Sirolimus Therapy to Halt the Progression of ADPKD

Norberto Perico,* Luca Antiga,* Anna Caroli,* Piero Ruggenenti,*† Giorgio Fasolini,‡
Mariateresa Cafaro,‡ Patrizia Ondei,† Nadia Rubis,* Olimpia Diadei,* Giulia Gherardi,*
Silvia Prandini,* Andrea Panozo,* Rodolfo Flores Bravo,* Sergio Carminati,*
Felipe Rodriguez De Leon,* Flavio Gaspari,* Monica Cortinovis,* Nicola Motterlini,*
Bogdan Ene-Iordache,* Andrea Remuzzi,*§ and Giuseppe Remuzzi*†

*Clinical Research Center for Rare Diseases, "Aldo e Cele Daccò," Mario Negri Institute for Pharmacological
Research, Bergamo, Italy; Units of †Nephrology and Dialysis and ‡Radiology, Azienda Ospedaliera Ospedali Riuniti
Bergamo, Bergamo, Italy; and §Department of Industrial Engineering, University of Bergamo, Bergamo, Italy

ABSTRACT

Activation of mammalian target of rapamycin (mTOR) pathways may contribute to uncontrolled cell proliferation and secondary cyst growth in patients with autosomal dominant polycystic kidney disease (ADPKD). To assess the effects of mTOR inhibition on disease progression, we performed a randomized, crossover study (The SIRENA Study) comparing a 6-month treatment with sirolimus or conventional therapy alone on the growth of kidney volume and its compartments in 21 patients with ADPKD and GFR ≥40 ml/min per 1.73 m². In 10 of the 15 patients who completed the study, aphthous stomatitis complicated sirolimus treatment but was effectively controlled by topical therapy. Compared with pretreatment, posttreatment mean total kidney volume increased less on sirolimus (46 ± 81 ml; $P = 0.047$) than on conventional therapy (70 ± 72 ml; $P = 0.002$), but we did not detect a difference between the two treatments ($P = 0.45$). Cyst volume was stable on sirolimus and increased by 55 ± 75 ml ($P = 0.013$) on conventional therapy, whereas parenchymal volume increased by 26 ± 30 ml ($P = 0.005$) on sirolimus and was stable on conventional therapy. Percentage changes in cyst and parenchyma volumes were significantly different between the two treatment periods. Sirolimus had no appreciable effects on intermediate volume and GFR. Albuminuria and proteinuria marginally but significantly increased during sirolimus treatment. In summary, sirolimus halted cyst growth and increased parenchymal volume in patients with ADPKD. Whether these effects translate into improved long-term outcomes requires further investigation.

Autosomal dominant polycystic kidney disease (ADPKD) is an inherited systemic disorder with major renal manifestations, which occurs in 1 of 400 to 1000 individuals.[1] ADPKD is genetically heterogeneous. Mutations of the two genes PKD1 (85% of the cases) and PKD 2 (15% of cases), encoding polycystin-1 (PC1) and polycystin-2 (PC2), respectively, are implicated in the disease development.[2] The functions of PC1 and PC2 have not been defined with certainty; however, PC1 is thought to interact with and regulate PC2, which is a member of a subfamily of transient receptor potential channels[3] and may act as a cation channel allowing Ca^{2+}

entry from the extracellular environment. Consistent with the PC1/PC2 complex having a role in Ca^{2+} regulation, PKD epithelial cells display altered

intracellular Ca^{2+} homeostasis,[4] which alters the response to increased levels of intracellular cAMP.[5–7]

Another change consistently found in PKD cells is activation of the Ser/Thr kinase mammalian target of rapamycin (mTOR), an enzyme that coordinates cell growth, cell-cycle progression, and proliferation.[8] mTOR is made up of two distinct complexes: mTORC1 and TORC2. The direct downstream targets of mTORC1, the eukaryotic initiation factor 4E-binding protein and ribosomal protein S6 kinase (p70S6K1),[9,10] tightly regulate the translational initiation machinery to control cell growth and proliferation.[8] *In vitro* studies demonstrated that the N-terminal cytoplasmic domain of PC1 co-localizes and interacts with tuberin.[11] Activated phospho-mTOR and p70S6K are induced in cyst-lining epithelial cells in cysts from human and mouse kidneys.[11] Moreover, p70S6K is increased in Han:SPRD rat kidneys with PKD.[12] These observations led to the hypothesis that defects in PC1 in ADPKD promote disruption of the tuberin-mTOR complex, leading to aberrant mTOR activation and signaling.[11] There is also evidence that IGF-1 by binding to its receptor is a major regulator of the mTOR pathway *via* signaling to phosphatidylinositol-3 kinase, protein kinase B (Akt), and mTOR.[8] Increase in IGF-1 mRNA levels in the kidneys of the pcy mouse model of PKD[13] and in IGF-1 protein in Han:SPRD rats[14] has been reported. In addition, the amount of phospho-Akt in cystic Pkd1$^{-/-}$ mouse kidneys was more than that in wild-type kidneys.[15] Thus, if mTOR is such a converging point in PKD cells, it would be worthwhile as a possible drug target for treatment of renal cystic disorders.

