Sulodexide Fails to Demonstrate Renoprotection in Overt Type 2 Diabetic Nephropathy

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ABSTRACT

Sulodexide, a mixture of naturally occurring glycosaminoglycan polysaccharide components, has been reported to reduce albuminuria in patients with diabetes, but it is unknown whether it is renoprotective. This study reports the results from the randomized, double-blind, placebo-controlled, sulodexide macroalbuminuria (Sun-MACRO) trial, which evaluated the renoprotective effects of sulodexide in patients with type 2 diabetes, renal impairment, and significant proteinuria (>900 mg/d) already receiving maximal therapy with angiotensin II receptor blockers. The primary end point was a composite of a doubling of baseline serum creatinine, development of ESRD, or serum creatinine ≥6.0 mg/dl. We planned to enroll 2240 patients over approximately 24 months but terminated the study after enrolling 1248 patients. After 1029 person-years of follow-up, we did not detect any significant differences between sulodexide and placebo; the primary composite end point occurred in 26 and 30 patients in the sulodexide and placebo groups, respectively. Side effect profiles were similar for both groups. In conclusion, these data do not suggest a renoprotective benefit of sulodexide in patients with type 2 diabetes, renal impairment, and macroalbuminuria.

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Diabetic nephropathy is now the most common cause of ESRD in developed nations.[1,2] Type 2 diabetic nephropathy accounts for the majority of patients with diabetes and ESRD.[3] The number of patients with type 2 diabetes mellitus globally is expected to double its current levels and reach 366 million persons worldwide by 2030.[4] Attempts to alleviate or avert this chronic disease will require long-term detection and prevention strategies.[5] In the short term, much attention is focused on developing proven intervention strategies to alter the course of diabetic nephropathy.

Studies show that angiotensin receptor blockers (ARBs) significantly reduce composite end points of disease progression in patients with type 2 diabetes with established nephropathy and renal impairment.[5,6] This effect is independent of the benefits of BP reduction and seems to correlate with reductions in albuminuria[7] and proteinuria.[8] Subsequently,

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ARB use in treatment of type 2 diabetic nephropathy is an established standard of care; however, ARB use does not abolish progression to ESRD. Accordingly, researchers have investigated, with varying success, the renoprotective effects of other agents acting through the renin-angiotensin system (RAS)[9,10] and of agents with a novel mode of action not involving the RAS.[11–13]

Sulodexide is a mixture of four naturally occurring glycosaminoglycan polysaccharide components isolated from porcine lung and liver. Manufacturers have marketed the drug in many parts of the world for more than 20 years for a range of vascular pathologies.[14] Preliminary studies show that sulodexide reduces urinary albumin excretion rates in patients with type 1 and type 2 diabetes.[15–22] A number of studies suggest different modes of action to explain this phenomenon.[23–29]

After conducting a previously reported pilot study,[30] the Collaborative Study Group (CSG) undertook two multinational, randomized, double-blind, placebo-controlled trials using sulodexide in patients with type 2 diabetes, and the study designs were reported in detail previously.[31] The sulodexide microalbuminuria (Sun-MICRO) trial[32] was conducted in patients with preserved renal function and microalbuminuria. This article reports the results of the sulodexide macroalbuminuria (Sun-MACRO) trial.

Table 1. Description of sulodexide and placebo groups in the sulodexide overt nephropathy trial

