

# Kidney Outcomes in Long-Term Studies of Ruboxistaurin for Diabetic Eye Disease

Katherine R. Tuttle,\* Janet B. McGill,<sup>†</sup> Douglas J. Haney,<sup>‡</sup> Toni E. Lin,<sup>‡</sup>  
Pamela W. Anderson;<sup>‡</sup> for the PKC-DRS, PKC-DMES, and PKC-DRS 2 Study Groups

\*Providence Medical Research Center and University of Washington School of Medicine, Spokane, Washington;

<sup>†</sup>Department of Medicine, Washington University, St. Louis, Missouri; and <sup>‡</sup>Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana

**Background:** A pilot study showed that ruboxistaurin (RBX), a protein kinase C  $\beta$  inhibitor, significantly decreased albuminuria and stabilized kidney function over 1 yr in patients who had diabetic nephropathy and persistent macroalbuminuria despite receiving the current standard of care, including renin-angiotensin system inhibition. In contrast, in a trial of patients with diabetic retinopathy, investigators reported the adverse event “diabetic nephropathy” more frequently in patients who received RBX.

**Design, setting, participants, and measurements:** The purpose of this study was to evaluate long-term effects of RBX on kidney outcomes among patients with diabetic eye disease in three diabetic retinopathy trials ( $n = 1157$ ). Baseline-to-study end changes in estimated GFR (eGFR) were calculated. Kidney outcomes included doubling of serum creatinine, development of advanced chronic kidney disease (stages 4 to 5), and death.

**Results:** Baseline eGFR was  $81.6 \pm 26.0$  ml/min per  $1.73 \text{ m}^2$ . In the combined placebo and RBX treatment groups, eGFR decreased by  $11.0 \pm 19.6$  ml/min per  $1.73 \text{ m}^2$  during median follow-up of 33 to 39 mo. At least one kidney outcome occurred in 11.3% of patients. Frequency of doubling of serum creatinine was 6.0%, progression to advanced chronic kidney disease was 4.1%, and death was 4.1%. Kidney outcome rates did not differ by treatment assignment.

**Conclusions:** Long-term kidney outcomes in patients with diabetic eye disease were similar in placebo and RBX groups. In conclusion, large-scale, prospective trials in patients with diabetic nephropathy are needed to confirm safety and potential benefits of RBX on clinical outcomes.

*Clin J Am Soc Nephrol* 2: 631-636, 2007. doi: 10.2215/CJN.00840207

In patients with diabetes, eye disease and kidney disease frequently coexist. Diabetic retinopathy develops in 75% of those who have had diabetes for at least 15 yr and is the most common cause of adult-onset blindness in developed nations (1,2). Blindness can also be attributed to macular edema, which can arise at any stage of diabetic retinopathy (3–5). Risk for nephropathy is high in these patients. The probability of developing diabetic nephropathy within 12 yr of the diagnosis of diabetic retinopathy is 75% (6). Diabetic nephropathy remains the leading cause of ESRD in the United States and the developed world despite widespread use of approved therapies (7,8). Therefore, novel therapies that target mechanisms of progression other than glycemic control, reduction of BP, and renin-angiotensin system inhibition are needed.

Protein kinase C (PKC) inhibition has been proposed as a treatment strategy for diabetic retinopathy and nephropathy. Hyperglycemia-stimulated activation of PKC contributes to the development of diabetic retinopathy and nephropathy *via* over-

activation of intracellular signaling pathways, leading to vascular injury (9–11). In particular, the  $\beta$  isoform of PKC has been implicated in the pathogenesis of both of these diabetic microvascular complications (12–14). Ruboxistaurin (RBX) mesylate is an oral PKC- $\beta$  inhibitor that prevents kidney disease in animal models of diabetes (13,14). In patients who had diabetic nephropathy and persistent macroalbuminuria despite receiving the current standard of care, including renin-angiotensin system inhibition, albuminuria decreased by  $24 \pm 9\%$  and estimated GFR (eGFR) did not decline after 1 yr of treatment with 32 mg/d RBX (15).