Sirolimus (originally referred to as rapamycin) is a macrocyclic lactone that is derived from *Streptomyces hygroscopicus* and exerts antiproliferative and growth-inhibiting effects as well as antifibrotic effect by inhibition of the mTOR enzyme.[16,17] The drug has been used in kidney transplant recipients as part of maintenance immunosuppressive therapy[18] and more recently as an antitumor agent[19,20] and in drug-eluting stents to prevent coronary artery stenosis.[21] Short-term treatment with sirolimus markedly reduced kidney size and lowered renal total cyst volume (TCV) density in PKD animal models.[11,12,22] In addition, in renal transplant recipients who had progressed to ESRD because of ADPKD, the size of native kidney and liver cysts decreased while on mTOR inhibitor therapy but did not change appreciably during treatment with other immunosuppressants.[11,23]

Thus, to assess formally the risk/benefit profile of mTOR inhibitor therapy in PKD, we designed the Sirolimus Treatment in Patients with Autosomal Dominant Polycystic Kidney Disease: Renal Efficacy and Safety (SIRENA; http://clinicaltrials.gov identifier NCT00491517), a proof-of-concept, randomized clinical trial aimed to compare the changes in total kidney volume (TKV) and in the kidney's various compartments. This was assessed by serial computed tomography (CT) scan evaluations during 6 months of treatment with sirolimus or conventional therapy alone in 21 patients with ADPKD and normal or moderately decreased kidney function. The study secondarily evaluated whether and to which extent treatment-induced changes in kidney volume and structure translated into concomitant changes in GFR as assessed by standard techniques. The results of these analyses formed the basis of this report.

RESULTS

Baseline Clinical and Laboratory Characteristics

Twenty-one patients, 20 with a family history of ADPKD, entered the study. Six patients were prematurely withdrawn from the study: One had an allergic reaction to the contrast agent during the first CT scan evaluation, two withdrew the consent, and three had treatment-related adverse effects (erythema nodosus and thrombocytopenia in two cases) that fully resolved after withdrawal. Thus 15 patients (12 men) completed the study. Among these, seven were randomly assigned to sirolimus followed by conventional treatment and eight to conventional followed by sirolimus therapy. Their main demographic and clinical characteristics at study entry are summarized in Table 1. Ten patients had hypertension and were on antihypertensive therapy with an angiotensin-converting enzyme inhibitor ($n = 8$) or an angiotensin receptor blocker ($n = 2$). Six patients were also on diuretic ($n = 3$) or calcium channel blocker therapy. Estimated GFR was $\geq$40 ml/min per 1.73 m^2, and 24-hour urinary protein excretion rate was $<$0.3 g in all cases. Total (left + right) kidney volumes were heterogeneous within the study group, ranging from 725 to 4605 ml (Table 1). In five patients, TKVs exceeded 2 L. Radiologically detectable cyst volumes ranged from 58 to 70% of TKVs (average 65%).

Safety and Tolerability

Adverse Events

Aphthous stomatitis, acne, and peripheral edema were reported in 10, three, and two patients, respectively, during the treatment period with sirolimus. Two patients also had a wa-

Table 1. Baseline demographic, clinical, and renal structural characteristics

Characteristic	Value
Age (years; mean [range])	39.1 (28 to 46)
Gender (M/F; *n*)	12/3
Body weight (kg)	73.8 ± 15.4 (51.0 to 115.0)
BMI (kg/m^2)	24.2 ± 4.3 (18.7 to 36.3)
Systolic BP (mmHg)	137.6 ± 11.0 (122.0 to 159.0)
Diastolic BP (mmHg)	88.3 ± 7.2 (76.0 to 103.0)
Serum creatinine (mg/dl)	1.27 ± 0.41 (0.79 to 2.00)
eGFR (ml/min per 1.73 m^2)	76.5 ± 28.3 (40.2 to 112.6)
Total cholesterol (mg/dl)	181.2 ± 23.7 (136.0 to 219.0)
Triglycerides (mg/dl)	71.1 ± 28.8 (26.0 to 137.0)
Proteinuria (g/24 h)	0.151 ± 0.055 (0.047 to 0.232)
Total kidney volume (ml)	1874 ± 1057 (725 to 4605)
Cyst volume (ml)	1102 ± 764 (239 to 2891)
Parenchymal volume (ml)	299 ± 81 (157 to 463)
Intermediate volume (ml)	473 ± 281 (162 to 1251)

Data are means ± SD (range), except where noted. BMI, body mass index; eGFR, estimated GFR.

Table 2. Clinical and laboratory parameters before and after sirolimus or conventional treatment

Parameter	Sirolimus		Conventional	
	Before	After	Before	After
Systolic BP (mmHg)	136.7 ± 10.8	133.3 ± 6.9	134.2 ± 7.7	132.9 ± 6.9
Diastolic BP (mmHg)	88.1 ± 6.6	87.1 ± 5.3	87.5 ± 5.0	86.4 ± 6.0
AST (U/L)	21.3 ± 6.1	26.5 ± 8.9[a]	23.5 ± 6.1	21.1 ± 6.1
ALT (U/L)	23.0 ± 12.9	32.8 ± 19.4	26.3 ± 16.7	22.0 ± 12.7
GGT (U/L)	20.3 ± 6.3	24.3 ± 11.3	22.8 ± 9.0	19.3 ± 8.5
Alkaline phosphatase (U/L)	44.2 ± 12.9	51.7 ± 13.4[b]	46.6 ± 14.7	45.3 ± 12.2
Calcium (mg/dl)	9.2 ± 0.2	9.0 ± 0.3	9.2 ± 0.3	9.1 ± 0.4
Phosphorus (mg/dl)	3.7 ± 0.5	3.3 ± 0.7[b]	3.2 ± 0.6	3.6 ± 0.6[a]
Sodium (mEq/L)	139.4 ± 1.3	140.2 ± 1.3	140.0 ± 1.1	140.0 ± 1.7
Potassium (mEq/L)	3.74 ± 0.20	3.66 ± 0.34	3.69 ± 0.31	3.75 ± 0.20
Blood glucose (mg/dl)	92.2 ± 5.9	94.2 ± 9.2	95.6 ± 9.9	90.1 ± 9.4
Uric acid (mg/dl)	5.8 ± 1.6	5.6 ± 1.3	5.7 ± 1.3	6.0 ± 1.7
Total cholesterol (mg/dl)	184.5 ± 27.6	220.5 ± 46.2[a]	202.9 ± 46.6	187.3 ± 30.9
LDL cholesterol (mg/dl)	123.2 ± 30.0	152.3 ± 40.8[c]	135.5 ± 39.1	119.5 ± 30.1[b]
HDL cholesterol (mg/dl)	48.30 ± 11.50	46.30 ± 9.80	45.80 ± 10.10	48.63 ± 13.10
Triglycerides (mg/dl)	77.4 ± 55.1	100.7 ± 34.4[b]	92.5 ± 33.1	88.7 ± 53.1
Leukocytes ($*10^3/\mu l$)	5.9 ± 1.1	5.3 ± 1.5	5.4 ± 1.4	5.9 ± 1.3
Hemoglobin (g/dl)	13.6 ± 1.3	12.7 ± 1.1[a]	13.1 ± 1.1	13.5 ± 1.4
Hematocrit (%)	39.5 ± 3.4	37.7 ± 3.2[b]	38.5 ± 3.2	39.3 ± 3.9
Platelets ($*10^3/\mu l$)	218.0 ± 58.9	225.7 ± 70.5	228.7 ± 61.7	216.6 ± 55.6

