CJASN ePress. Published on February 22, 2016 as doi: 10.2215/CJN.09900915

Effect of Sirolimus on Disease Progression in Patients with Autosomal Dominant Polycystic Kidney Disease and CKD Stages 3b-4

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Abstract

Background and objectives The effect of mammalian target of rapamycin (mTOR) inhibitors has never been tested in patients with autosomal dominant polycystic kidney disease (ADPKD) and severe renal insufficiency.

Design, setting, participants, & measurements In this academic, prospective, randomized, open label, blinded end point, parallel group trial (ClinicalTrials.gov no. NCT01223755), 41 adults with ADPKD, CKD stage 3b or 4, and proteinuria ≤0.5 g/24 h were randomized between September of 2010 and March of 2012 to sirolimus (3 mg/d; serum target levels of 5–10 ng/ml) added on to conventional therapy (n=21) or conventional treatment alone (n=20). Primary outcome was GFR (iohexol plasma clearance) change at 1 and 3 years versus baseline.

Results At the 1-year preplanned interim analysis, GFR fell from 26.7±5.8 to 21.3±6.3 ml/min per 1.73 m^2 (P<0.001) and from 29.6±5.6 to 24.9±6.2 ml/min per 1.73 m^2 (P<0.001) in the sirolimus and conventional treatment groups, respectively. Albuminuria (73.8±81.8 versus 154.9±152.9 μg/min; P=0.02) and proteinuria (0.3±0.2 versus 06±0.4 g/24 h; P<0.01) increased with sirolimus. Seven patients on sirolimus versus one control had *de novo* proteinuria (P=0.04), ten versus three patients doubled proteinuria (P=0.02), 18 versus 11 patients had peripheral edema (P=0.04), and 14 versus six patients had upper respiratory tract infections (P=0.03). Three patients on sirolimus had angioedema, 14 patients had aphthous stomatitis, and seven patients had acne (P<0.01 for both versus controls). Two patients progressed to ESRD, and two patients withdrew because of worsening of proteinuria. These events were not observed in controls. Thus, the independent data and safety monitoring board recommend early trial termination for safety reasons. At 1 year, total kidney volume (assessed by contrast–enhanced computed tomography imaging) increased by 9.0% from 2857.7±1447.3 to 3094.6±1519.5 ml on sirolimus and 4.3% from 3123.4±1695.3 to 3222.6±1651.4 ml on conventional therapy (P=0.12). On follow-up, 37% and 7% of serum sirolimus levels fell below or exceeded the therapeutic range, respectively.

Conclusions Finding that sirolimus was unsafe and ineffective in patients with ADPKD and renal insufficiency suggests that mTOR inhibitor therapy may be contraindicated in this context.

Clin J Am Soc Nephrol 11: ●●●–●●●, 2016. doi: 10.2215/CJN.09900915

Introduction

In total, 8%–10% of patients with ESRD have autosomal dominant polycystic kidney disease (ADPKD) (1), an inherited systemic disorder of relentless cyst enlargement caused by fluid transport into the cavities generated by uncontrolled renal tubular cell proliferation. cAMP accumulation and Ser/Thr kinase mammalian target of rapamycin (mTOR) activation mediate cyst expansion (2–5), whereas mTOR inhibition with sirolimus or everolimus slowed cyst growth and preserved renal function in a variety of animal models of polycystic kidney disease (4,6–8).

After observational findings that, in patients with ADPKD receiving a kidney transplant, cyst growth was slowed by sirolimus–based immunosuppressive therapy (4), a pilot, prospective, randomized, crossover trial found that 6-month sirolimus therapy, unlike conventional therapy, halted the growth of total cyst volume in 15 patients with normal renal function or mild to moderate renal dysfunction (9). However, two subsequent large clinical trials (10,11) failed to show a clear beneficial effect of either sirolimus or everolimus in patients with CKD stages 2–3b renal function.

To address whether mTOR inhibitors might have any therapeutic role in more advanced phases of the disease, we tested the effect of sirolimus on disease progression in patients with ADPKD and severe renal insufficiency (SIRENA 2 Study) in the context of a single–center, randomized, 3-year clinical trial

registered in June of 2007 with the ClinicalTrials.gov number NCT01223755 (Supplemental Appendix 1).

Materials and Methods

Patients aged ≥18 years old with ADPKD and eGFR (by Modification of Diet in Renal Disease equation) =15–40 ml/min per 1.73 m^2 and proteinuria ≤0.5 g/24 h were eligible. Those with concomitant glomerular or urinary tract disease, diabetes, cancer, psychiatric disorders, and any condition that might confound data interpretation or prevent full comprehension of the purposes and risks of the study were excluded as well as pregnant or breastfeeding women and women of childbearing potential without effective contraception (the protocol is at http://clintrials.marionegri.it/index.php/electronictrials/completed-electronic-trials.html). Eligible participants identified among patients referring to the Outpatient Clinic of the Unit of Nephrology of the Azienda Ospedaliera Papa Giovanni XXIII who provided written informed consent were randomized between September of 2010 and March of 2012. The study conformed to the principles of the Declaration of Helsinki and was approved by the local ethical committee. It was coordinated, monitored, and reported by the Clinical Research Center for Rare Diseases "Aldo e Cele Daccò," IRCCS—Istituto di Ricerche Farmacologiche "Mario Negri" according to the Consolidated Statement of Reporting Trials guidelines (Supplemental Table 1). Data were recorded locally by an electronic case report form implemented by the Biomedical Technologies Laboratory of the Clinical Research Center. Locations of the source data were specified and listed at the center initiation visit.

Objectives

This single–center, academic, prospective, randomized, open label, blind end point, parallel group trial was organized into two phases. A core study primarily aimed to assess whether 12-month treatment with sirolimus added on to conventional treatment significantly reduced measured GFR decline (12,13) versus conventional treatment alone and was safe. Evidence that sirolimus may safely slow GFR decline would have provided the background for an extension phase to evaluate treatment effect on kidney and cystic growth and progression to ESRD over an additional 2-year follow-up. Because of the discouraging results of the core study, the extension phase was aborted.

Randomization, Allocation Concealment, and Follow-Up

An independent investigator (G. Giuliano) centrally randomized patients by telephone call to sirolimus (Rapamune; Pfizer Inc., New York, NY) or conventional treatment. A computer–generated randomization list (1:1 ratio and four or eight random block size) was created at the Laboratory of Biostatistics of the Clinical Research Center by using SAS software, version 9 (SAS Institute Inc., Cary, NC). Patients and their physicians were aware of treatment allocation, whereas outcome assessors were blinded. Sirolimus was started at 3 mg/d and subsequently titrated to target blood trough levels between 5 and 10 ng/ml. Drug levels were measured by HPLC (14).

BP (mean of three consecutive measurements) and laboratory parameters were evaluated at baseline and every 3 months thereafter. GFR was measured every 6 months by iohexol plasma clearance (12,13). Computed tomography images were acquired and analyzed at baseline and 12 months as previously reported (15,16) (Supplemental Appendix 2).

Stopping Rules

Interim analyses were preplanned at core study end to assess whether, on the basis of predefined safety and efficacy criteria, patients could enter the extension phase (Supplemental Table 2). Statistical stopping criteria were on the basis of analyses of the efficacy outcome. The critical value for the test was set to have a value of 0.005 (analysis 1) or 0.049 (analysis 2). The Data Safety and Monitoring Board (DSMB) (Supplemental Appendices 2 and 3), however, could also stop the study on the basis of clinical judgment of safety and efficacy outcome variables, including treatment–related side effects, new onset (urinary protein excretion >0.5 g/24 h in patients without preexisting proteinuria) or worsening (doubling of 24-hour urinary protein excretion compared with previous values) of proteinuria, and serum creatinine increases >25% compared with previous levels.

Sample Size Estimation

On the basis of data from patients with ADPKD and severe renal insufficiency maintained on conservative therapy in the context of the Ramipril Efficacy in Nephropathy Study (17), we predicted a 1-year mean (SD) GFR reduction versus baseline of 6.31 (±4.47) ml/min per 1.73 m^2. Assuming a 65% reduction from 6.31 to 2.2 ml/min per 1.73 m^2 by sirolimus treatment, we calculated that 20 patients per group had to complete the study to provide the analysis with an 80% power to detect a significantly (two-sided test; α=0.05) different change in GFR between treatment groups.