In a 36-mo study of patients with moderate to severe diabetic retinopathy—the PKC Diabetic Retinopathy Study 2 (PKC-DRS2)—RBX reduced the risk for experiencing sustained moderate vision loss by approximately 40% (16). However, investigators reported the adverse event of “diabetic nephropathy” more frequently in RBX-treated patients than in those who received placebo ( $n = 7$  [2%] *versus*  $n = 0$ ;  $P = 0.015$ ) in the PKC-DRS2 trial. This finding was investigated further because of concerns about subjectivity in adverse event reporting, the small number of such reports, and evidence for favorable effects on the kidney in patients with diabetic nephropathy and in animal models. Furthermore, safety is a primary concern in development of novel therapies for diabetic nephropathy or

Received February 16, 2007. Accepted April 24, 2007.

Published online ahead of print. Publication date available at [www.cjasn.org](http://www.cjasn.org).

**Address correspondence to:** Dr. Katherine R. Tuttle, 122 W. 7th Avenue, Suite 230, Spokane, WA 99204-2340. Phone: 509-474-4345; Fax: 509-474-4325; E-mail: [ktuttle@this.org](mailto:ktuttle@this.org)

other diseases. The purpose of this study was to evaluate effects of RBX on kidney outcomes using objective, quantifiable measurements from the large, long-term trials in diabetic retinopathy (16–18).

## Concise Methods

### Study Design

The PKC-Diabetic Retinopathy Study (PKC-DRS), PKC-Diabetic Macular Edema Study (PKC-DMES), and PKC-DRS2 were multicenter, randomized, double-blind, placebo-controlled, phase 3 trials of RBX. Study designs and primary results have been published (16–18). In PKC-DRS and PKC-DRS2, patients had either type 1 or type 2 diabetes, moderate to severe nonproliferative diabetic retinopathy, and best corrected visual acuity of 20/125 or better with no previous panretinal photocoagulation in at least one eye. For the PKC-DMES, patients were required to have type 1 or type 2 diabetes, mild to moderate nonproliferative diabetic retinopathy, best corrected visual acuity of 20/32 or better, and diabetic macular edema not involving the center of the macula with no previous panretinal or focal/grid photocoagulation in at least one eye.

The PKC-DRS was a dose-ranging trial in which 252 patients were randomly assigned to one of four study arms (placebo [ $n = 61$ ], 8 mg/d RBX [ $n = 60$ ], 16 mg/d RBX [ $n = 64$ ], or 32 mg/d RBX [ $n = 67$ ]) and treated for 36 mo (17). Primary end points were progression of diabetic retinopathy or need for panretinal photocoagulation. The PKC-DMES evaluated effects of treatment with three dosages of RBX for 30 mo on progression of macular edema and need for photocoagulation in 686 patients who were followed up to 52 mo (placebo [ $n = 176$ ], 4 mg/d RBX [ $n = 168$ ], 16 mg/d RBX [ $n = 174$ ], or 32 mg/d RBX [ $n = 168$ ]) (18). Patients were excluded from these trials when serum creatinine at screening was  $>2.5$  mg/dl (221  $\mu$ mol/L). The PKC-DRS2 was a later trial that randomly assigned 685 patients to two study arms (placebo [ $n = 340$ ] or 32 mg/d RBX [ $n = 345$ ]) for a 36-mo treatment period (16). PKC-DRS2 entry criteria were similar to the other studies except that serum creatinine level was not an exclusion criterion. The primary end point was the development of sustained moderate visual loss (16).

BP, weight, serum creatinine, serum albumin, and glycosylated hemoglobin were measured during the screening phase, at randomization, and at each study visit. PKC-DRS and PKC-DMES included measurement of baseline urine protein by dipstick (17,18). In PKC-DRS2, urine albumin-to-creatinine ratio (ACR) was measured only at study end (36 mo) (16). Laboratory analyses were performed in a central laboratory (Covance, Indianapolis, IN). In all three diabetic retinopathy studies, serum creatinine was measured by the same assay, the modified Jaffe reaction, using Roche Modular Analyzers (Roche Diagnostics, Indianapolis, IN).