Data are means ± SD. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ glutaryl transaminase.
[a]$P < 0.01$; [b]$P < 0.05$; [c]$P < 0.001$ *versus* before.

tery diarrhea that spontaneously recovered within the first few days of sirolimus treatment. Two episodes of urinary tract infection were reported on sirolimus and one of hematuria on conventional therapy alone.

Clinical and Laboratory Parameters
Systolic and diastolic BP did not significantly change during sirolimus or conventional therapy (Table 2). Thus, the antihypertensive therapy remained unchanged during the study interval. Total cholesterol levels significantly increased from 184.5 ± 27.6 to 220.5 ± 46.2 mg/dl ($P < 0.01$) and exceeded the upper limit of the normal range in nine patients during sirolimus therapy (Table 2). Urinary albumin and protein excretion significantly ($P < 0.001$) increased during sirolimus therapy, whereas it did not appreciably change during conventional therapy alone (Table 3). Conversely, blood levels decreased from 202.9 ± 46.6 to 187.3 ± 30.9 mg/dl on conven-

tional therapy alone. One patient was treated with omega-3 polyunsaturated fatty acids because of increasing triglyceride levels during sirolimus therapy. A transient and marked increase in creatine-phosphokinase (9330 U/L), with transient increase in alanine aminotransferase level (170 U/L), was occasionally documented at a scheduled visit after gym exercise in one asymptomatic patient who was on sirolimus. These changes resolved spontaneously within 2 weeks. Mild and self-limiting leucopenia was observed in five patients who were on sirolimus, and mild anemia was reported in three patients who were on sirolimus and in two who were on conventional treatment alone.

Volumetric Analysis
Kidney Volume
On average, TKV tended to increase less on sirolimus (46 ± 81 ml; $P = 0.047$ *versus* pretreatment) than on conventional therapy alone (70 ± 72 ml; $P = 0.002$; Figure 1, Table 3), although

Table 3. Kidney functional and structural parameters before and after sirolimus or conventional treatment

Parameter	Sirolimus		Conventional	
	Before	After	Before	After
Diuresis (ml/24 h)	2150 ± 670	2167 ± 556	2149 ± 591	2055 ± 728
GFR (ml/min per 1.73 m²)	75.8 ± 23.4	73.4 ± 25.1	73.8 ± 22.2	74.3 ± 24.4
Urinary albumin excretion (μg/min)	36.5 ± 26.8	76.3 ± 57.3[a]	43.7 ± 24.5	39.0 ± 31.8
Urinary protein excretion (g/24 h)	0.136 ± 0.063	0.223 ± 0.125[a]	0.164 ± 0.057	0.140 ± 0.075
Total kidney volume (ml)	1914 ± 1051	1960 ± 1095[b]	1907 ± 1107	1977 ± 1133[c]
Cyst volume (ml)	1129 ± 759	1132 ± 773	1112 ± 780	1167 ± 815[b]
Parenchymal volume (ml)	301 ± 78	327 ± 91[c]	314 ± 93	312 ± 87
Intermediate volume (ml)	484 ± 280	501 ± 307	480 ± 304	498 ± 293

Data are means ± SD.
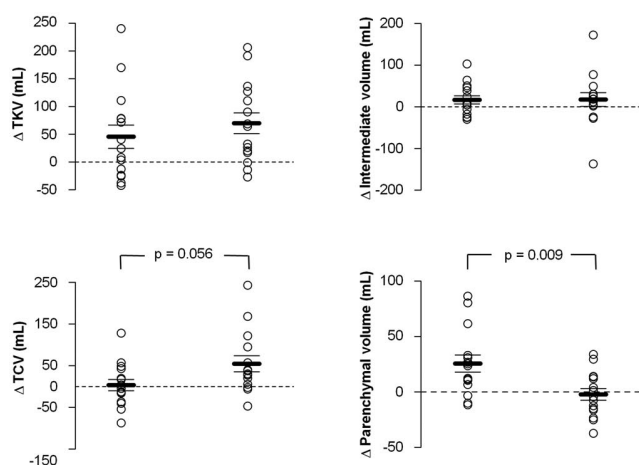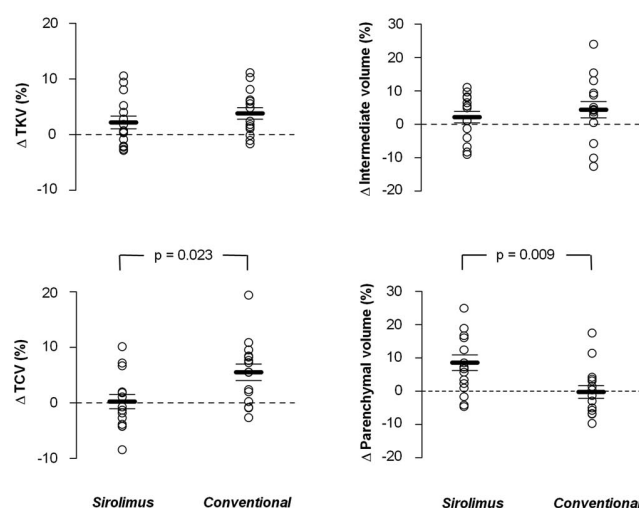[a]$P < 0.001$; [b]$P < 0.05$; [c]$P < 0.01$ *versus* before.