Statistical Analyses

Statistical analyses were performed according to a modified intention to treat approach (18) without replacing missing data (19) by using the SAS software, version 9 (SAS Institute Inc.) and the STATA software, version 13 (StataCorp., College Station, TX). Between-group changes in clinical and laboratory parameters before and after sirolimus or conventional treatment were assessed by analysis of covariance adjusted for baseline measurements (at randomization). Within-group changes in clinical and laboratory parameters were assessed by paired t test or Wilcoxon rank sum test (for continuous variables) and repeated measures ANOVA or McNemar test (for categorical variables) as appropriate. Relationships between continuous variables were assessed by means of Pearson r or Spearman rho correlation coefficient. Data were expressed as means±SDs or medians and interquartile ranges as appropriate. As per protocol, multiplicity adjustments were not planned for secondary efficacy and safety variables, subgroup analyses, supportive analyses, or sensitivity analyses. All tests were two sided, and $P<0.05$ was deemed statistically significant.

Results

Of 47 assessed patients, one withdrew consent, and five had eGFRs out of range. Of 41 included participants,

21 were randomized to sirolimus added on to conventional treatment, and 20 were randomized to conventional treatment alone (Figure 1). Main patient characteristics were similar between groups (Table 1): 20 patients on sirolimus and 19 patients on conservative therapy only were on antihypertensive therapy, with average numbers of 2.2 and 2.0 medications per patient, respectively.

Safety and Tolerability

In >1 year of follow-up, proteinuria ensued *de novo* in seven patients (33.3%) on sirolimus versus one patient (5.0%) on conventional therapy (P=0.04). Ten patients on sirolimus (47.6%), including seven with new onset of proteinuria, doubled their proteinuria versus baseline compared with three patients (15.0%) on conventional therapy (P=0.02). Among patients on sirolimus, two were prematurely withdrawn because of worsening of proteinuria, and two progressed to ESRD. Serum creatinine increased by >25% versus baseline in ten patients on sirolimus and eight patients on conventional therapy (P=0.62).

Serious adverse events were observed in six patients on sirolimus and six patients on conventional treatment. One event in the sirolimus group (severe peripheral edema) was considered as treatment related (Table 2). There were 81 nonserious adverse events in the sirolimus group and 37 nonserious adverse events in the control group. Treatment-related events included aphthous stomatitis (n=14), acne (n=7; $P<0.001$ and $P<0.01$ versus conventional therapy, respectively), transient watery diarrhea (n=4), and angioedema (n=3). All patients with angioedema were on angiotensin–converting enzyme (ACE) inhibitor therapy. There were also significantly more cases of peripheral edema (18 versus 11; P=0.04) and upper respiratory tract infection (14 versus 6; P=0.03) in patients on sirolimus than in controls (Table 2). Other events were similarly distributed between groups.

GFR Interim Analyses

The above alarming safety parameters prompted the DSMB to anticipate the preplanned interim analyses of the primary efficacy variables with the prespecified decision to stop the study in the case that the analyses would not have detected a statistically significant benefit of sirolimus on the primary efficacy variable of the study. One-year data for interim GFR assessments were available from 16 participants on sirolimus and 17 controls (Figure 1). GFR fell from 26.7±5.8 ml/min per 1.73 m^2 at baseline to 23.3±6.4 ml/min per 1.73 m^2 at 6 months (−13.4±9.3%) and 21.3±6.3 ml/min per 1.73 m^2 at 1 year (−20.7±13.5%) in the sirolimus group ($P<0.001$ versus baseline for both) and from 29.6±5.6 to 26.9±5.4 ml/min per 1.73 m^2 at 6 months (−9.1±7.6%) and 24.9±6.2 ml/min per 1.73 m^2 (−6.5±7.6%) at 1 year in controls ($P<0.001$ versus baseline for both) (Figure 2). At both time points, changes versus baseline did not differ significantly between groups (−0.68 ml/min per 1.73 m^2; 95% confidence interval, −2.35 to 0.99 ml/min per 1.73 m^2; P=0.25 at 6 months and −0.61 ml/min per 1.73 m^2; 95% confidence interval, −2.57 to 1.35 ml/min per 1.73 m^2; P=0.53 at 1 year). Over the whole observation period, the GFRs similarly declined by 0.4±0.3 and 0.4±0.2 ml/min per 1.73 m^2 per month in the sirolimus and conventional treatment groups, respectively (between-group difference: 0.05 ml/min per 1.73 m^2;

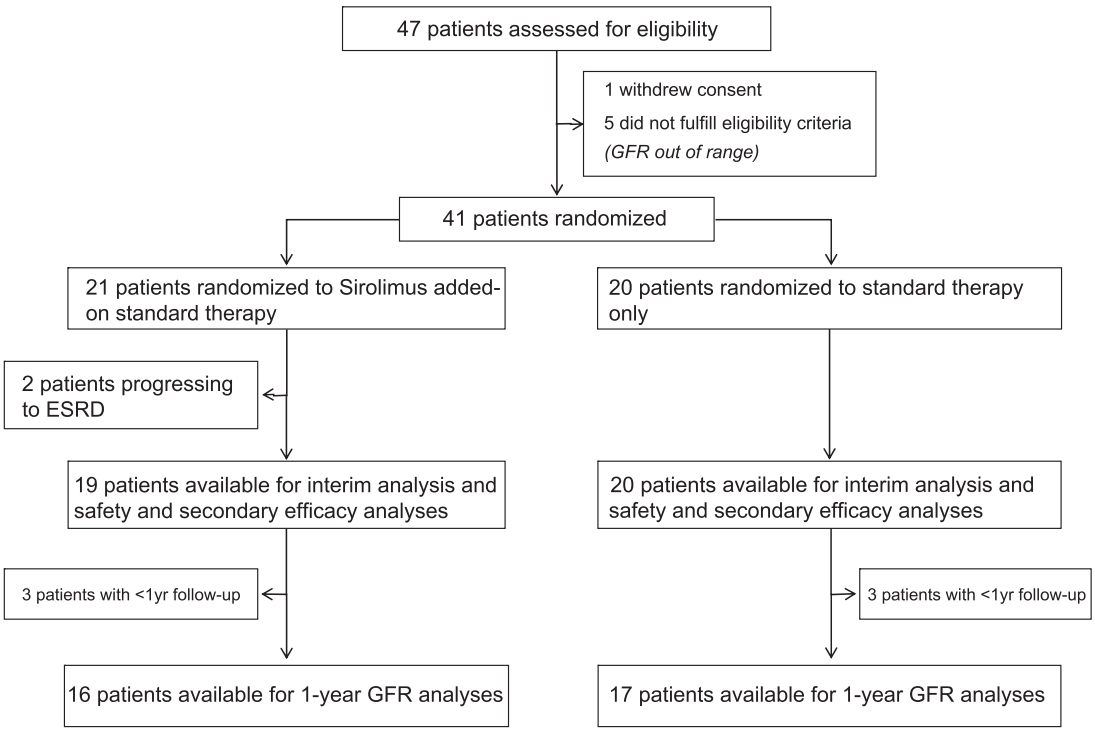

Figure 1. | **Study flow diagram.**

Table 1. Demographic, anthropometric, clinical, laboratory, and kidney function parameters and concomitant medications at baseline according to randomization to sirolimus added on to conventional therapy (sirolimus) or conventional therapy only (conventional)

