1.6)	0		
Pollakiuria	0	2 (1.6)	0	1 (0.5)
Polyuria	1 (1.6)	0	1 (1.6)	0
Proteinuria	0	1 (0.5)		
Renal cyst	0	1 (0.5)		
Renal failure	0	1 (0.5)		
Renal failure acute	1 (1.6)	1 (0.5)		
Renal impairment	1 (1.6)	0		
Urinary incontinence	1 (1.6)	0		
Urinary retention	0	1 (0.5)		
REPRODUCTIVE SYSTEM AND BREAST DISORDERS	1 (1.6)	2 (1.0)	0	2 (1.0)
Prostatitis	1 (1.6)	0		
Spontaneous penile erection	0	2 (1.0)	0	2 (1.0)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	2 (3.1)	8 (4.2)	0	2 (1.0)
Chronic obstructive pulmonary disease	0	1 (0.5)		
Cough	1 (1.6)	0		
Dyspnoea	0	1 (0.5)	0	1 (0.5)
Dyspnoea exertional	0	1 (0.5)		
Epistaxis	0	1 (0.5)		
Nasal congestion	0	2 (1.0)	0	1 (0.5)
Pulmonary embolism	0	1 (0.5)		
Pulmonary oedema	0	1 (0.5)		
Respiratory failure	1 (1.6)	0		
Sinus congestion	0	1 (0.5)		

Number (%) of Subjects with Treatment-Emergent Adverse Events by: System Organ Class and MedDRA (v16.0) preferred term	Placebo (n=64) N (%) (All Causality)	PF-00489791 20 mg (n=192) N (%) (All Causality)	Placebo (n=64) N (%) (Treatment Related)	PF-00489791 20 mg (n=192) N (%) (Treatment Related)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	3 (4.7)	9 (4.7)	1 (1.6)	1 (0.5)
Alopecia	0	1 (0.5)		
Dry skin	2 (3.1)	0		
Hyperhidrosis	0	1 (0.5)	0	1 (0.5)
Neurodermatitis	0	1 (0.5)		
Neuropathic ulcer	0	1 (0.5)		
Night sweats	0	1 (0.5)		
Pruritus	0	3 (1.6)		
Pruritus generalised	0	1 (0.5)		
Rash	1 (1.6)	0	1 (1.6)	0
Rash pruritic	0	1 (0.5)		
SOCIAL CIRCUMSTANCES	0	1 (0.5)		
Immobile	0	1 (0.5)		
SURGICAL AND MEDICAL PROCEDURES	1 (1.6)	0		
Prostatectomy	1 (1.6)	0		
VASCULAR DISORDERS	5 (7.8)	14 (7.3)	2 (3.1)	2 (1.0)
Accelerated hypertension	0	1 (0.5)		
Aortic aneurysm	0	1 (0.5)		
Flushing	0	1 (0.5)	0	1 (0.5)
Hot flush	0	1 (0.5)	0	1 (0.5)
Hypertension	2 (3.1)	9 (4.7)		
Hypotension	2 (3.1)	0	2 (3.1)	0
Orthostatic hypotension	0	1 (0.5)		
Venous insufficiency	1 (1.6)	0		
Total preferred term events	**94**	**294**	**16**	**78**

**** If the same subject in a given treatment had more than one occurrence in the same preferred term category, only the most severe occurrence is presented. Subjects are counted only once per treatment in each row.***