A Absolute Volume Change

B Relative Volume Change

Figure 1. Renal volume changes during sirolimus and conventional treatment. (A) Absolute changes in TKV, TCV, and parenchymal and intermediate volumes during the two treatment periods in 15 patients with ADPKD. Kidney volumes are computed using a specifically designed volumetric quantification method based on Otsu's thresholding classification method. ○, Individual values; heavy lines, average values; thin lines, SEM. (B) Relative changes in TKV, TCV, and parenchymal and intermediate volumes during the two treatment periods in 15 patients with ADPKD. Relative changes are computed with respect to absolute volumes at the start of each treatment period. ○, Individual values; heavy lines, average values; thin lines, SEM.

the difference between the two treatment periods was not statistically significant ($P = 0.45$). Six-month changes in TKV during conventional therapy (69.4 ml; 3.7%) were in line with changes observed in the CRISP cohort for matching patients' characteristics (TKV $\geq$1500 ml, age $\geq$30 years).[24]

Cyst Volume

TCV did not change appreciably during sirolimus treatment (4 ± 52 ml; $P = 0.808$), whereas it increased significantly ($55 \pm$

75 ml; $P = 0.013$) during conventional therapy alone (Figure 1). The difference between absolute changes observed during the two treatment periods approximated the statistical significance ($P = 0.055$; Table 4); however, when relative changes *versus* pretreatment values were considered (Figure 1, Table 4), the increase in cyst volume was significantly lower on sirolimus than on conventional therapy alone ($P = 0.023$).

Parenchymal Volume

Parenchymal volume increased significantly during sirolimus (26 ± 30 ml; $P = 0.005$) but did not change appreciably during the conventional treatment period (-2 ± 20 ml; $P = 0.677$). The difference between absolute changes observed during the two treatment periods achieved statistical significance ($P = 0.008$; Figure 1, Table 4). The difference was significant ($P = 0.009$) even when relative changes *versus* pretreatment values were considered (Table 4).

Intermediate Volume

The intermediate volume, previously defined as the fraction of PKD kidney tissue appearing hypoenhanced (intensity approximately 100 Hounsfield units) at contrast-enhanced CT relative to parenchyma,[25] showed a similar trend to increase during sirolimus (17 ± 38 ml; $P = 0.110$) and conventional treatment alone (18 ± 64 ml; $P = 0.305$; Figure 1).

Carryover Analyses

In patients who were randomly assigned to sirolimus treatment followed by conventional therapy alone, the effect of sirolimus on TCV tended to carry over during the subsequent treatment with conventional therapy ($P = 0.063$), whereas conventional therapy alone had no appreciable carryover effect during the subsequent sirolimus therapy. The marginal carryover effect of sirolimus on TCV is not of concern for the validity of this analysis. Indeed, because cyst volume increases at a faster rate under conventional therapy than during sirolimus, carryover leads to an artificially lower cyst volume increase during the conventional therapy period after sirolimus. Eventually, it may attenuate the differences between the two treatments.

Functional Analysis

No changes in measured GFR were observed throughout both treatment periods. No significant correlation was found between changes in TKV, TCV, parenchymal volume, and GFR during both treatment periods.

Sirolimus Levels and Outcomes

The sirolimus daily dosage averaged 3.6 ± 0.7 mg and ranged from 1 to 6 mg throughout the treatment period (Figure 2). Trough blood levels averaged 7.7 ± 2.2 ng/ml and ranged from 2.5 to 14.8 ng/ml (Figure 2). Trough blood level normalized for concomitant sirolimus dosages widely ranged from 2.5 to 4.9 ng/ml per mg.
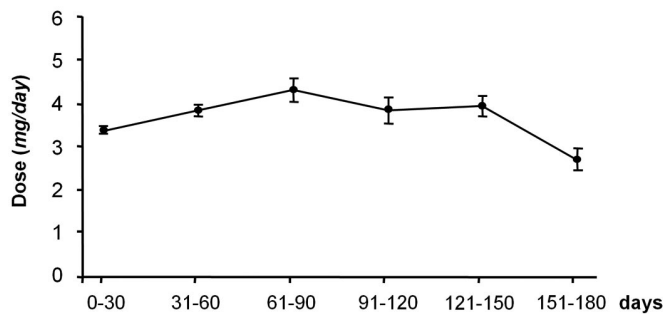
We used receiver operating characteristic (ROC) curve analysis to determine the ability of sirolimus dosage or blood trough levels to predict a beneficial effect of drug treatment on cyst vol-

Table 4. Adjusted mean changes of structural parameters after sirolimus or conventional treatment