Variable	Sulodexide (*n*=619)[a]	Placebo (*n*=629)[a]
Male, *n* (%)	383 (79)	376 (74)
Age (y), mean (SD)	62.3 (9.4)	63.6 (9.5)
Height (cm), mean (SD)	168.8 (12.4)	168.5 (11.1)
Weight (kg), mean (SD)	93.7 (21.7)	91.1 (21.9)
Body mass index (kg/m^2), mean (SD)	33.6 (19.3)	32.3 (12.4)
Total cholesterol (mg/dl), mean (SD)	173.7 (41.5)	179.6 (54.8)
HbA1c (%), mean (SD)	8.0 (1.6)	7.9 (1.5)
Prior CVD, *n* (%)	227 (47)	233 (46)
Family history of CVD, *n* (%)	304 (63)	335 (66)
Race, *n* (%)		
African descent	59 (12)	61 (12)
Asian descent	67 (14)	71 (14)
Caucasian descent	334 (69)	347 (69)
other	21 (4)	26 (5)
Use of medication before screening for overt trial, *n* (%)		
ACE inhibitor only	131 (28)	148 (31%)
ARB only	239 (52)	247 (51%)
ACE inhibitor and ARB	62 (13)	62 (13%)
none	28 (6)	28 (6%)
SeSBP (mmHg), mean (SD)	138.0 (14.0)	138.0 (14.8)
SeDBP (mmHg), mean (SD)	73.6 (9.9)	73.0 (10.0)
Pulse rate (sitting; beats per min), mean (SD)	69.9 (12.3)	69.3 (11.4)
Estimated GFR (ml/min per 1.73 m^2), mean (SD)	31.4 (8.7)	31.4 (8.6)
Baseline serum creatinine (mg/dl), mean (SD)	2.19 (0.53)	2.16 (0.50)
PCR,[b] (mg/g), median (25th–75th percentile)	1836 (990, 3088)	1802 (965, 2966)
ACR,[b] (mg/g), median (25th–75th percentile)	1415 (714, 2400)	1364 (685, 2319)

[a]Data were not available for all participants, and the number with missing data varied for each variable.
[b]Geometric mean of three values.

RESULTS

We conducted laboratory screening in 2828 patients, with a large proportion failing to meet creatinine and/or protein inclusion criteria. Inability to satisfy run-in and baseline visit criteria excluded additional patients. Table 1 presents characteristics of the 1248 participants who were randomized (629 to placebo and 619 to sulodexide) and indicates that the two groups were well balanced at randomization, with no important differences in patient demographics. Figure 1 presents the clinical trial randomization flow chart. Figure 2 shows the numbers of patients followed up beyond baseline to subsequent visit times. Twenty-seven patients withdrew consent, and five others were lost to follow-up. Overall, there were 1029 person-years of follow-up available. Mean (SD) follow-up was 11.2 (6.6) months in the sulodexide group and 10.7 (6.6) months in the placebo group. Medians were similar to these means at 11.7 and 9.7 months in the sulodexide and placebo groups, respectively.

During follow-up, 29 participants doubled their baseline serum creatinine, 28 reached a serum creatinine of ≥6.0 mg/dl, and 44 progressed to chronic dialysis. Some participants experienced more than one of these events, and Table 2 shows the number of first events for the primary end point (*n*=56). The sulodexide group had a lower number of primary end point events (*n*=26) than the placebo group (*n*=30), but this comparison was not statistically significant (hazard ratio: 0.85 [95% confidence interval: 0.50–1.44]; *P*=0.54). Thirty-six patients died: 16 in the sulodexide group and 20 in the placebo group (*P*=0.49; Table 3). As shown in Table 3, there was no evidence to attribute any observed imbalances in time to death or time to first cardiovascular event to a benefit or harm of treatment, nor was there a statistically significant difference in changes in serum creatinine, proteinuria/creatinine ratio (PCR), or albumin/creatinine ratio (ACR). Table 4 shows that serious adverse events were similar in the placebo and sulodexide groups.

A posteriori analysis identified an early divergence in mean seated systolic BP (SeSBP) between the groups. Three months after randomization, mean SeSBP had increased to 139.5 in the placebo group but decreased to 137.1 in the sulodexide group (two-sample *t* test, *P*=0.04).