eGFR was calculated using the re-expressed, four-component Modification of Diet in Renal Disease (MDRD) equation for standardized creatinine assays (19):

$$\text{eGFR (ml/min per } 1.73 \text{ m}^2) = (175)(\text{serum creatinine})^{-1.154} (\text{age})^{-0.203} (0.742 \text{ if female})(1.212 \text{ if black})$$

Creatinine clearance was calculated with the Cockcroft-Gault equation (20):

$$\text{Cl}_{\text{Cr}} \text{ (ml/min)} = \frac{(1.23)(140 - \text{age})(\text{weight (kg)})(0.85 \text{ if female})}{S_{\text{Cr}}} \quad (1)$$

Kidney outcomes were defined as (1) doubling of serum creatinine, (2) progression to advanced chronic kidney disease (CKD; stages 4 to 5: eGFR  $\leq 30$  ml/min per 1.73 m<sup>2</sup>), and (3) death. Advanced CKD was used instead of ESRD because these events were rare in patients who were selected for the diabetic retinopathy trials.

All three clinical trials received approval from the institutional review boards of their respective sites and strictly adhered to the ethical principles of the Declaration of Helsinki and the guidelines on good clinical practice (16–18). Written informed consent was obtained from all patients.

### Statistical Analyses

Analyses were confined to patients who received placebo or 32 mg/d RBX ( $n = 1157$ ) because there were insufficient data to analyze effects of RBX on kidney outcomes at each of the lower dosages. In addition, an increased rate of the investigator-ascribed adverse event diabetic nephropathy was reported only in the PKC-DRS2 trial, in which the RBX dosage was 32 mg/d. Data were analyzed according to the intention-to-treat principle. The last-observation-carried-forward method was used to account for any missing end-point values. Baseline patient characteristics were compared across treatment groups using  $\chi^2$  tests or ANOVA. Spearman rank correlations were used to assess the relationships between eGFR and baseline variables. Analysis of adverse event data were performed using  $\chi^2$  or Fisher exact test. Continuous safety parameters were analyzed using ANOVA. All analyses were conducted using the statistical package SAS (version 8; SAS Institute, Cary, NC).  $P < 0.05$  was considered to be statistically significant. Two-sided significance tests were used for all analyses. Continuous data are expressed as means  $\pm$  SD.

## Results

### Patient Characteristics

In the combined data from the three trials, 577 patients received placebo and 580 received 32 mg/d RBX. In the RBX groups, 28% of patients discontinued treatment during the trials, compared with 26% in the placebo groups ( $P = 0.59$ ). Study participants were predominantly men (64%) with type 2 diabetes (86%; Table 1). The mean duration of diabetes was  $16 \pm 8$  yr, and the mean body mass index was  $32.1 \pm 7.1$  kg/m<sup>2</sup>. Angiotensin-converting enzyme inhibitors were used in 50% of patients, and 12% were taking an angiotensin receptor blocker; these two medicines were used concurrently in 2% of patients. Overall, baseline characteristics and the use of concomitant medicines were similar in placebo and RBX groups. The only borderline statistically significant difference between groups at baseline was a slightly higher systolic BP in those who were treated with placebo compared with RBX ( $139 \pm 18$  versus  $137 \pm 18$  mmHg,  $P = 0.05$ ; Table 1). At end point, there was no difference between placebo- and RBX-treated groups in either systolic or diastolic BP ( $135 \pm 20/75 \pm 11$  and  $134 \pm 19/75 \pm 11$  mmHg;  $P = 0.54$  and  $P = 0.98$ , respectively).

### Kidney Function

Baseline eGFR by the MDRD equation ( $81.6 \pm 26.0$  ml/min per 1.73 m<sup>2</sup>) and calculated creatinine clearance by the Cockcroft-Gault formula ( $114.3 \pm 46.6$  ml/min) were similar between treatment groups in the combined data from the three clinical trials (Table 2). Neither baseline eGFR nor prevalence of CKD stage 3 or greater defined by eGFR  $\leq 60$  ml/min per 1.73 m<sup>2</sup> (19%) differed by treatment assignment. From baseline to end point, patients who received placebo lost a similar amount of eGFR compared with patients who were treated with RBX ( $11.3 \pm 19.4$  versus  $10.7 \pm 19.8$  ml/min per 1.73 m<sup>2</sup>;