Parameter	Sirolimus	Conventional	Difference	P
Absolute changes (ml)				
total kidney volume	47.1 (3.8 to 90.5)	69.4 (26.1 to 112.8)	−22.3 (−83.6 to 39.0)	0.4463
cyst volume	4.5 (−32.1 to 41.2)	54.9 (18.3 to 91.6)	−50.4 (−102.2 to 1.4)	0.0558
parenchymal volume	26.0 (11.8 to 40.3)	−2.7 (−17.0 to 11.6)	28.7 (8.5 to 48.9)	0.0089
intermediate volume	16.6 (−13.8 to 47.0)	17.2 (−13.2 to 47.6)	−0.7 (−43.6 to 42.3)	0.9738
Relative changes (%)				
total kidney volume	2.2 (−0.2 to 4.6)	3.7 (1.3 to 6.1)	−1.5 (−4.9 to 1.9)	0.3482
cyst volume	0.3 (−2.8 to 3.4)	5.5 (2.4 to 8.6)	−5.2 (−9.6 to −0.8)	0.0231
parenchymal volume	8.7 (4.1 to 13.3)	−0.4 (−5.0 to 4.2)	9.1 (2.7 to 15.6)	0.0094
intermediate volume	2.1 (−2.6 to 6.8)	4.3 (−0.4 to 9.0)	−2.2 (−8.8 to 4.5)	0.4949

Data are means (95% confidence intervals), adjusted for the period and carryover effect.
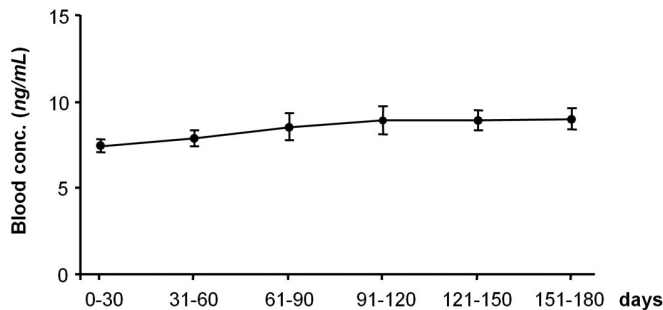

Figure 2. Trend to increase the blood trough level of sirolimus despite mean dosage drug reduction during the 180 days of follow-up. (A and B) Daily sirolimus doses (A) and blood sirolimus trough levels (B) averaged over 30-day time intervals during the sirolimus treatment period in 15 patients with ADPKD. Data are means ± SE.
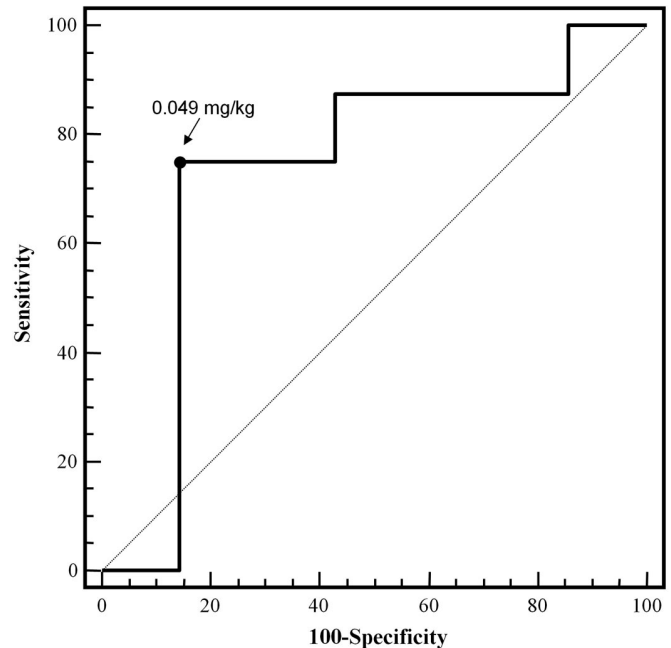
Figure 3. ROC curve identifies the cutoff threshold of sirolimus dosage normalized to patient body weight that predicts treatment effect in reducing or reversing cyst volume growth. The vertical axis, sensitivity, represents the percentage probability that the reduction or reversal in cyst volume growth is detected by sirolimus dosage ratio higher than a given value. The horizontal axis, specificity, represents the percentage probability that sirolimus dosage ratio lower than a given value does not result in reduction or limitation in cyst volume growth. According to the ROC curve analysis, the highest accuracy in predicting treatment effect is at a threshold of 0.049 mg/kg sirolimus dosage to body weight ratio (sensitivity 75%, specificity 86%). The 45° dotted line indicates failure to identify a sirolimus dosage ratio that discriminates between effect and no effect of treatment. AUC, area under the curve for ROC.

ume growth. As shown in Figure 3, the area under curve for ROC for sirolimus dosage normalized by patient body weigh to predict reduction or reversal in cyst volume growth was 0.732 (95% confidence interval 0.448 to 0.920; sensitivity 75%, specificity 86%). Although not statistically significant ($P = 0.0798$), the high area under curve value allowed us to identify 0.049 mg/kg as the cutoff threshold of normalized sirolimus dosage for prediction of treatment effect. Above this dosage, the mean changes in cyst and parenchymal volumes were −12.16 and 25.86 ml, respectively. Below this dosage, the changes in cyst and parenchymal volumes were 16.99 ml ($P = 0.12$) and 25.35 ml ($P = 0.61$), respectively. No predictive cutoff sirolimus trough level was identified.