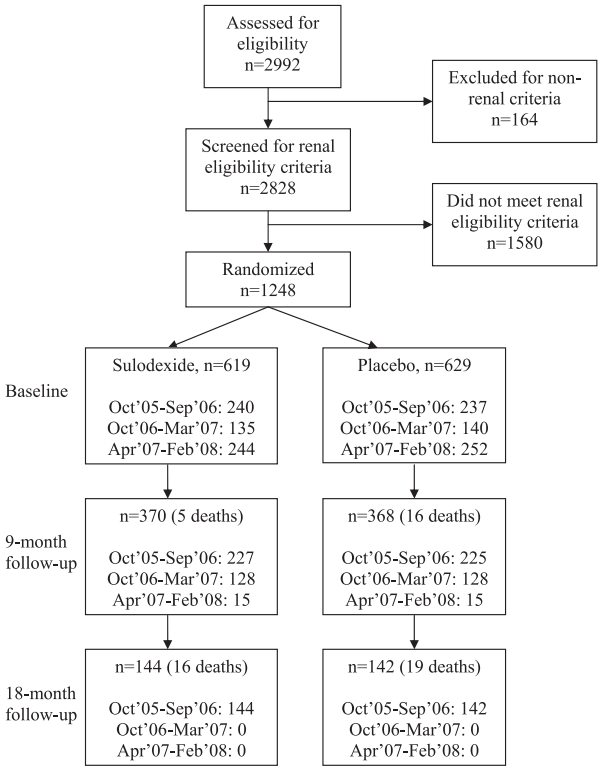

Figure 1. Sun-MACRO clinical trial randomization flowchart.

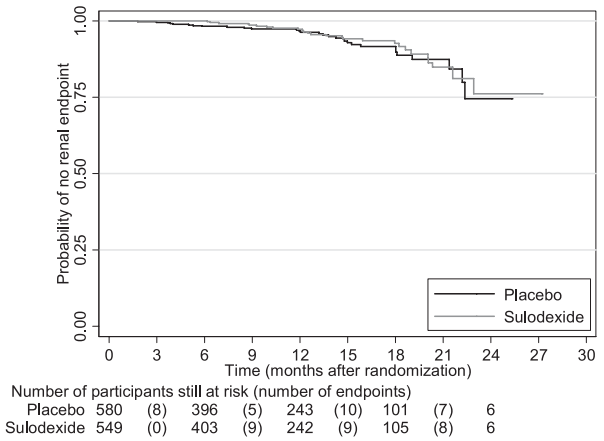

Figure 2. Kaplan-Meier survival curves comparing the placebo and sulodexide groups in the overt study with the 6-month interval number of participants followed beyond baseline and still at risk, with the number of end points in each 6 months.

DISCUSSION

At the time of early termination of this study, we found no significant differences between the treatment and placebo groups in the incidence of primary or secondary end points. However, less than half of the required sample size and one-tenth of the expected end points were available for analysis. The slight difference in SeSBP observed in the sulodexide group was not regarded as clinically significant. No differences were found in the side effect profiles of sulodexide and placebo and, importantly, no serious adverse effects were ascribed to the trial drug. It is important to emphasize that the decision to curtail this trial was not because the Data Safety Monitoring Committee of the CSG had concerns regarding drug toxicity or adverse clinical outcomes.

Although the study design required 2240 patients to detect a 20% effect regarding the primary endpoint of doubling initial serum creatinine or ESRD, it is important to note that the secondary outcome of a decrease in proteinuria only required 300–500 patients, a number well below the 1248 patients randomized and followed in this trial. This trial had adequate power to detect a 20% effect in the surrogate outcome of decreased proteinuria. It follows that this trial did not confirm the potentially beneficial effect of sulodexide in reducing proteinuria that was previously reported in smaller, underpowered studies.