Table 1. Baseline characteristics by assignment to placebo or RBX in long-term studies of diabetic eye disease<sup>a</sup>

Characteristic	Placebo	RBX 32 mg/d	Total	P
<i>n</i>	577	580		
Age (yr)	57.8 ± 11.0	57.7 ± 11.3	57.8 ± 11.2	0.87
Male ( <i>n</i> [%])	373 (65)	362 (63)	735 (64)	0.43
Type 2 diabetes ( <i>n</i> [%])	491 (85)	499 (86)	990 (86)	0.65
Duration of diabetes (yr)	16 ± 8	16 ± 8	16 ± 8	0.58
HbA <sub>1c</sub> (%)	8.4 ± 1.4	8.5 ± 1.5	8.4 ± 1.5	0.38
Non-smokers ( <i>n</i> [%])	525 (91)	526 (91)	1051 (91)	0.86
BMI (kg/m <sup>2</sup> )	32.2 ± 6.7	32.1 ± 7.4	32.1 ± 7.1	0.91
ACEI ( <i>n</i> [%])	286 (50)	288 (50)	574 (50)	0.98
ARB ( <i>n</i> [%])	69 (12)	70 (12)	139 (12)	0.97
ACEI and ARB ( <i>n</i> [%])	11 (2)	12 (2)	23 (2)	0.84
Systolic BP (mmHg)	139 ± 18	137 ± 18	138 ± 18	0.05
Diastolic BP (mmHg)	78 ± 10	78 ± 10	78 ± 10	0.93

<sup>a</sup>ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; HbA<sub>1c</sub>, glycosylated hemoglobin; RBX, ruboxistaurin.

Table 2. Kidney function by assignment to placebo or RBX in long-term studies of diabetic eye disease<sup>a</sup>

Measurement	Placebo		RBX 32 mg/d		Total		P
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
Baseline							
eGFR (ml/min per 1.73 m <sup>2</sup> )	576	82.4 ± 26.0	579	80.8 ± 26.0	1155	81.6 ± 26.0	0.30
calculated creatinine clearance (ml/min)	570	115.6 ± 46.4	575	113.0 ± 46.8	1145	114.3 ± 46.6	0.35
serum creatinine (mg/dl)	576	1.0 ± 0.3	579	1.0 ± 0.4	1155	1.0 ± 0.3	0.22
Study end (after 33 to 39 mo of treatment)							
eGFR (ml/min per 1.73 m <sup>2</sup> )	561	71.1 ± 26.8	557	70.4 ± 26.3	1118	70.8 ± 26.5	0.67
calculated creatinine clearance (ml/min)	555	101.4 ± 44.2	553	100.1 ± 45.0	1108	100.7 ± 44.6	0.63
serum creatinine (mg/dl)	561	1.2 ± 0.7	557	1.2 ± 0.8	1118	1.2 ± 0.7	0.56
Change (after 33 to 39 mo of treatment)							
eGFR (ml/min per 1.73 m <sup>2</sup> )	560	-11.3 ± 19.4	556	-10.7 ± 19.8	1116	-11.0 ± 19.6	0.61
calculated creatinine clearance (ml/min)	555	-14.2 ± 26.0	552	-13.6 ± 27.3	1107	-13.9 ± 26.6	0.69
serum creatinine (mg/dl)	560	0.2 ± 0.5	556	0.2 ± 0.6	1116	0.2 ± 0.6	0.90

<sup>a</sup>eGFR, estimated GFR; MDRD, Modification of Diet in Renal Disease. eGFR estimated by the MDRD formula. Creatinine clearance calculated by the Cockcroft-Gault formula.

$P = 0.61$ ; Table 2). The decrease in creatinine clearance as determined using the Cockcroft equation was also similar in placebo- and RBX-treated patients ( $14.2 \pm 25.9$  versus  $13.6 \pm 22.3$  ml/min;  $P = 0.69$ ; Table 2). Median follow-up time was 33 to 39 mo in PKC-DMES, PKC-DRS, and PKC-DRS2.