Patients Parameters and Medications	Sirolimus, *n*=21	Conventional, *n*=20
Age, yr	49.0 (7.1)	47.6 (8.1)
Men, no. (%)	9 (42.9)	8 (40.0)
Height, cm	168.7 (10.1)	168.5 (10.3)
Weight, kg	73.5 (14.3)	73.8 (17.8)
BP, mmHg		
Systolic	136.3 (10.6)	133.8 (14.4)
Diastolic	86.1 (7.7)	85.5 (8.4)
Mean	102.8 (7.8)	101.6 (9.8)
Laboratory parameters		
AST, U/L	18.5 (3.5)	19.4 (5.1)
ALT, U/L	16.7 (4.6)	16.4 (4.9)
GGT, U/L	24.0 (14.9)	22.2 (9.7)
Alkaline phosphatase, U/L	66.2 (14.4)	58.5 (18.5)
Calcium, mg/dl	9.3 (0.3)	9.2 (0.5)
Phosphorus, mg/dl	4.0 (0.5)	3.7 (0.4)
Sodium, mEq/L	139.9 (1.8)	140.0 (1.6)
Potassium, mEq/L	4.3 (0.4)	4.1 (0.6)
Blood glucose, mg/dl	89.8 (11.4)	88.4 (12.2)
Uric acid, mg/dl	6.6 (1.3)	7.1 (1.5)
Total cholesterol, mg/dl	201.7 (27.6)	203.9 (25.7)
LDL cholesterol, mg/dl	125.3 (23.9)	127.5 (32.4)
HDL cholesterol, mg/dl	47.5 (10.3)	51.8 (14.3)
Triglycerides, mg/dl	120.4 (37.2)	105.8 (45.6)
Leukocytes, $\times 10^3/\mu$l	5.7 (1.5)	5.6 (1.8)
Hemoglobin, g/dl	12.3 (1.6)	12.4 (1.2)
Hematocrit, %	37.1 (5.0)	37.5 (3.5)
Platelets, $\times 10^3/\mu$l	194.2 (56.4)	188.1 (46.6)
Kidney function parameters		
Serum creatinine, mg/dl	2.89 (0.62)	2.52 (0.49)
GFR, ml/min per 1.73 m^2	26.8 (5.6)	30.8 (6.6)
Albuminuria, μg/min	43.0 (23.8–84.1)	53.4 (42.8–131.7)
Proteinuria, g/24 h	0.25 (0.16–0.36)	0.24 (0.15–0.45)
Concomitant medications, no. (%)		
ACE inhibitors	12 (57.1)	11 (55.0)
ARBs	7 (33.3)	8 (40.0)
CCBs	9 (42.9)	5 (25.0)
α-Blocking agents	2 (9.5)	6 (30.0)
β-Blockers	7 (33.3)	3 (15.0)
Diuretics	6 (28.6)	5 (25.0)
Statins	2 (9.5)	3 (15.0)
Anticoagulants	0 (–)	2 (10.0)
Iron	1 (4.8)	1 (5.0)
ESAs	3 (14.3)	1 (5.0)
Calcium	1 (4.8)	1 (5.0)
Vitamin D	5 (23.8)	6 (30.0)
Bicarbonate	2 (9.5)	2 (10.0)
PPIs	3 (14.3)	3 (15.0)

Values are mean (SD), median (interquartile range), or number (percentage). GFR was by the iohexol plasma clearance technique. Mean BP = (systolic BP +2× diastolic BP)/3. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl-transpeptidase; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; ESA, erythropoiesis-stimulating agent; PPI, proton pump inhibitor.

95% confidence interval, −0.99 to 2.35 ml/min per 1.73 m^2 per month).

Study Interruption

On the basis of observed adverse events and GFR data, the DSMB decided to stop the study because of safety and futility. The Steering Committee accepted this recommendation and on July 16, 2012, instructed the investigators to stop treatment but complete all of the planned evaluations at the 1-year follow-up whenever feasible.

Kidney Function

Albuminuria significantly increased in the sirolimus group at 6 ($P<0.01$) and 12 months ($P<0.01$) after

Table 2. Number (percentage) of patients with at least one serious or nonserious adverse event over the 1-year follow-up period according to randomization to sirolimus added on to conventional therapy (sirolimus) or conventional therapy only (conventional)

Adverse Events	Sirolimus, *n*=21	Conventional, *n*=20
Serious		
ESRD	2 (9.5)	0
Acute diverticulitis	1 (4.8)	0
Anal fissures, broncopneumonia[a]	1 (4.8)	0
Peripheral edema[b]	1 (4.8)	0
Renal cyst rupture	1 (4.8)	0
Inguinal hernia, gastroenteritis, pneumonia[a]	0	1 (5)
Chest pain	0	1 (5)
Acute kidney function worsening	0	1 (5)
Atrial fibrillation, ventricular extrasystoles[a]	0	1 (5)
Acute bronchitis	0	1 (5)
Hematuria	0	1 (5)
Nonserious		
Peripheral edema	18 (85.7)	11 (55.0)[c]
Aphthous stomatitis[b]	14 (66.7)	0[d]
Upper respiratory tract infections	14 (66.7)	6 (30.0)[c]
Acne[b]	7 (33.3)	0[c]
Dyspepsia	5 (23.8)	2 (10.0)
Diarrhea[b]	4 (19.0)	0
Dysmenorrhea	4 (19.0)	1 (5.0)
Arrhythmias	4 (19.0)	4 (20.0)
Dermatitis	3 (14.3)	1 (5.0)
Urinary tract infections	3 (14.3)	5 (25.0)
Hematuria	2 (9.5)	7 (35.0)
Angioedema[b]	3 (14.3)	0

[a]Events observed in the same patient.
[b]Treatment-related events according to the investigators' judgment.
[c]$P<0.05$ versus sirolimus.
[d]$P<0.001$ versus sirolimus.

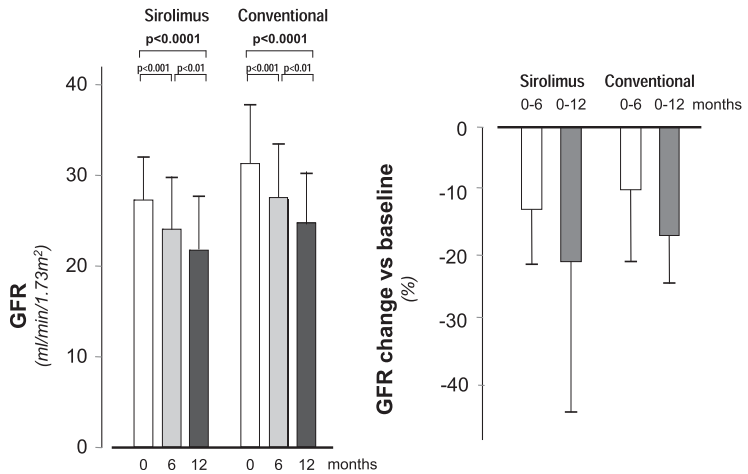

Figure 2. | **GFR changes during the study according to treatment groups.** Mean±SD. GFR at baseline and 6 and 12 months of follow-up (left panel) and percentage GFR changes at 6 and 12 months of follow-up versus baseline (right panel). GFR values at different time points and GFR changes versus baseline did not differ significantly between treatment groups.

randomization compared with baseline, whereas an opposite trend to decrease was observed in controls (Figure 3, left panel). At study end, changes between the two treatment groups were significantly different (P=0.003). Consistently, proteinuria progressively increased on sirolimus at 6 (P=0.04) and 12 months (P=0.01) versus baseline and did not change appreciably on conventional treatment (Figure 3, right panel). At 12 months, changes between groups were significantly different ($P<0.01$). Serum creatinine similarly increased in both groups (Table 3).

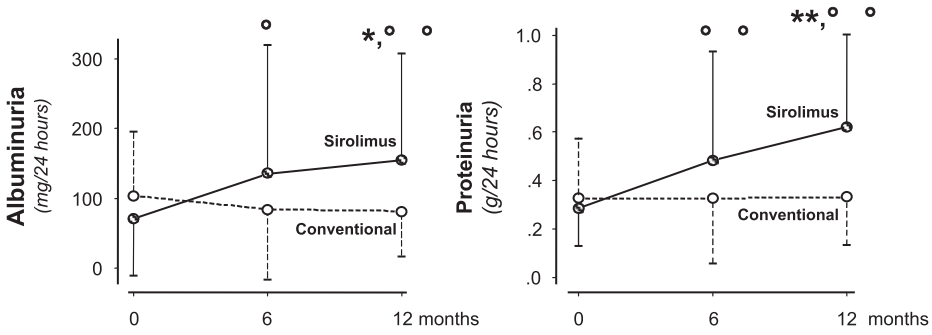

Figure 3. | Changes in twenty-four–hour albuminuria and proteinuria during the study according to treatment groups. Median (interquartile range) 24-hour albuminuria (left panel) and proteinuria (right panel) at baseline and 6 and 12 months of follow-up. Albuminuria and proteinuria both increased in the sirolimus group. At 12 months, changes in both parameters were significantly different between the two treatment groups. *$P<0.05$ versus conventional therapy (analysis of covariance); **$P<0.01$ versus conventional therapy (analysis of covariance); °$P<0.05$ versus baseline; °°$P<0.01$ versus baseline.

Other Parameters

Body weight significantly ($P=0.04$) increased in the conventional treatment compared with the sirolimus group (Table 4). Changes in BP did not significantly differ between groups (Table 4). At 1 year, all study participants were on antihypertensive therapy, with an average number of medications (2.4 in the sirolimus group and 2.3 in the conventional treatment group) that was similar between groups. HDL cholesterol, hemoglobin, and hematocrit values similarly decreased within each group compared with baseline. Changes in the other parameters were unremarkable in both groups, with the exception of serum calcium, which significantly decreased in patients on sirolimus compared with controls ($P=0.002$).