DISCUSSION

In this prospective, randomized clinical trial, we found that in adult patients with ADPKD and normal or slightly impaired kidney function, 6 months of treatment with the mTOR inhibitor sirolimus added on conventional therapy halted the growth of

kidney cysts and achieved a significant increase in parenchymal volume, whereas 6 months of conventional therapy alone was associated with a significant increase in cyst volume with no appreciable changes in kidney parenchyma. The intermediate kidney volume slightly and similarly increased during both treatment periods. Notably, no serious adverse event was observed throughout the study period, and, after the sirolimus dosage was titrated to target trough blood levels between 5 and 10 ng/ml, no patient had to withdraw because of adverse effects of treatment.

Safety

A major aim of this pilot, explorative study was to assess the safety profile of sirolimus therapy in patients with ADPKD and to verify that the possible adverse effects of the study drug will not offset the benefits expected from preventing or limiting renal disease progression in this population. Three patients were prematurely withdrawn from the study because of the onset of an erythema nodosus in one case and thrombocytopenia in two cases after a few days of sirolimus therapy, when sirolimus dosage was titrated to target trough levels between 10 and 15 ng/ml (all events fully resolved with treatment withdrawal). The poor tolerability of this high-dosage regimen led us to reduce the target levels to 5 to 10 ng/ml. With this approach, no serious event requiring treatment interruption was observed.

The most frequent and disturbing adverse effect, which affected 10 of our patients during sirolimus therapy, was the development of aphthous stomatitis. Lack of concomitant steroid therapy most likely explained why aphthous mouth ulcers were more frequent and severe in our series than in the average population of organ transplant recipients.[26] In all cases, however, symptoms were relieved with topical treatment alone using a mouthwash (Alovex). Lesions did not require premature treatment interruption and fully resolved without sequelae after completion of the treatment period.

Hypercholesterolemia is another widely known adverse effect of mTOR inhibitors.[27] Our data, however, show that in patients without concomitant medications that may also adversely affect the lipid profile, such as steroids or calcineurin inhibitors, hypercholesterolemia is mild and can be easily managed just with dietary counseling. Thus, this adverse effect does not seem to be a major drawback of sirolimus therapy, even in the case of prolonged exposure. Bone marrow toxicity was also negligible, because only a few patients had mild and self-limiting abnormalities in blood cell counts that promptly and fully recovered after completion of sirolimus therapy.

Efficacy

Morphologic Outcomes
Finding that kidney cyst growth was substantially reduced during the 6 months on sirolimus therapy corroborated our working hypothesis that uncontrolled mTOR activation may play a pathogenic role in ADPKD[11,23] and that effective mTOR inhibition may help prevent or limit the phenotypic manifestations of the disease. Inhibition of mTOR activity has been shown to lead to G1 cell-cycle arrest and apoptosis.[28–30] Consistently, the

mTOR inhibitors sirolimus and everolimus significantly retarded cyst development and expansion and prevented renal function loss in Han:SPRD rats,[12,17,22,31] as well as in other mouse models of dominant or recessive PKD.[11] In addition, small retrospective studies found a significant reduction in polycystic kidney or liver volumes in renal transplant recipients who were on long-term sirolimus therapy but not in control subjects who were given other immunosuppressants.[11,23]

An unexpected finding was the significant growth in parenchyma volume during sirolimus therapy but not during conventional therapy alone. We hypothesize that while on sirolimus therapy, the parenchyma was allowed to expand upon relief of the mass effect of surrounding cysts. Indeed, the compression of growing cysts on residual parenchymal tissue and the stretching of kidney microvessels with secondary tissue hypoperfusion[32] have been suggested to play a role in the progressive disruption of the normal tissue architecture, with secondary atrophy and shrinking.[32] This sequence of events would be prevented or limited when cyst growth is halted by sirolimus therapy. Another and not necessarily alternative explanation is that mTOR inhibitors may increase capillary permeability through decreased endothelial-cadherin expression, in particular when the oxidative stress is increased.[33] This would allow enhanced fluid ultrafiltration through the capillary wall with secondary interstitial imbibition and edema, which, in turn, might account for the increasing parenchyma volume detected by CT scan evaluation during sirolimus therapy. Of note, noncystic parenchymal enlargement after discontinuation of the mTOR inhibitor everolimus was recently reported in rat polycystic kidney disease model.[34] At variance with these findings, the analysis of parenchymal volume changes in each of the four treatment phases of our study did not show a similar effect in conventional treatment phase after sirolimus withdrawal (parenchymal volume changes: Phase 1 sirolimus 32.9 ± 28.2 ml, Phase 2 conventional -9.2 ± 19.1 ml; Phase 1 conventional 3.9 ± 20.6 ml, Phase 2 sirolimus 19.20 ± 31.86 ml).

Functional Outcomes
Finding that GFR was relatively stable throughout the study period allowed us to exclude any appreciable nephrotoxicity of sirolimus therapy, at least in the short term. The follow-up was too short to draw any conclusion on the possible effects of sirolimus therapy on the relentless renal function loss that invariably accompanies renal cyst growth, in particular in more advanced stages of the disease.