Mean decrease in eGFR for patients with type 1 diabetes ( $n = 162$ ) was 6.3 ml/min per 1.73 m<sup>2</sup>, whereas that for patients with type 2 diabetes ( $n = 954$ ) was 11.8 ml/min per 1.73 m<sup>2</sup> ( $P < 0.001$ ). Patients who reported alcohol usage ( $n = 376$ ) experienced a mean decrease in eGFR of 9.2 ml/min per 1.73 m<sup>2</sup> compared with 11.9 ml/min per 1.73 m<sup>2</sup> ( $P = 0.02$ ) in those who classified themselves as nondrinkers ( $n = 740$ ). Correlations between the following baseline variables and baseline-to-end point changes in eGFR in the combined clinical trials population were as follows: Duration of diabetes ( $r = 0.13$ ,  $n = 1115$ ,  $P < 0.001$ ), baseline systolic BP ( $r = -0.09$ ,  $n = 994$ ,  $P = 0.004$ ),

baseline diastolic BP ( $r = -0.08$ ,  $n = 994$ ,  $P = 0.009$ ), age ( $r = 0.05$ ,  $n = 1116$ ,  $P = 0.07$ ), baseline body mass index ( $r = -0.02$ ,  $n = 1107$ ,  $P = 0.56$ ), and glycosylated hemoglobin ( $r = -0.07$ ,  $n = 1099$ ,  $P = 0.02$ ).

#### Albuminuria/Proteinuria

On the basis of urine dipstick, 37% of PKC-DRS patients and 35% of PKC-DMES patients had a positive test for proteinuria. At study end after 36 mo of treatment, 34% of the PKC-DRS2 patients had an ACR of  $>30$  to  $\leq 300$  mg/g, and 20% of PKC-DRS2 patients had urinary ACR of  $>300$  mg/g. ACR was missing at end point for 33% of the patients in the study. There were no statistically significant differences between the two treatment groups with respect to the proportion of patients with end-point ACR  $>30$  mg/g (placebo 57%, RBX 53%;  $P = 0.46$ ) or with end-point ACR  $>300$  mg/g (placebo 21%, RBX 19%;  $P = 0.64$ ).

### Kidney Outcomes

During the course of PKC-DMES, PKC-DRS, and PKC-DRS2, 6.0% of patients (placebo 6.1%, RBX 5.9%;  $P = 0.88$ ; Table 3) experienced doubling of serum creatinine. Progression to advanced CKD was observed in 4.1% of patients (placebo 4.3%, RBX 3.8%;  $P = 0.64$ ). Death occurred in 4.1% of patients (placebo 4.7%, RBX 3.6%;  $P = 0.37$ ). At least one kidney outcome was reached in 11.3% of patients (placebo 11.8%, RBX 10.9%;  $P = 0.62$ ). When each trial was examined individually, there was no difference between treatment groups in the rate of kidney outcomes.

### Discussion

This analysis of data from the long-term diabetic retinopathy trials was performed to assess the effect of RBX on kidney outcomes in patients with diabetic eye disease and relatively normal kidney function at study entry. After approximately 3 yr, the number of RBX-treated patients who reached at least one kidney outcome was low and similar to that of patients who received placebo. The most common outcome was doubling of serum creatinine. Each of the three kidney outcomes (doubling of serum creatinine, progression to advanced CKD, or death) occurred with nearly equal frequency in the placebo- and RBX-treated groups. Change in kidney function, as evaluated by eGFR or calculated creatinine clearance, did not differ by treatment assignment.

In the 1-yr pilot study, RBX improved albuminuria by  $24 \pm 9\%$ , and eGFR did not decline significantly in patients who had type 2 diabetes and persistent macroalbuminuria despite already receiving renin-angiotensin system inhibition (15). In rodent models of diabetes, RBX has similarly been shown to protect the kidney (13,14). Even in a model of severe hyperglycemia and hypertension (streptozotocin-induced diabetes in rats that were transgenic for renin), RBX prevented various indices of kidney damage, including albuminuria, glomerulosclerosis, and tubulointerstitial injury (21). Therefore, the biologic rationale for PKC- $\beta$  inhibition to treat diabetic nephropathy is substantial. PKC is composed of at least 12 isoforms that signal a number of responses in resident kidney cells, including oxidative stress, activation and/or expression of inflammatory mediators, proliferation, and fibrosis (22). Various PKC isoforms, particularly  $\beta$ , are overactivated by hyperglycemia acting through generation of cellular diacylglycerol, advanced glycation end products, and other aberrant metabolic products (22). Taken together, multiple lines of experimental evidence and the recent pilot study in patients with diabetic nephropathy

indicate that PKC- $\beta$  inhibition with RBX may protect the kidney.