Volumetric Analyses

At study closure, total kidney volume (TKV) data were available from eight and 11 patients on sirolimus or conventional treatment, respectively. TKV slightly increased from 2857.7±1447.3 to 3094.6±1519.5 ml and from 3123.4±1695.3 to 3222.6±1651.4 ml in the sirolimus and conventional treatment groups, respectively (between-group difference: 137.6 ml; 95% confidence interval, −27.7 to 303.0 ml; $P=0.12$) (Figure 4). The percentage TKV increases (8.99±7.06% versus 4.30±5.01%) tended to be larger in the sirolimus group than in the conventional treatment group ($P=0.13$). Cystic volumes increased by 10.4±10.7% on sirolimus and 3.8±4.0% on conservative therapy ($P=0.31$), whereas parenchymal volumes were relatively stable in both groups (Supplemental Appendix 3).

Sirolimus Pharmacokinetic Parameters

Throughout 1 year of follow-up, the total and body weight–adjusted doses of sirolimus averaged 2.2±0.7 mg/d (range =0.5–3 mg/d) and 0.03±0.01 mg/kg per day (0.01–0.07 mg/kg per day), respectively. Trough blood

Table 3. Renal function parameters at baseline and the end of the 1-year treatment period according to randomization to sirolimus added on to conventional therapy (sirolimus) or conventional therapy only (conventional)

Renal Function Parameters	Sirolimus		Conventional	
	Baseline, n=16	1 yr, n=16	Baseline, n=17	1 yr, n=17
Diuresis, ml/24 h	2268.8 (648.3)	2245.6 (851.2)	2287.7 (560.4)	2508.4 (639.8)
Serum creatinine, mg/dl	3.02 (0.59)	4.03 (1.03)[a]	2.63 (0.45)	3.35 (0.83)[a]
GFR, ml/min per 1.73 m^2	26.7 (5.8)	21.3 (6.3)[a]	29.6 (5.6)	24.9 (6.2)[b]
Albuminuria, μg/min	46.3 (26.0–80.9)	101.7 (50.5–194.6)[b]	53.7 (48.3–120.9)	69.5 (33.6–103.1)[a,c]
Proteinuria, g/24 h	0.28 (0.17–0.37)	0.49 (0.39–0.70)[a]	0.27 (0.15–0.45)	0.29 (0.17–0.44)[d]

Data are means (SDs) or medians (interquartile ranges). GFR was measured by the iohexol plasma clearance technique.
[a]$P<0.01$ versus baseline (paired t test).
[b]$P<0.05$ versus baseline (paired t test).
[c]$P<0.05$ versus sirolimus adjusted for baseline value (analysis of covariance).
[d]$P<0.01$ versus sirolimus adjusted for baseline value (analysis of covariance).

Table 4. Clinical and laboratory parameters at randomization and the end of the 1-year treatment period according to randomization to sirolimus added on to conventional therapy (sirolimus) or conventional therapy only (conventional)

Patients Parameters	Sirolimus		Conventional	
	Baseline, *n*=16	1 yr, *n*=16	Baseline, *n*=17	1 yr, *n*=17
Weight, kg	73.5 (15.2)	72.0 (13.9)	74.2 (17.2)	75.4 (19.0)[a]
SBP, mmHg	138.3 (9.5)	132.8 (12.7)	134.1 (13.3)	127.9 (11.6)[b]
DBP, mmHg	87.1 (8.3)	84.8 (7.3)	85.9 (7.2)	81.9 (6.4)[b]
MAP, mmHg	102.8 (7.8)	100.8 (8.8)	101.6 (9.8)	97.2 (7.1)[b]
AST, U/L	19.1 (3.1)	17.6 (2.8)	19.6 (5.5)	17.4 (4.6)[c]
ALT, U/L	17.8 (4.4)	17.3 (3.6)	16.6 (5.2)	15.1 (6.0)
GGT, U/L	27.4 (14.4)	24.6 (12.9)	23.7 (9.8)	21.5 (9.7)
Alkaline phosphatase, U/L	67.0 (14.4)	63.9 (11.3)	61.4 (18.3)	57.6 (17.7)
Calcium, mg/dl	9.4 (0.3)	8.9 (0.5)[b]	9.2 (0.5)	9.2 (0.5)[d]
Phosphorus, mg/dl	3.9 (0.5)	4.0 (0.8)	3.7 (0.4)	4.0 (0.6)[b]
Sodium, mEq/L	140.3 (1.6)	139.5 (1.1)	140.0 (1.5)[e]	139.5 (2.0)
Potassium, mEq/L	4.3 (0.4)	4.2 (0.5)	4.1 (0.7)	4.3 (0.6)
Blood glucose, mg/dl	90.1 (12.3)	88.4 (8.7)	89.4 (11.7)	88.6 (8.9)
Uric acid, mg/dl	6.4 (1.4)	6.3 (1.0)	7.3 (1.4)	6.3 (1.0)[c]
Total cholesterol, mg/dl	198.4 (23.2)	189.9 (24.0)	201.6 (26.8)	188.7 (34.8)
LDL cholesterol, mg/dl	123.6 (18.1)	121.6 (22.6)	124.4 (31.8)	116.1 (28.5)
HDL cholesterol, mg/dl	46.7 (10.4)	42.9 (8.8)[c]	51.3 (14.0)	46.6 (13.5)[c]
Triglycerides, mg/dl	116.3 (39.9)	114.4 (25.3)	108.1 (46.7)	103.9 (43.9)
Leukocytes, $\times 10^3/\mu$l	5.3 (1.5)	5.2 (1.3)	5.1 (0.8)	5.0 (1.0)
Hemoglobin, g/dl	12.7 (1.5)	12.0 (1.3)[c]	12.4 (1.3)	11.6 (1.3)[b]
Hematocrit, %	38.5 (4.6)	36.4 (3.5)[c]	37.6 (3.7)	34.6 (3.6)[b]
Platelets, $\times 10^3/\mu$l	188.1 (56.8)	176.5 (53.2)	182.8 (38.0)	184.2 (37.7)

Data are means (SDs). SBP, systolic BP; DBP, diastolic BP; MAP, mean arterial pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl-transpeptidase.
[a]$P<0.05$ versus sirolimus adjusted for baseline value (analysis of covariance).
[b]$P<0.01$ versus baseline (paired *t* test).
[c]$P<0.05$ versus baseline (paired *t* test).
[d]$P<0.01$ versus sirolimus adjusted for baseline value (analysis of covariance).
[e]XXX.

levels averaged 6.1±2.8 ng/ml (2.5–20.7 ng/ml), whereas levels normalized for concomitant sirolimus dosages averaged 3.1±1.6 ng/ml per milligram (0.9–10.6 ng/ml per milligram). On follow-up, 37% and 7% of sirolimus trough blood levels fell below or exceeded the therapeutic range (5–10 ng/ml), respectively (Supplemental Figure 1). In the sirolimus treatment arm, we found no significant relationship between sirolimus trough levels—averaged throughout the whole treatment period or considered separately at each single time point—and different considered outcomes, including side effects and changes in GFR, albuminuria, and proteinuria.

Discussion

We primarily aimed to analyze whether sirolimus added on to conventional therapy allowed slowing of GFR decline and secondarily, kidney volume growth in ADPKD with severe renal insufficiency. The 1-year interim analysis had been planned to establish whether the trial could be continued or had to be stopped because of efficacy, futility, or safety reasons. Actually, the alarming cumulative incidence of treatment–related adverse events prompted the Safety Board to anticipate main efficacy analyses to assess whether an even initial benefit on GFR decline could be detected that could justify study prosecution, despite the excess of side effects in the sirolimus arm. Among major reasons of concern were progression to ESRD of two patients in addition to three patients with angioedema and two premature discontinuations from the study because of worsening of proteinuria in the sirolimus group. Moreover, proteinuria ensued *de novo* in seven patients on sirolimus but only one control. Thus, on the basis of safety outcomes and 1-year GFR data, the Safety Board decided to stop the study.