Limitations and Strengths

This was an explorative study with a relatively small sample size and short follow-up. The short duration of the trial did not allow us to demonstrate any beneficial effects on GFR. Moreover, the statistical power was not sufficient to evaluate the effects of sirolimus therapy on left ventricular mass[35]; however, main outcome variables—including different considered morphologic parameters, GFR, and albuminuria—were measured

and recorded by widely recognized standard procedures in the setting of a prospective, controlled trial. The small sample size also did not allow us to test formally the various dosages of sirolimus for efficacy on cyst growth; however, the dosage was adjusted to target blood trough levels, thus resulting in individual sirolimus dosing. Despite this limitation, ROC curve analysis allowed us to identify 0.049 mg/kg as a specific cutoff threshold of sirolimus dosage normalized by patient body weight to predict reduction or reversal in TCV growth. This finding may be of help for the design of future clinical trials with sirolimus in patients with ADPKD. The availability of a sensitive and reproducible technique such as spiral CT allowing for precise volumetric evaluations and intrapatient comparisons in the setting of a cross-sectional design increased the power of the analysis and allowed us to achieve robust findings despite the relatively small sample size and short follow-up. Another major strength of our study is that the analyses considered the treatment effect on all kidney tissue components beyond the sole TKV.[36] Indeed, no specific treatment effects were observed on TKV, possibly because this volume increased less than expected during conventional therapy, which decreased the power of the analysis to detect a slower growth during sirolimus therapy; however, a segregate analysis of the various kidney compartments allowed us to detect opposite effects on cyst and parenchymal volumes we observed during sirolimus therapy. This might open novel lines of research aimed to assess whether preventing or retarding progressive tissue disruption may protect patients with ADPKD from relentless renal function loss and progression to terminal kidney failure.

In this proof-of-concept study, we provided clear-cut evidence that in patients with ADPKD, 6 months of sirolimus therapy halted the growth of renal cysts and increased the volume of seemingly healthy kidney parenchyma with acceptable adverse effects. These findings provide the rationale for adequately powered trials aimed to assess whether and to which extent long-term sirolimus therapy may improve clinical outcomes of patients with ADPKD in the long run. These long-term efficacy trials, however, should require careful patient monitoring for ensuring tolerability of sirolimus therapy.

CONCISE METHODS

Patient Population

Patients who were aged ≥18 years and had a clinical and ultrasonographic diagnosis of ADPKD and estimated GFR of ≥40 ml/min per 1.73 m² were eligible for study participation.[37] Those with concomitant systemic renal parenchymal (proteinuria ≥1 g/24 h) or urinary tract disease, diabetes, cancer, psychiatric disorders, and any condition that could prevent full comprehension of the purposes and risks of the study were excluded as well as pregnant or lactating women or fertile women without effective contraception. Patients were retrieved from the Outpatient Clinic of the Unit of Nephrology of the Ospedali

Riuniti di Bergamo. Eligible patients who provided written informed consent to study participation were included according to Declaration of Helsinki guidelines. The study protocol was approved by the ethical committee of Azienda Ospedaliera Ospedali Riuniti di Bergamo. The study was performed between April 2007 and August 2009.

Objectives of the Study

The broad aim of this study was to assess the risk/benefit profile of a relatively short treatment period with sirolimus in a well-characterized cohort of adult patients with ADPKD. In the setting of a prospective, randomized, crossover clinical trial, we aimed to compare the effects of 6 months of treatment with sirolimus added on conventional therapy or with conventional therapy alone on TKV (main efficacy variable) and renal cyst, intermediate, and parenchymal volumes[25] in patients with normal renal function or mild to moderate renal insufficiency. Secondarily, we evaluated the treatment effects on GFR[38] and whether these effects were correlated with the concomitant changes in kidney volume or structure during the two treatment periods.

Study Design

Baseline Evaluations
The BP was recorded in sitting position as the mean of three readings 2 minutes apart. Blood and urine samples were taken in the morning under fasting conditions for routine laboratory evaluations, including hematochemistry, renal and liver function tests, and peripheral blood cell counts. GFR was measured by the iohexol plasma clearance technique, as described previously,[38] and TKV, renal cyst volume, and intermediate and parenchymal volumes were measured by spiral CT and morphometric analysis.[25]

Treatment and Monitoring
After baseline evaluation, patients were randomly allocated to two sequences of 6 months of treatment with sirolimus (Rapamune; Wyeth-Lederle S.p.A., Aprilia, Latina, Italy) added on conventional therapy followed by conventional therapy alone or *vice versa*. Sirolimus was started at the daily oral dosage of 3 mg. The dosage was adjusted to target blood trough levels between 10 and 15 ng/ml (subsequently amended to 5 to 10 ng/ml). Drug levels were measured by the HPLC method[39] at days 5 through 7 of treatment, every week for the first month, and subsequently at monthly intervals. BP and routine laboratory tests, including blood glucose, transaminases, total cholesterol and triglycerides, serum creatinine, complete blood cell count and 24-hour urinary protein excretion, were evaluated every 2 months for all patients. At 6 months, all of the measurements performed at baseline (including CT and GFR evaluations) were repeated. Then each patient crossed over to the other treatment arm. The same measurements performed during the first treatment period were repeated during the second 6-month follow-up period. At completion of the second treatment period, all baseline evaluations, including CT scans and GFR measurements, were repeated.

No specific change in pharmacologic treatment was introduced throughout the study period. Only adjustments in dosages of the ongoing antihypertensive therapy were allowed to target systolic/diastolic BP <130/80 mmHg during the study period.

Image Acquisition, Processing, and Analysis
CT Imaging
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 512 × 512; collimation 2.5 mm; slice pitch 0.984; increment 2.46 mm) was initiated 80 seconds after injection of 100 ml of an iodinated nonionic contrast agent (Iomeron 350; Bracco, Italy) at a rate of 2 ml/s. The risk for nephrotoxicity as a result of contrast agent injections was expected to be negligible, because none of the patients had severe renal insufficiency at enrollment or during the study follow-up. Once acquired, images were transferred in DICOM 16-bit format from the clinical scanner onto PC workstations for subsequent processing.