Failure to demonstrate a therapeutic effect of sulodexide in this trial is in marked contrast to numerous previously published clinical studies[18–23] and a pilot study undertaken by the CSG.[30] Results from the Diabetic Nephropathy and Albuminuria Sulodexide Study[22] showed that, in 223 patients with either type 1 or type 2 diabetes and with either microalbuminuria or more advanced nephropathy, sulodexide-assigned patients showed a significant decrease in albumin excretion rate, regardless of the stage of their independent diabetic nephropathy and effects of angiotensin-converting enzyme (ACE) inhibitors. Effects were maximal after 4 months and seemed to persist for at least another 4 months after cessation of sulodexide. Similarly, Achour *et al.* reported, again in patients with type 1 and type 2 diabetes, that treatment with oral sulodexide significantly reduced albuminuria compared with controls.[23] The CSG undertook a pilot study in 149 patients with type 2 diabetes with microalbuminuria, and they reported a 25% incidence of a primary end point defined as a normalization on 50% reduction in baseline urinary ACRs in sulodexide-treated individuals compared with a 15% incidence in a placebo group. Differences, however, between the treatment and placebo groups in the pilot study did not reach clinical significance.[30]

In view of these earlier experiences with sulodexide, it is important to publish the outcome of this trial despite its early termination. In all, 1248 patients worldwide were randomized to the study, 619 of whom received sulodexide. With 1029 person-years of follow-up, approximately half in patients receiving the active drug, this represents clinical experience with sulodexide that is much more extensive than in previously reported trials.

The lack of any significant difference in the primary study end point at the time of termination is noted; however, because only one-tenth of the end points required by the study design had occurred at termination, this could represent a type 2 error. Thus, the statistical analysis of the primary end point of the trial at the time of discontinuation was rendered grossly

Table 2. Description and comparison of primary end point events (serum creatinine doubling or ESRD) in the sulodexide and placebo groups in the sulodexide overt nephropathy trial

	Sulodexide (No. First Events)[a]	Placebo (No. First Events)[a]	Sulodexide Compared With Placebo (*P* Value)[b]
Serum creatinine ≥6.0 mg/dl	12	16	
Dialysis	12	9	
Serum creatinine doubling	2	5	
Total	26	30	
Total person-years of follow-up to primary event or censoring (death or end of trial)	513.9	514.8	
Primary endpoint event rate (per 1000 person-years) with 95% confidence intervals	51 (34–74)	58 (41–83)	0.54
Hazard ratio of time to first occurrence of a primary end point event with 95% confidence intervals	0.85 (0.50–1.44)	1.00 (Ref)	

[a]When two events occurred on the same date (*e.g.*, serum creatinine doubling and serum creatinine ≥6.0 mg/dl), the first event was considered as the first listed event in the table.
[b]*P* value from log-rank test.

underpowered. In addition, early termination resulted in relatively short mean follow-up, and we cannot exclude a significant difference in primary end points between treatment arms after more prolonged follow-up.

It is important to note that the decision to discontinue (which followed the negative findings of the Sun-MICRO study) was based on analysis of change in serum creatinine, a surrogate marker for the primary outcomes of doubling of baseline creatinine or ESRD, and a secondary surrogate end point, which is mean change in quarterly urine PCR. Although discontinuing a trial on this basis may be open to criticism, the statistical validity of the data analysis on serum creatinine and urinary PCR up to trial termination is sufficient to exclude a significant difference in serum creatinine and PCR change between treatment and placebo groups by virtue of the narrow confidence intervals. Specifically, we estimated the relative change in urinary PCR per annum to be between 9% lower and 3% higher with sulodexide than the corresponding change with placebo. Thus, any effect of sulodexide on primary end points must be independent of any effect on annualized rates of change in PCR, widely regarded as a surrogate marker for improved renal outcome.