Safety

The three patients with angioedema were on concomitant treatment with ACE inhibitors. The excess risk of angioedema (ranging from minor facial edema up to life–threatening throat and mouth swelling) associated with mTOR and ACE inhibitor combination therapy could be explained by defective degradation of the vasoactive peptides bradykinin or substance P when ACE is inhibited (20,21). Bradykinin is inactivated by aminopeptidase P (22), whereas substance P is inactivated by dipeptidyl peptidase IV (23). Decreased dipeptidyl peptidase IV activity has been observed in patients with ACE inhibitor–associated

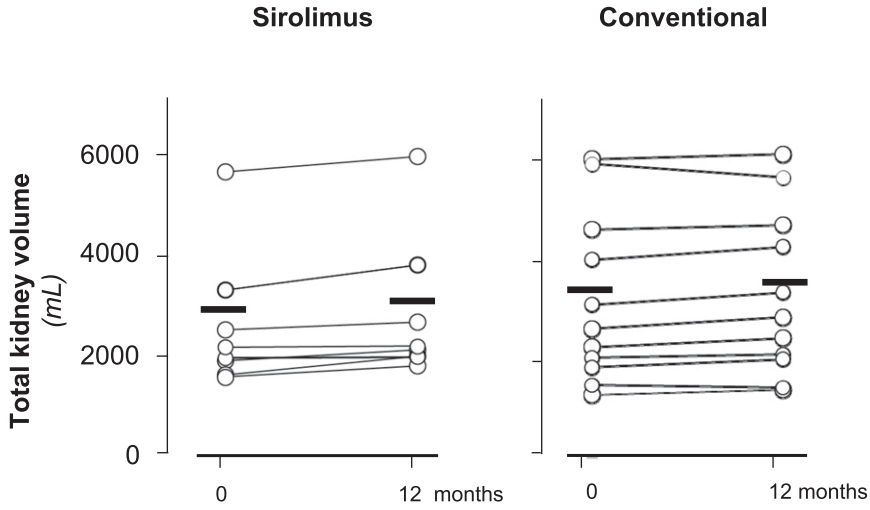

Figure 4. | **Individual patient total kidney values at baseline and 12 months of follow-up according to treatment groups.** No significant change was observed in the sirolimus (left panel) and conventional therapy (right panel) groups. Circles identify single-patient data, and horizontal thick lines denote mean values.

angioedema. A 60% additional decrease can be observed with sirolimus (24), which might explain the increased risk of angioedema in patients receiving sirolimus and ACE inhibitor combination therapy. Thus, the possibility that sirolimus treatment might hinder the safe continuation of ACE inhibitor therapy might have major clinical implications in this context, because ACE inhibitors, in addition to exerting specific cardioprotective effects, have been reported to be renoprotective in children with ADPKD and glomerular hyperfiltration (25) as well as adults, particularly those with more severe proteinuria (26).

Increasing proteinuria led to premature withdrawal of two patients from the sirolimus group. Proteinuria doubled in ten patients on sirolimus versus three controls and ensued *de novo* in seven patients on sirolimus versus only one control. Proteinuria was reported to increase in renal transplant recipients with chronic allograft dysfunction who had been shifted to sirolimus treatment after withdrawal of a calcineurin inhibitor (27). Proteinuria was typically of glomerular origin (28) and could not be explained just by an increase in GFR associated with cyclosporin withdrawal (29). Finding that sirolimus exacerbated both proteinuria and different markers of podocyte damage in a model of severe puromycin–induced glomerular injury (30) can be taken to suggest that sirolimus may have a direct nephrotoxic effect, particularly in patients with advanced renal disease, such as our patients with ADPKD. Independent of the underlying mechanisms, worsening of proteinuria must be considered as a clinically relevant adverse effect, because proteinuria is a well established risk factor for the progression of chronic nephropathies, including ADPKD (31,32).

Finally, sirolimus therapy was associated with a series of nonserious but disturbing side effects, such as watery diarrhea, abdominal swelling, upper respiratory tract infections, and in particular, aphthous stomatitis, that caused consent withdrawal because of subjective distress for six patients. Down titration of the drug was often necessary to control symptoms. Consequently, in about 40% of measurements, sirolimus trough blood levels failed to fit the target range. This is a major limitation to sirolimus therapy, because underdosage or poor compliance to the drug dictated by its poor safety profile and tolerability is one of the possible explanations for treatment failure. The narrow therapeutic window of sirolimus might be an even more stringent limitation in everyday clinical practice, in particular in a fragile population of patients with ADPKD and severe renal insufficiency, such as those under consideration here.

Efficacy

At the 1-year interim analysis, sirolimus showed no appreciable protective effect against progressive GFR loss. GFR reduction even tended to be larger in the sirolimus group than in controls, particularly over the first 6 months after randomization. Previous large trials with mTOR inhibitors in patients with ADPKD and relatively preserved renal function (10,11) showed that sirolimus or everolimus did not affect renal function decline. Thus, available data converge to indicate that mTOR inhibitors have no appreciable protective effect against progressive renal function loss, independent of the level of initial GFR. Within the limitations of the small sample size, finding that sirolimus did not seem to appreciably affect TKV increase provided additional evidence that mTOR inhibition is devoid of any specific renoprotective effect, at least in this context.

Limitations and Strengths

Because of reduced exposure to radiocontrast agents, reliable data for subanalyses of different components of kidney volumes could be obtained only from a minority of patients (Supplemental Appendices 2 and 3). This limitation, however, did not affect TKV analyses as well as safety and GFR analyses. Failure to detect significant associations between sirolimus levels and considered outcomes was most likely explained by the relatively small

sample size, the wide data fluctuations (particularly in sirolimus levels), and the confounding effect of changes introduced in sirolimus dosing to target therapeutic range and limit side effects. Direct measurement of GFR by a gold standard technique (12) was a major strength that allowed powerful analyses and avoided the limitations of estimation equations (13). In the context of the prospective, randomized, open label, blind end point design, the assessors of outcome variables were blinded to treatment. Moreover, all study participants were given the best available therapy. Finally, the two treatment regimens were evaluated in the context of daily clinical practice, which increases the generalizability of study findings to the average population of patients with ADPKD and advanced renal involvement.

Altogether, our findings and those from previous trials in patients with normal or mildly reduced renal function (10,11) can be taken to suggest that mTOR inhibitors do not seem to offer a valuable therapeutic option for patients with ADPKD, independent of their residual kidney function. Although some data suggest that low doses of sirolimus might offer some benefit (33) and ongoing trials are investigating whether pulsed oral administration may improve the drug risk/benefit profile (NCT02055079), future research efforts should probably focus on much safer medications with larger therapeutic windows and stronger evidence of efficacy in this context (34,35).

Acknowledgments

We thank the participants of the SIRENA 2 Study; all trial investigators; the nephrologists, radiologists, and nurses of the participating centers for their invaluable assistance; the laboratory, medical imaging, and regulatory affairs staff; the trial monitors, data managers, and statisticians; and everyone at the Clinical Research Center for Rare Diseases "Aldo e Cele Daccò," IRCCS—Istituto di Ricerche Farmacologiche "Mario Negri" for their efforts in making this study possible.

Part of this work was funded by TranCYST Marie Curie Initial Training Networks project within 7th European Community Framework programme EU-FP7/2007-2013 grant 317246 (TranCYST).

Pfizer Italia (Latina, Italy) and formerly, Wyeth Lederle (Aprilia, Italy) freely supplied sirolimus but did not fund the study, and they were not involved in the planning and conducting of the study or the data analyses and reporting.

Members of the SIRENA 2 Study organization are listed in Supplemental Appendix 1.

Disclosures

None.