The studies of diabetic eye disease included 580 patients at multiple sites who were taking 32 mg/d RBX and had a median follow-up of 33 to 39 mo (16–18). Reports of the adverse event diabetic nephropathy in the PKC-DRS2 trial are not consistent with results of previous studies of diabetic kidney disease (humans and animal models) or the albuminuria data in PKC-DRS2 itself. The proportion of PKC-DRS2 participants with either microalbuminuria or macroalbuminuria at study end did not differ between placebo and RBX groups. In addition, kidney function declined at the same rate over time in both groups. Patients who have diabetes and increasing albuminuria are more likely to have declining kidney function than those with stable or decreasing albuminuria (23). As a whole, the objective measures that are available in PKC-DRS2 do not support an increased frequency of new-onset diabetic nephropathy in the RBX group. Furthermore, a recent safety analysis of patients from a large portfolio of RBX studies (not limited to diabetic eye disease trials) did not confirm an increased rate of investigator-ascribed diabetic nephropathy (24). Investigator subjectivity and lack of specific criteria for diagnoses are important limitations to the accuracy of adverse event reporting. However, it is valuable to analyze safety data from the combined RBX trials for patient protection and planning new studies.

The kidney outcome definitions in this analysis were based on end points that were used in large clinical trials of diabetic nephropathy (25–27). Patients who were enrolled in the studies of diabetic eye disease had essentially normal kidney function at baseline; consequently, very few ESRD events (dialysis or kidney transplant) occurred during follow-up (16–18). Therefore, advanced CKD (stage 4 or greater) was used as a kidney outcome. eGFR was calculated from the serum creatinine level using the re-expressed, four-component MDRD equation for standardized creatinine assays, as currently recommended by the Chronic Kidney Disease Epidemiology Collaboration (19). The MDRD equation was designed for use in patients with low GFR and underestimates kidney function in populations with eGFR  $>60$  ml/min per  $1.73$  m<sup>2</sup> (19,28). Therefore, the Cockcroft-Gault equation, which calculates creatinine clearance using serum creatinine levels, was used as a complementary indicator of kidney function. Because patients in these studies were typically obese, kidney function may have been lower than that reflected in the calculated creatinine clearance because of the weight term in the numerator. Therefore, actual

Table 3. Kidney outcomes by assignment to placebo or RBX long-term studies of diabetic eye disease<sup>a</sup>

Characteristic	Placebo	RBX 32 mg/d	Total	<i>P</i>
Doubling of serum creatinine	6.1% (35/577)	5.9% (34/580)	6.0% (69/1157)	0.88
Progression to advanced CKD	4.3% (25/577)	3.8% (22/580)	4.1% (47/1157)	0.64
Death	4.7% (27/577)	3.6% (21/580)	4.1% (48/1157)	0.37
At least one kidney outcome	11.8% (68/577)	10.9% (63/580)	11.3% (131/1157)	0.62

<sup>a</sup>CKD, chronic kidney disease.

baseline GFR was likely somewhere between the eGFR and calculated creatinine clearance ( $81.6 \pm 26.0$  ml/min per  $1.73 \text{ m}^2$  and  $114.3 \pm 46.6$  ml/min, respectively). Regardless, changes in kidney function as measured by either method were similar in the placebo and RBX groups. However, a benefit of RBX on kidney outcomes was not detected, possibly because this group of patients had relatively normal kidney function at entry into the studies. Furthermore, incomplete ascertainment for kidney disease in the diabetic retinopathy trials limited the extent to which effects of RBX on the kidney could be evaluated.