In view of the negative results of Sun-MICRO trial[32] and the data analysis from this study, it is tempting to conclude that

Table 3. Secondary and tertiary outcomes in the sulodexide and placebo groups in the sulodexide overt nephropathy trial

Event	Sulodexide (No. Events)	Placebo (No. Events)	Sulodexide Compared with Placebo	
			Hazard Ratio (95% CI)	*P* Value
All-cause mortality	16	20	0.79 (0.41, 1.53)	0.49
CV death	13	16		
Non-CV death	3	4		
First CV fatal or nonfatal event	80	74	1.12 (0.82, 1.54)	0.48
Event type that was first CV event				
CV death	6	11		
nonfatal myocardial infarction	11	9		
heart failure hospitalization	34	29		
stroke	2	3		
trans-ischemic attack	3	4		
peripheral vascular disease	15	11		
revascularization	9	7		
	Sulodexide Change (95% CI)	Placebo Change (95% CI)	Difference in Change (95% CI)[a]	*P* Value
Serum creatinine change from baseline to 18 mo (mean mg/dl per year)	+0.38 (0.34, 0.41)	+0.35 (0.32, 0.38)	0.03 (−0.02, 0.07)	0.29
PCR change from baseline to 18 mo				0.27
mean log per year (mg/g)	−0.02 (−0.07, 0.02)	+0.01 (−0.03, 0.06)	−0.04 (−0.10, 0.03)	
geometric mean ratio per year	0.98 (0.94, 1.02)	1.01 (0.97, 1.06)	0.97 (0.91, 1.03)	
relative increase per year (%)	−2% (−6, +2)	+1% (−3, +6)	−3% (−9, +3)	
ACR change from baseline to 3 mo				0.56
mean log per year (mg/g)	−0.03 (−0.09, 0.04)	−0.07 (−0.13, 0.00)	0.04 (−0.05, 0.14)	
geometric mean ratio per year	0.97 (0.91, 1.04)	0.93 (0.88, 1.00)	1.04 (0.94, 1.14)	
relative increase per year (%)	−3% (−34, +11)	−7% (−12%, 0)	+4% (−6, +14)	

CI, confidence interval; CV, cardiovascular.
[a]Difference of mean mg/dl or mean log (mg/g) per year change; ratio of geometric mean per year ratios.

Table 4. Serious adverse events

	Sulodexide (*n*=619)	Placebo (*n*=629)
Total events	218	203
Cardiovascular	55	46
Renal and urinary disorders	26	28
Gastrointestinal and hepatobiliary disorders	31	24
Infections and infestations	20	21
Respiratory, thoracic, and mediastinal disorders	14	21
Injury, poisoning, and procedural complications	12	15
Metabolism and nutrition disorders	12	11
Neoplasms, benign, malignant, and unspecified	9	5
Musculoskeletal and connective tissue disorders	7	5
General disorders and administration site conditions	5	6
Eye disorders	6	3
Nervous system disorders	5	4
Vascular	5	4
Endocrine, reproductive, and breast disorders	4	3
Skin and subcutaneous tissue disorders	0	3
Blood and lymphatic system disorders	1	0
Ear and labyrinth disorders	1	0
Immune system disorders	0	1

sulodexide has no therapeutic benefit in type 2 diabetic nephropathy.

Patients in this study all had stage 3–4 CKD, and proteinuria represented "residual" proteinuria on a maximal dose of ARB. Patients in previous studies of sulodexide included individuals with lesser degrees of renal impairment and patients on submaximal or no inhibition of the RAS. Therefore, a comparison between trials cannot be performed. Recent experimental models suggest that sulodexide may ameliorate early but not late kidney disease.[33] As discussed previously,[32] potential confounding factors must be mentioned. First, although previous pharmacokinetic studies demonstrated oral absorption of sulodexide from the gastrointestinal tract, this was at levels that did not alter hemostasis.[34,35] The inability to detect factor Xa generation concomitant with oral administration of sulodexide makes pharmacokinetic studies difficult, if not impossible. It is possible that the low rate of absorption of sulodexide from the gastrointestinal tract failed to provide enough agent to achieve an effect at the glomerular level. Sulodexide is used as a therapeutic agent in Europe in a variety of settings associated with the treatment of occlusive vascular disease. There has been no report of altered hemostasis concomitant with sulodexide use in these settings.[36–38]