References

1. Gabow PA: Autosomal dominant polycystic kidney disease. *N Engl J Med* 329: 332–342, 1993
2. Yamaguchi T, Nagao S, Wallace DP, Belibi FA, Cowley BD, Pelling JC, Grantham JJ: Cyclic AMP activates B-Raf and ERK in cyst epithelial cells from autosomal-dominant polycystic kidneys. *Kidney Int* 63: 1983–1994, 2003
3. Bhunia AK, Piontek K, Boletta A, Liu L, Qian F, Xu PN, Germino FJ, Germino GG: PKD1 induces p21(waf1) and regulation of the cell cycle via direct activation of the JAK-STAT signaling pathway in a process requiring PKD2. *Cell* 109: 157–168, 2002
4. Shillingford JM, Murcia NS, Larson CH, Low SH, Hedgepeth R, Brown N, Flask CA, Novick AC, Goldfarb DA, Kramer-Zucker A, Walz G, Piontek KB, Germino GG, Weimbs T: The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. *Proc Natl Acad Sci U S A* 103: 5466–5471, 2006
5. Fingar DC, Blenis J: Target of rapamycin (TOR): An integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. *Oncogene* 23: 3151–3171, 2004
6. Wu M, Arcaro A, Varga Z, Vogetseder A, Le Hir M, Wüthrich RP, Serra AL: Pulse mTOR inhibitor treatment effectively controls cyst growth but leads to severe parenchymal and glomerular hypertrophy in rat polycystic kidney disease. *Am J Physiol Renal Physiol* 297: F1597–F1605, 2009
7. Tao Y, Kim J, Schrier RW, Edelstein CL: Rapamycin markedly slows disease progression in a rat model of polycystic kidney disease. *J Am Soc Nephrol* 16: 46–51, 2005
8. Zafar I, Belibi FA, He Z, Edelstein CL: Long-term rapamycin therapy in the Han:SPRD rat model of polycystic kidney disease (PKD). *Nephrol Dial Transplant* 24: 2349–2353, 2009
9. Perico N, Antiga L, Caroli A, Ruggenenti P, Fasolini G, Cafaro M, Ondei P, Rubis N, Diadei O, Gherardi G, Prandini S, Panozo A, Bravo RF, Carminati S, De Leon FR, Gaspari F, Cortinovis M, Motterlini N, Ene-Iordache B, Remuzzi A, Remuzzi G: Sirolimus therapy to halt the progression of ADPKD. *J Am Soc Nephrol* 21: 1031–1040, 2010
10. Serra AL, Poster D, Kistler AD, Krauer F, Raina S, Young J, Rentsch KM, Spanaus KS, Senn O, Kristanto P, Scheffel H, Weishaupt D, Wüthrich RP: Sirolimus and kidney growth in autosomal dominant polycystic kidney disease. *N Engl J Med* 363: 820–829, 2010
11. Walz G, Budde K, Mannaa M, Nürnberger J, Wanner C, Sommerer C, Kunzendorf U, Banas B, Hörl WH, Obermüller N, Arns W, Pavenstädt H, Gaedeke J, Büchert M, May C, Gschaidmeier H, Kramer S, Eckardt KU: Everolimus in patients with autosomal dominant polycystic kidney disease. *N Engl J Med* 363: 830–840, 2010
12. Gaspari F, Perico N, Matalone M, Signorini O, Azzollini N, Mister M, Remuzzi G: Precision of plasma clearance of iohexol for estimation of GFR in patients with renal disease. *J Am Soc Nephrol* 9: 310–313, 1998
13. Ruggenenti P, Gaspari F, Cannata A, Carrara F, Cella C, Ferrari S, Stucchi N, Prandini S, Ene-Iordache B, Diadei O, Perico N, Ondei P, Pisani A, Buongiorno E, Messa P, Dugo M, Remuzzi G; GFR-ADPKD Study Group: Measuring and estimating GFR and treatment effect in ADPKD patients: Results and implications of a longitudinal cohort study. *PLoS One* 7: e32533, 2012
14. Cattaneo D, Perico N, Gaspari F: Assessment of sirolimus concentrations in whole blood by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 774: 187–194, 2002
15. Ibanez L, Schroeder W, Ng L, Cates J: *The ITK SoftwareGuide*, 2nd Ed., Albany, NY, Kitware Inc., 2005
16. Chapman AB, Bost JE, Torres VE, Guay-Woodford L, Bae KT, Landsittel D, Li J, King BF, Martin D, Wetzel LH, Lockhart ME, Harris PC, Moxey-Mims M, Flessner M, Bennett WM, Grantham JJ: Kidney volume and functional outcomes in autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol* 7: 479–486, 2012
17. Ruggenenti P, Perna A, Gherardi G, Benini R, Remuzzi G: Chronic proteinuric nephropathies: Outcomes and response to treatment in a prospective cohort of 352 patients with different patterns of renal injury. *Am J Kidney Dis* 35: 1155–1165, 2000
18. Gupta SK: Intention-to-treat concept: A review. *Perspect Clin Res* 2: 109–112, 2011
19. Carpenter JR, Kenward M: Missing Data in Randomised Controlled Trials—A Practical Guide. Birmingham: National Institute for Health Research, Publication RM03/JH17/MK, 2008. Available at: http://www.missingdata.org.uk. Accessed February 8, 2016
20. Burdese M, Rossetti M, Guarena C, Consiglio V, Mezza E, Soragna G, Gai M, Segoloni GP, Piccoli GB: Sirolimus and ACE-inhibitors: A note of caution. *Transplantation* 79: 251–252, 2005
21. Duerr M, Glander P, Diekmann F, Dragun D, Neumayer HH, Budde K: Increased incidence of angioedema with ACE inhibitors in combination with mTOR inhibitors in kidney transplant recipients. *Clin J Am Soc Nephrol* 5: 703–708, 2010
22. Ward PE, Chow A, Drapeau G: Metabolism of bradykinin agonists and antagonists by plasma aminopeptidase P. *Biochem Pharmacol* 42: 721–727, 1991

23. Ahmad S, Wang L, Ward PE: Dipeptidyl(amino)peptidase IV and aminopeptidase M metabolize circulating substance P in vivo. *J Pharmacol Exp Ther* 260: 1257–1261, 1992
24. Byrd JB, Woodard-Grice A, Stone E, Lucisano A, Schaefer H, Yu C, Eyler AE, Salloum NE, Brown NJ: Association of angiotensin-converting enzyme inhibitor-associated angioedema with transplant and immunosuppressant use. *Allergy* 65: 1381–1387, 2010
25. Cadnapaphornchai MA, McFann K, Strain JD, Masoumi A, Schrier RW: Prospective change in renal volume and function in children with ADPKD. *Clin J Am Soc Nephrol* 4: 820–829, 2009
26. Jafar TH, Stark PC, Schmid CH, Strandgaard S, Kamper AL, Maschio G, Becker G, Perrone RD, Levey AS; ACE Inhibition in Progressive Renal Disease (AIPRD) Study Group: The effect of angiotensin-converting-enzyme inhibitors on progression of advanced polycystic kidney disease. *Kidney Int* 67: 265–271, 2005
27. Kuypers DR: Benefit-risk assessment of sirolimus in renal transplantation. *Drug Saf* 28: 153–181, 2005
28. Rangan GK: Sirolimus-associated proteinuria and renal dysfunction. *Drug Saf* 29: 1153–1161, 2006
29. Saurina A, Campistol JM, Piera C, Diekmann F, Campos B, Campos N, de las Cuevas X, Oppenheimer F: Conversion from calcineurin inhibitors to sirolimus in chronic allograft dysfunction: Changes in glomerular haemodynamics and proteinuria. *Nephrol Dial Transplant* 21: 488–493, 2006
30. Torras J, Herrero-Fresneda I, Gulias O, Flaquer M, Vidal A, Cruzado JM, Lloberas N, Franquesa M, Grinyó JM: Rapamycin has dual opposing effects on proteinuric experimental nephropathies: Is it a matter of podocyte damage? *Nephrol Dial Transplant* 24: 3632–3640, 2009
31. Chapman AB, Johnson AM, Gabow PA, Schrier RW: Overt proteinuria and microalbuminuria in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 5: 1349–1354, 1994
32. Gabow PA, Johnson AM, Kaehny WD, Kimberling WJ, Lezotte DC, Duley IT, Jones RH: Factors affecting the progression of renal disease in autosomal-dominant polycystic kidney disease. *Kidney Int* 41: 1311–1319, 1992
33. Braun WE, Schold JD, Stephany BR, Spirko RA, Herts BR: Low-dose rapamycin (sirolimus) effects in autosomal dominant polycystic kidney disease: An open-label randomized controlled pilot study. *Clin J Am Soc Nephrol* 9: 881–888, 2014
34. Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Grantham JJ, Higashihara E, Perrone RD, Krasa HB, Ouyang J, Czerwiec FS; TEMPO 3:4 Trial Investigators: Tolvaptan in patients with autosomal dominant polycystic kidney disease. *N Engl J Med* 367: 2407–2418, 2012
35. Caroli A, Perico N, Perna A, Antiga L, Brambilla P, Pisani A, Visciano B, Imbriaco M, Messa P, Cerutti R, Dugo M, Cancian L, Buongiorno E, De Pascalis A, Gaspari F, Carrara F, Rubis N, Prandini S, Remuzzi A, Remuzzi G, Ruggenenti P; ALADIN study group: Effect of longacting somatostatin analogue on kidney and cyst growth in autosomal dominant polycystic kidney disease (ALADIN): A randomised, placebo-controlled, multicentre trial. *Lancet* 382: 1485–1495, 2013

Received: September 23, 2015 **Accepted:** January 26, 2016

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Published online ahead of print. Publication date available at www.cjasn.org.

This article contains supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.09900915/-/DCSupplemental.