Diabetic eye disease was associated with considerable loss of eGFR during approximately 3 yr, despite good kidney function at study entry. This observation may have important implications for kidney disease risk stratification and management. In addition, kidney function was lost more rapidly in patients with type 2 compared with type 1 diabetes and in those who were abstinent from alcohol. Other baseline variables (BP, age, glycemic control, and obesity) were weakly correlated with eGFR. These findings suggest that in populations that are selected for clinical trials in diabetic retinopathy, some variables that typically are associated with progression of kidney disease may be less predictive than in other populations.

## Conclusion

Long-term RBX treatment did not influence rates of kidney outcomes among patients who were in clinical trials for diabetic eye disease and had relatively normal kidney function. Loss of kidney function occurred in these patients, irrespective of placebo or RBX treatment, during approximately 3-yr duration. Large-scale, prospective trials are needed to confirm safety and the potential benefits of RBX with regard to clinical outcomes in patients with diabetic nephropathy.

## Acknowledgments

These studies were funded by Eli Lilly and Company.

These data were presented in abstract form at annual meetings of the American Diabetes Association (Washington, DC; June 11, 2006), the European Association for the Study of Diabetes (Copenhagen, Denmark; September 16, 2006), and the International Diabetes Federation (Cape Town, South Africa; December 7, 2006).

This article was reviewed by the Study Executive Committee: Lloyd P. Aiello, Boston, MA (Chair); Matthew D. Davis, Madison, WI; Aniz Girach, Surrey, United Kingdom; Keri A. Kles Poi, Indianapolis, IN; Roy C. Milton, Rockville, MD; Matthew J. Sheetz, Indianapolis, IN; Louis Vignati, Indianapolis, IN; and Xin (Eric) Zhi, Indianapolis, IN.

We thank the study participants and clinical investigators and their support staff. Members of the PKC-DRS2 Study Group, PKC-DRS Study Group and PKC-DMES Study Group can be found in the articles that describe those clinical trials (16–18). We thank Keri A. Kles Poi, Tim Mason, Justin Northrup, Jianyu Zhu, Natalie Hall, and Lilly Research Laboratories for valuable assistance with the preparation of this manuscript.

## Disclosures

D.J.H., P.W.A., and T.E.L. were employed by Lilly Research Laboratories at the time this article was written. K.R.T. is on the scientific advisory board for Eli Lilly and Company, which involves ruboxistaurin and diabetic nephropathy. She has received consulting fees from Eli Lilly and Company regarding ruboxistaurin and diabetic

nephropathy. J.B.M. was the principal investigator at Washington University for another study investigating ruboxistaurin and as such received grant support through the university. She has served both as a consultant and on an advisory panel for Eli Lilly and has received honoraria for speaking on behalf of Eli Lilly.

## References

1. Klein R, Klein BEK, Moss SE: The Wisconsin epidemiological study of diabetic retinopathy: A review. *Diabetes Metab Rev* 5: 559–570, 1989
2. Kempen JH, O'Colmain BJ, Leske MC, Haffner SM, Klein R, Moss SE, Taylor HR, Hamman RFL: Eye Diseases Prevalence Research Group: The prevalence of diabetic retinopathy among adults in the United States. *Arch Ophthalmol* 122: 552–563, 2004
3. Javitt JC, Aiello LP, Chiang Y, Ferris FL 3rd, Canner JK, Greenfield S: Preventive eye care in people with diabetes is cost-saving to the federal government. Implications for health-care reform. *Diabetes Care* 17: 909–917, 1994
4. Henricsson M, Sellman A, Tyrberg M, Groop L: Progression to proliferative retinopathy and macular oedema requiring treatment. Assessment of the alternative classification of the Wisconsin Study. *Acta Ophthalmol Scand* 77: 218–223, 1999
5. Klein R, Klein BEK, Moss SE, Davis MD, DeMets DL: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. IV. Diabetic macular edema. *Ophthalmology* 91: 1464–1474, 1984
6. Ballone E, Colagrande V, DiNicola M, DiMascio R, DiMascio C, Capani F: Probabilistic approach to developing nephropathy in diabetic patients with retinopathy. *Stat Med* 22: 3889–3897, 2003
7. United States Renal Data System: Annual data report: Incidence and prevalence. *Am J Kidney Dis* 49: S68–S84, 2007
8. National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) Clinical Practice Guidelines for Diabetes and Chronic Kidney Disease. *Am J Kidney Dis* 49: S42–S61, 2007
9. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414: 813–820, 2001
10. Candido R, Allen TJ: Haemodynamics in microvascular complications in type 1 diabetes. *Diabetes Metab Res Rev* 18: 286–304, 2002
11. Sheetz MJ, King GL: Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA* 288: 2579–2588, 2002
12. Curtis TM, Scholfield CN: The role of lipids and protein kinase Cs in the pathogenesis of diabetic retinopathy. *Diabetes Metab Res Rev* 20: 28–43, 2004
13. Ishii H, Jirousek MR, Koya D, Takagi C, Xia P, Clermont A, Bursell SE, Kern TS, Ballas LM, Heath WF, Stramm LE, Feener EP, King GL: Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. *Science* 272: 728–731, 1996
14. Koya D, Haneda M, Nakagawa H, Ishiki K, Sato H, Maeda S, Sugimoto T, Yasuda H, Kashiwagi A, Wada DK, King GL, Kikkawa R: Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. *FASEB J* 14: 439–447, 2000
15. Tuttle KR, Bakris GL, Toto RD, McGill JB, Hu K, Anderson