The chemical content of sulodexide is characteristic of heparin compounds, and the sulfate content, sulfate/carbonyl ratios, and *in vitro* antifactor Xa activity reported to the US Food and Drug Administration (FDA) for sulodexide are well within the required ranges.[39] However, differences between glycosaminoglycan formulations between the drug used in previous studies and that used in this study cannot be ruled out. The virtual identity in the results between the control and sulodexide groups in this trial not only raises the question of whether the therapeutic agent used was absorbed from the gastrointestinal tract, but also advances consideration of whether the agent had pharmacologic activity. The manufacturing requirements of sulodexide are complex.[39] Previous studies used sulodexide (Vessel) developed by Alfa Wassermann; however, Keryx Biopharmaceuticals developed the sulodexide (Sulonex) used in this study (see Concise Methods). There is no direct evidence that there was a lack of adherence to the required quality controls for the manufacture of the low molecular weight heparin–containing compound used in the study. However, failures in heparin manufacture have occurred in other circumstances, which must be considered in the face of a negative clinical trial.

In summary, the early termination of this trial precludes a definitive answer as to whether sulodexide is an effective therapeutic agent in type 2 diabetic nephropathy characterized by renal impairment and overt proteinuria (despite established treatment with ARBs). However, we found no evidence of an effect on change in serum creatinine or urinary PCR with sulodexide treatment over 1029 person-years of follow-up.

CONCISE METHODS

Entry criteria for this study included women and men ≥18 years of age with type 2 diabetes mellitus and clinically overt proteinuria (total protein ≥0.9 g/24 h), the diagnosis of which we obtained from 24-hour urine collection. Patients with additional nondiabetic renal disease were excluded. In women, serum creatinine in women was between 1.3 and 3.0 mg/dl (115–265 μmol/L) inclusive. In men, serum creatinine was between 1.5 and 3.0 mg/dl (133–265 μmol/L) inclusive or an estimated GFR was between 25 and 45 ml/min, as calculated by the Modification of Diet in Renal Disease formula.[40]

Recruitment occurred between August 1, 2005 and February 29, 2008 at clinical centers worldwide, including 114 in Europe and Israel, 77 in the United States and Canada, and 27 in Asia and the Pacific. The clinical coordinating center in the United States and the various CSG committees managed and monitored the study conduct. The institutional ethics committee of each center approved the trial, and all patients provided written informed consent. Keryx Biopharmaceuticals (New York, NY) developed sulodexide (Sulonex) for phase 3 and phase 4 trials under a special protocol assessment with the FDA. Manufacture was assigned to Scientific Protein Laboratories LLC (Waunakee, WI) in 2004. This trial is registered with ClinicalTrials.gov (NCT00130312).

This study consisted of screening, run-in, qualifying, randomization, and maintenance phases. During the screening phase, we selected

patients with type 2 diabetes mellitus on the basis of their serum creatinine and 24-hour protein excretion. All urine and blood assessments were performed at a central laboratory. After screening, patients who took losartan 100 mg/d or irbesartan 300 mg/d for at least 60 days and had adequate and stable BP control could bypass the run-in period. Patients not already taking losartan 100 mg/d or irbesartan 300 mg/d, or using these ARBs but not meeting the BP goals, proceeded to a 60-day run-in period. The target BP goal was a SeSBP $\leq$130 mmHg and a seated diastolic BP (SeDBP) $\leq$80 mmHg. The BP had to be controlled to SeSBP $\leq$160 mmHg and SeDBP $\leq$100 mmHg to proceed to randomization. We allowed patients to take concomitant antihypertensive medication for BP control, but they could not use additional ARBs, ACE inhibitors, or aldosterone antagonists. After achieving adequate and stable BP control, we randomized patients to receive sulodexide 200 mg/d or placebo. We assigned patients to study treatments using a central computer-based allocation service. After randomization, patients were seen at 1 month and every 3 months thereafter to assess BP, renal function, and adverse events.