Supplementary Table 1

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4, 5-6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	2,5-6, 7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	7-8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	7,10, Suppl Tab 2
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	6
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	6
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to	6

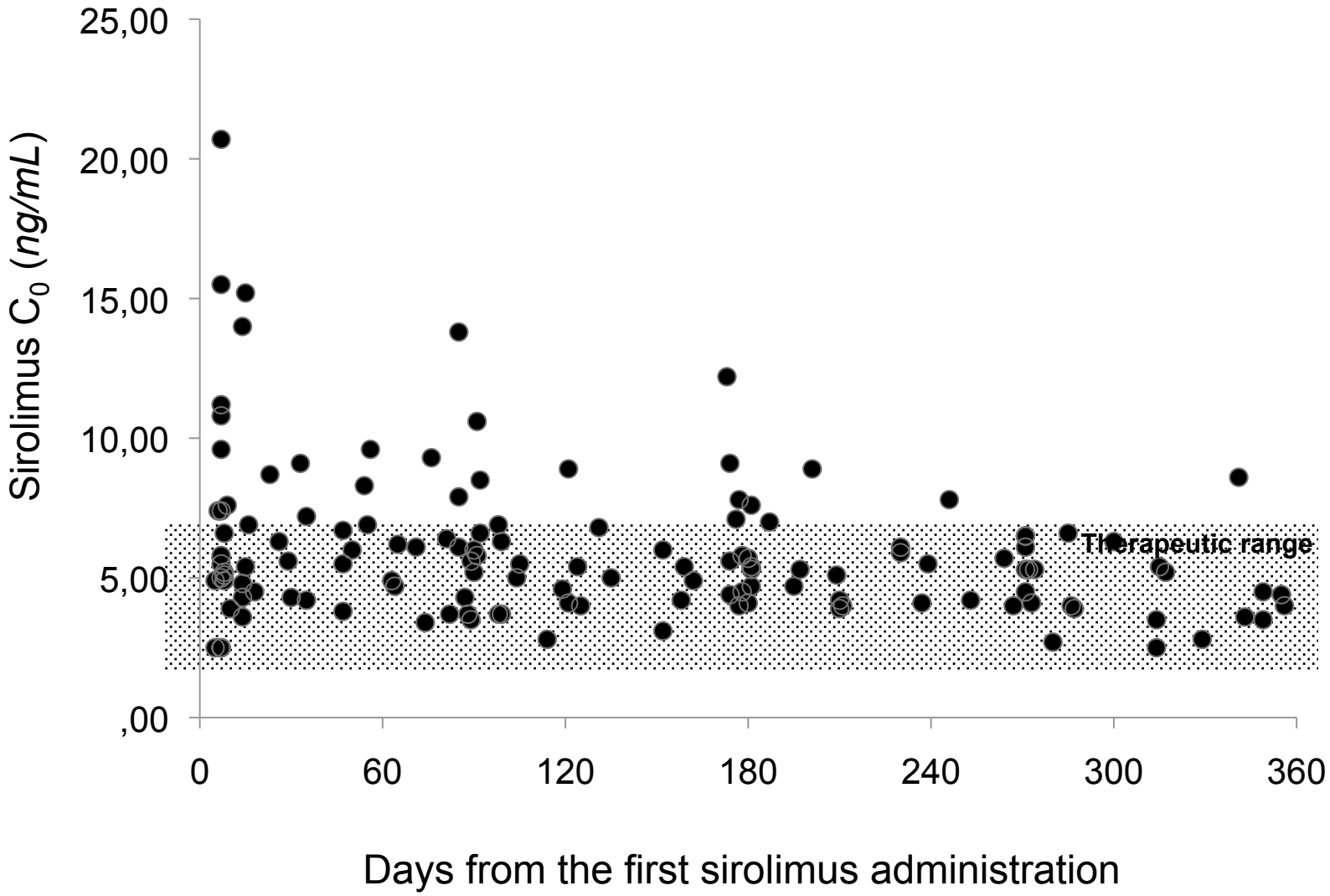
		interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	9, figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	9, figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	9, 10, 11
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8,9-12
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	9-12
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	9-10
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	9-12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	9-11
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13-16
Other information			
Registration	23	Registration number and name of trial registry	2,4
Protocol	24	Where the full trial protocol can be accessed, if available	5
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	18,19

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Supplementary Table 2. Stopping rules for the interim analysis of the SIRENA 2 trial.

Difference in favour of	**Interim analysis p-value**	**Stopping rule**
Sirolimus	$p < 0.005$	Stop the study early
Sirolimus	$p \geq 0.005$ (unless $p \geq 0.049$ and Δ excluded from the 99% CI	Complete the study as planned
Sirolimus	$p \geq 0.049$ and Δ excluded from the 99% CI	Stop the trial for futility
Conventional treatment	$p \geq 0.05$ and Δ included in the 99% CI	Stop the trial for futility (impossibility to obtain a reversal of the results at this point of the study)
Conventional treatment	$p < 0.05$ or $p \geq 0.05$ and Δ excluded from the 99% CI	Stop the trial for emerging evidence of the superiority of conventional treatment

Δ = minimum important difference of the primary end point in favour of sirolimus of 0.95 mL/min/1.73 m^2 (i.e. from -6.31 to -5.36 mL/min/1.73 m^2). 99% CI= 99% confidence interval for the difference in the primary end point



Supplemental Figure 1: Serum sirolimus levels at different time points during the study period. The gray area encompasses the therapeutic range.

Appendix 1

SIRENA 2 STUDY ORGANIZATION

Members of the SIRENA 2 Study Organization *(all in Italy unless otherwise noted):*

Principal investigator — G. Remuzzi (Bergamo, Italy); *Study coordinators* —Norberto Perico (Bergamo, Italy) and P. Ruggenenti (Bergamo, Italy); *Coordinating Center* - IRCCS - Mario Negri Institute for Pharmacological Research , Clinical Research Center for Rare Diseases Aldo e Cele Daccò , Villa Camozzi, Ranica (Bergamo, Italy); *Partecipating Center* - G. Remuzzi, N. Perico, P. Ruggenenti, M. Trillini, S. Rota, S. Prandini, V. Lecchi, G. Gherardi, L. Barcella, G. Fasolini (Azienda Ospedaliera Papa Giovanni XXIII, Unit of Nephrology and Dialysis, and Unit of Radiology, and Clinical Research Center for Rare Diseases Aldo e Cele Daccò of the IRCCS - Mario Negri Institute for Pharmacological Research); *Monitoring and Drug Distribution* (Mario Negri Institute) - N. Rubis, O. Diadei, A. Villa; *Database and Data Validation* (Mario Negri Institute) - D. Martinetti, S. Carminati, B. Ene-Iordache; *Data Analysis* (Mario Negri Institute) - A. Perna, A. Russo, G. Gentile and G. A. Giuliano; *Medical Imaging* (Mario Negri Institute) – A. Remuzzi, A. Caroli, L. Antiga, K. Sharma, C. P. Ferrer Siles, J. A. Reyes Loaeza and M. C. Aparicio; *Laboratory Measurements* (Mario Negri Institute) – F. Gaspari, F. Carrara and M. Cortinovis ; *Regulatory Affairs* (Mario Negri Institute) – P. Boccardo and S. Peracchi; *Data and Safety Monitoring Board* – E. Porrini MD (Hospital Universitario de Canarias, Tenerife, Spain), A. Schieppati MD (Ethics Committee, Ospedale Papa Giovanni XXIII, Bergamo), Alejandro Jiménez Sosa StatSciD (Hospital Universitario de Canarias, Tenerife, Spain)

Appendix 2

CT Image Acquisition and Kidney Volumes measurement

CT images were acquired with a 64-slice CT scanner (LightSpeed VCT; GE Healthcare, Milwaukee, WI). A single breath-hold scan (120 kV; 150 to 500 mAs; matrix 512x512; collimation 2.5 mm; slice pitch 0.984; increment 2.5 mm) was initiated 80 seconds after the injection of 100 ml non-ionic iodinated contrast agent (Iomeron 350; Bracco, Italy) at a rate of 2 ml/s, followed by 20 ml physiologic solution at the same injection rate. Once acquired, images were transferred in DICOM 16-bit format from the clinical scanner on digital media for subsequent processing (18) (Appendix 2). Height-adjusted TKV (ht-TKV) was computed by dividing TKV by individual patient height (19). Kidneys were first manually outlined on all acquired digital images by trained operators, who were blind to treatment, using an interactive image editing software (ImageJ, Image processing and Analysis in Java, National Institutes of Health, http://rsbweb.nih.gov/ij/). Main renal blood vessels and hilum were carefully excluded from the outlines, and special attention was given to regions where kidneys and liver were adjacent. Tracing accuracy was double-checked and, whenever needed, manual outlines were corrected by a single operator (K.S.), also blind to treatment, in order to limit potential inter-operator variability. Renal masks were created from manual outlining, and TKV was computed by multiplying the voxel count of the masks by voxel volume, as determined by the acquisition protocol. Volume computation was performed with in-house software based on the Insight Toolkit version 4.5 and developed in the C++ programming language.