- PW: The effect of ruboxistaurin on nephropathy in type 2 diabetes. *Diabetes Care* 28: 2686–2690, 2005
16. The PKC-DRS2 Study Group: Effect of ruboxistaurin on visual loss in patients with diabetic retinopathy. *Ophthalmology* 113: 2221–2230, 2006
  17. The PKC-DRS Study Group: The effect of ruboxistaurin on visual loss in patients with moderately severe to very severe nonproliferative diabetic retinopathy: Initial results of the protein kinase C beta inhibitor diabetic retinopathy study (PKC-DRS) multicenter randomized clinical trial. *Diabetes* 54: 2188–2197, 2005
  18. The PKC-DMES Study Group: Effect of ruboxistaurin, a PKC beta isoform-selective inhibitor, in patients with diabetic macular edema: 30-month results of the randomized PKC-DMES clinical trial. *Arch Ophthalmol* 125: 318–324, 2007
  19. Levey AS, Coresh J, Greene T, Stevens LA, Zhang Y, Hendriksen S, Kusek JW, Van Lente F; for the Chronic Kidney Disease Epidemiology Collaboration: Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 145: 247–254, 2006
  20. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16: 31–41, 1976
  21. Kelly DJ, Zhang Y, Hepper C, Gow RM, Jaworski K, Kemp BE, Wilkinson-Berka JL, Gilbert RE: Protein kinase C beta inhibition attenuates the progression of experimental diabetic nephropathy in the presence of continued hypertension. *Diabetes* 52: 512–518, 2003
  22. Tuttle KR, Anderson PW: A novel potential therapy for diabetic nephropathy and vascular complications: Protein kinase C beta inhibition. *Am J Kidney Dis* 42: 456–465, 2003
  23. de Zeeuw D, Remuzzi G, Parving HH, Keane WF, Zhang Z, Shahinfar S, Snapinn S, Cooper ME, Mitch WE, Brenner BM: Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: Lessons from RENAAL. *Kidney Int* 65: 2309–2320, 2004
  24. McGill JB, King GL, Berg PH, Price KL, Kles KA, Bastyr EJ, Hyslop DL: Clinical safety of the selective PKC beta inhibitor, ruboxistaurin. *Expert Opin Drug Saf* 5: 835–845, 2006
  25. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD: The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. *N Engl J Med* 329: 1456–1462, 1993
  26. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345: 861–869, 2001
  27. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I: Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Engl J Med* 345: 851–860, 2001
  28. Rule AD, Gussak HM, Pond GR, Bergstralh EJ, Stegall MD, Cosio FG, Larson TS: Measured and estimated GFR in healthy potential kidney donors. *Am J Kidney Dis* 43: 112–119, 2004

See the related editorial, "Protein Kinase C- $\beta$  Inhibition: A Promise Not Yet Fulfilled," on pages 619–620.