Treatment compliance was assessed with pill counts at each scheduled study visit during the maintenance phase of the trial. Compliance was defined per protocol as the ingestion of at least 80% of the prescribed medication.

During the maintenance phase, we permitted the use of adjunctive antihypertensive agents, except additional ARBs and ACE inhibitors or other renin-angiotensin-aldosterone system inhibitors, to achieve the target BP ($\leq$130/80 mmHg). Data on the specific antihypertensive medications used during follow-up were not available. Patients with symptomatic orthostatic hypotension at any time during the study had adjunctive antihypertensive medications tapered or withdrawn first. If orthostatic symptoms persisted, the ARB dose was lowered or the ARB was discontinued after consultation with the clinical coordinating center.

The study investigator or the individual patient's diabetologist/primary care physician managed glycemic control in accordance with locally accepted standards of care for diabetes management, including a goal to achieve hemoglobin A1c levels $<$7% (American Diabetes Association standard) or $<$6.5% (European Diabetes Association standard). Patients could use any therapy for diabetes, including lifestyle/diet modification, oral antihyperglycemic agents, and insulin, to achieve and maintain these goals throughout the study. The specific agents used were not recorded.

We used the modified Jaffe, rate-blanked, alkaline picrate method incorporated on the automated Roche/Hitachi Modular System to determine serum and urine creatinine clearance. Protein concentration was determined by turbidimetric assay including the alkaline benzethonium chloride method incorporated on the automated Roche/Hitachi Modular System. Urinary albumin was measured by immunoturbidimetry (Cobas Mira Plus; Roche, Montclair, NJ).

The primary end point was time until the first occurrence of a confirmed doubling of baseline serum creatinine or ESRD, defined as renal transplantation, need for dialysis, or a serum creatinine $\geq$6.0 mg/dl (530 μmol/L). Baseline serum creatinine was defined as the mean of the serum creatinine levels obtained at the time of randomization and the preceding visit. To detect, with 80% power, a 20% reduction in the incidence of the primary end point in the treatment group compared with the placebo group, we aimed to recruit 2240 patients and to observe 500 end points in 3 years of follow-up per patient.[31] Secondary outcomes included the following: time to all-cause mortality, time to ESRD, time to doubling of baseline serum creatinine, changes in PCR, and changes in urine ACR. Tertiary outcomes included the following: time to occurrence of the composite end point of cardiovascular death, nonfatal myocardial infarction, myocardial infarction, hospitalization for congestive heart failure, heart failure, stroke, transient ischemic attack, trans-ischemic attack, resuscitated sudden death, coronary revascularization procedure, and peripheral vascular procedure. An experienced end points committee of the CSG adjudicated all end points. ARF episodes were excluded from primary end point analysis.

The results of the Sun-MICRO study, which began concurrently but concluded before this trial, showed no evidence of benefit from sulodexide.[32] An interim analysis of this study revealed no difference between the placebo and active drug groups with regard to change in serum creatinine from baseline. This was not a prespecified end point. Quarterly urine PCR data, a secondary en dpoint of the trial, were also analyzed and failed to indicate a difference between the placebo and active drug groups. This trial was terminated because no effect on change in serum creatinine or proteinuria was observed. We conducted an analysis of primary and secondary end points up to the time of termination, and we reported the results in this study.

We used a log-rank test and Cox proportional hazards regression models to compare the time to the first occurrence of an end point between study groups in intention-to-treat analyses. A *post hoc* analysis extended the Cox model to one with a shared frailty term to allow for an effect across the clinical centers. We used the Kaplan-Meier method to plot survival curves. Changes from randomization in serum creatinine, PCR, ACR, and SeSBP over time were compared between groups to the 18-month visit using random-intercept mixed models.[41] The analysis was based on natural log-transformed values for PCR and ACR. We performed *post hoc* analyses of these four measures using random slopes models and two-sample *t* tests of baseline to 3-month changes. We used Stata statistical software for all analyses (Release 10; StataCorp, College Station, TX).

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DISCLOSURES

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