Appendix 3

ADDITIONAL VOLUMETRIC ANALYSES

Renal cyst and parenchyma volumes were computed on all contrast-enhanced CT images, on the basis of manually traced kidney outlines, using a volumetric quantification method previously described in detail (Antiga 2006), and adopted in previous ADPKD clinical trials (Ruggenenti 2005, Perico 2010). Briefly, anisotropic diffusion filtering was first used to remove noise while preserving relevant features, such as the boundary between cysts and parenchyma. A histogram-based statistical classification method known as Otsu's thresholding (Otsu 1979*)* was then used to subdivide the outlined kidneys into tissue classes, so as to maximize the between class variance of image intensity values.

As patients involved in the current study had severe renal insufficiency, the exposure to the radiocontrast agent was minimized in order to prevent the risk of contrast nephrotoxicity. Because of this approach, however, some contrast-enhanced CT images were noisy and/or not enough contrasted to reliably differentiate cyst and parenchyma volumes. Moreover, some patients had hemorrhagic cysts (appearing bright on contrast-enhanced CT images), which confounded the tissue classification. For the above reasons, in this patient cohort, renal tissue segmentation required a number of additional processing steps.

Preliminary to Otsu's thresholding, all available acquisitions (n=60) underwent acquisition-specific tuning of the enhancement parameters. Six out of 60 acquisitions did not require any additional step. Twenty acquisitions, displaying up to 5 well-defined misclassified hemorrhagic cysts, required manual correction of the segmented images. On the remaining 34 acquisitions, renal cyst could not be computed due to the presence of several (more than 5) hemorrhagic cysts and heavy mix-up in the classified images, which could not be reliably manually corrected. On 15 out of these 34 acquisitions, parenchymal volume was identified by thresholding enhanced images based on a fixed

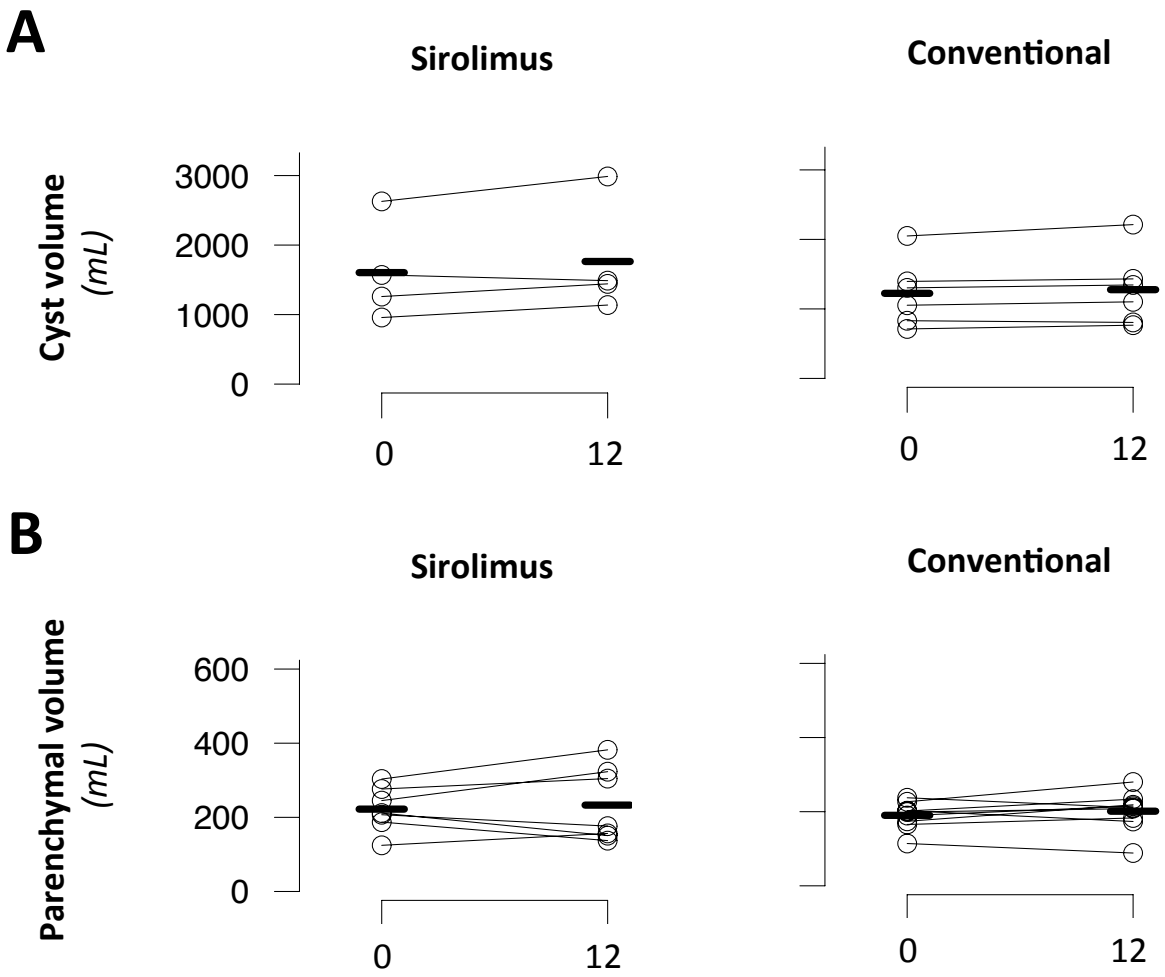
threshold (≥ 74 Hounsfield Units), defined as average parenchymal intensity on acquisitions with no processing problems; on 9 additional acquisitions, thresholding was followed by manual correction, in order to change label of well-defined hemorrhagic cysts misclassified as parenchyma. On the remaining 10 acquisitions parenchymal volume could not be reliably identified, and only total kidney volume could be computed.

From the segmented images, cyst and parenchymal volumes were computed by multiplying the voxel count of each class by voxel volume, as determined by the acquisition protocol. All image processing steps were performed with in-house software based on Insight Toolkit version 4.5 (Ibanez 2005) and developed in the C++ programming language. Manual correction of the classified images was performed with 3DSlicer (Fedorov 2012), using the editor Module.

For each tissue component, only patients with available baseline and 12-month follow-up volume data were included in the analyses.

Both renal cyst and parenchymal volumes did not significantly increase during 12 months of Sirolimus (cyst: from 1604 ± 727 to 1764 ± 831 mL, p=0.18, n=4; parenchyma: from 222 ± 59 to 233 ± 59 ml, p=0.64, n=7) or conventional therapy (cyst: from 1224 ± 482 to 1277 ± 533 mL, p=0.086, n=6; parenchyma: from 190 ± 36 to 201 ± 52 mL, p=0.32, n=9). (Figure A-1).

Figure A-1. Individual cyst (Panel A), and parenchymal (Panel B) volume volumes at baseline at at 12 months of follow-up in the two treatment groups. Horizontal thick segments denote mean values.



REFERENCES

A-1 Antiga L, Piccinelli M, Fasolini G, Ene-Iordache B, Ondei P, Bruno S, Remuzzi G, Remuzzi A: Computed tomography evaluation of autosomal dominant polycystic kidney disease progression: A progress report. Clin J Am Soc Nephrol 2006; 1: 754–760

A-2 Ruggenenti P, Remuzzi A, Ondei P, Fasolini G, Antiga L, Ene-Iordache B, Remuzzi G, Epstein FH. Safety and efficacy of long-acting somatostatin treatment in autosomal-dominant polycystic kidney disease. Kidney Int. 2005; 68:206-216.

A-3 Perico N, Antiga L, Caroli A, Ruggenenti P, Fasolini G, Cafaro M, Ondei P, Rubis N, Diadei O, Gherardi G, Prandini S, Panozo A, Bravo RF, Carminati S, De Leon FR, Gaspari F, Cortinovis M, Motterlini N, Ene-Iordache B, Remuzzi A, Remuzzi G. Sirolimus therapy to halt the progression of ADPKD. J Am Soc Nephrol. 2010; 21(6):1031-1040.

A-4 Otsu N: A threshold selection method from gray-level histogram. IEEE Trans Syst Man Cybern 1979; 9: 62–66

A-5 Ibanez L, Schroeder W, Ng L, Cates J: The ITK Software Guide, 2nd Ed., Albany, Kitware Inc., 2005

A-6 Fedorov A., Beichel R., Kalpathy-Cramer J., Finet J., Fillion-Robin J-C., Pujol S., Bauer C., Jennings D., Fennessy F., Sonka M., Buatti J., Aylward S.R., Miller J.V., Pieper S., Kikinis R. 3D Slicer as an Image Computing Platform for the Quantitative Imaging Network. Magn Reson Imaging. 2012; 30: 1323-1341.