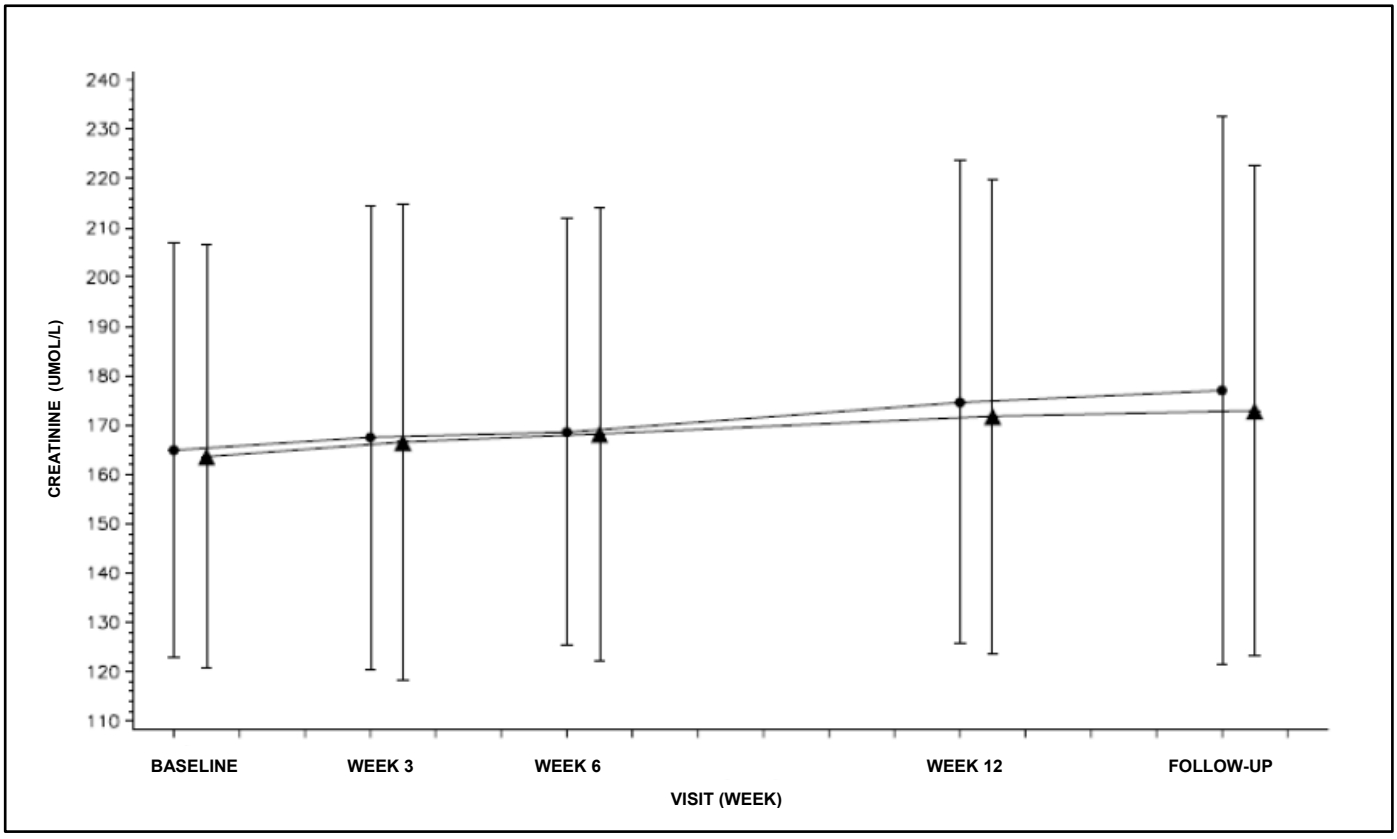
SUPPLEMENTAL FIGURE 1



Supplemental Figure 1 displays ratio change from baseline on the x-axis. A value of one indicates no change from baseline, positive values indicate increases from baseline and negative values indicate decreases from baseline. For each curve the first of each pair of values in the legend represents the geometric mean and the second number represents the percentage change from baseline. The curve labelled “prior” represents the informative prior on the placebo geometric mean response, which has a geometric mean of 0.964 corresponding to a 3.6% reduction from baseline. The curve labelled “likelihood” shows the range of geometric mean values consistent with the placebo data (ignoring prior belief) and has a geometric mean of 1.07 corresponding to a 7% increase from baseline. The curve labeled “posterior” represents the range of values for the placebo geometric mean response from the Bayesian analysis (i.e. utilizing both the prior and the data). This resultant posterior distribution of UACR for placebo subjects upon which the primary analysis is has a geometric mean ratio of 1.004 corresponding

to an increase of 0.4% from baseline. The mean effect for the treatment group was a 0.846 relative change from baseline. In the Bayesian analysis, the mean UACR response from the posterior distribution for placebo (1.004) shifts this result slightly to 0.843.

SUPPLEMENTAL FIGURE 2



Supplemental Figure 2. Plot of serum creatinine observed mean (± standard deviation) over time for PF-00489791 (▲) compared to Placebo (●).