Image Processing and Renal Volume Measurements
Kidneys were first manually outlined on all acquired digital images by a trained operator (A.C.), who was blind to the treatment phase, using an interactive image editing software (ImageJ, Image processing and Analysis in Java, National Institutes of Health, http://rsbweb.nih.gov/ij/). For facilitation of this task, an in-house ImageJ plug-in was created to allow automated interpolation of tracings between nonconsecutive slices. 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 by an expert radiologist (M. Cafaro), who also was blind to the treatment phase.

On the basis of the outlined images, cyst, intermediate, and parenchyma volumes were computed using a volumetric quantification method previously described in detail and validated by means of a physical phantom.[25] 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[40] was then used to subdivide the outlined kidneys into four tissue classes (fat, cyst, intermediate, or parenchyma), so as to maximize the between-class variance of image intensity values. From the segmented images, cyst, intermediate, and parenchymal volumes were computed by multiplying the voxel count of each class by voxel volume, as determined by the acquisition protocol. A segmented CT image for one of the data sets included in the study is presented in Figure 4. It is worth noting that manually traced kidney outlines were allowed to lie within perirenal fat, which was then automatically identified and excluded from TKV to minimize the effect of outlining on volume quantification.[41] All image processing steps were performed with in-house software based on the Insight Toolkit version 3.10[42] and developed in the C++ programming language.
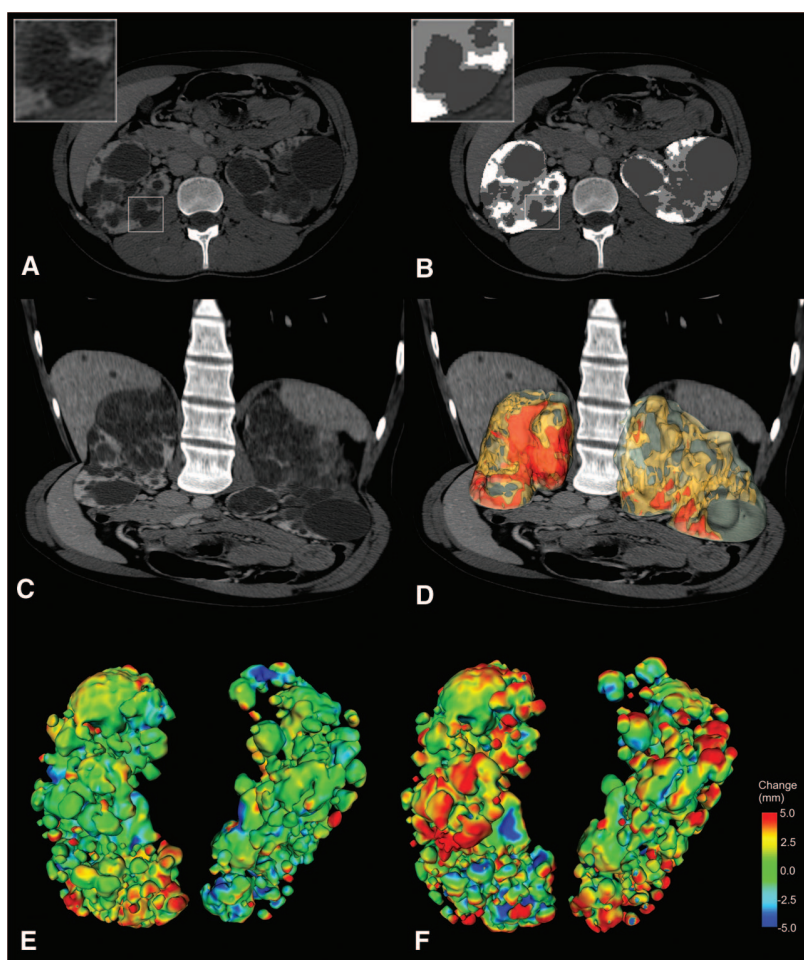

Figure 4. Contrast-enhanced CT volumetric quantification technique and visualization of TCV changes. (A and B) CT image of a representative patient (A) and result of image analysis for the same slice (B), showing voxels classified as cysts (dark gray), parenchyma (white), and intermediate volume (light gray). Upper left corner insets show the regions enclosed by white boxes at high magnification. (C and D) Three-dimensional representation of the image data for the same patient (C) and the resulting volumetric quantification (D), showing the spatial distribution of renal cysts (translucent), intermediate volume (orange), and parenchyma (red). (E and F) Local changes in cyst volume occurring during sirolimus (E) and conventional treatment (F) for a representative patient, color-coded over the outer surface of the identified cyst volume (red for expansion, blue for shrinkage). Local changes are computed as the distance between the cyst volume surfaces at the start and end of the two treatment periods. TCV changes for the patient are −40.27 ml during sirolimus and 121.7 ml during conventional treatment.

Sample Size Estimation
This was a pilot, explorative study, and no data on the effect of sirolimus therapy on kidney volume growth (primary efficacy variable) in patients with ADPKD were available at the time the study protocol was designed. Thus, to estimate the sample size for this study, we assumed a kidney volume growth during 6 months of active treatment with sirolimus similar to that previously observed during an identical treatment

period with the long-acting somatostatin analogue octreotide in a similar experimental setting.[41] Along the same line, we also assumed a kidney volume growth during the 6 months on conventional therapy alone similar to that previously reported during 6 months of placebo.[41] On the basis of these assumptions, we predicted a kidney volume growth of 162 ± 114 ml during conventional therapy alone and entered in the model an expected reduction in kidney volume growth to 71 ml during the sirolimus treatment period. Thus, we calculated that a total of 15 patients had to complete the two treatment periods to provide the study with 80% power to detect ($P < 0.05$, two-tailed test, log-rank test) the expected reduction in kidney volume growth on sirolimus as compared with conventional therapy alone. To account for a 25% dropout rate, we aimed recruitment at inclusion of at least 21 patients.

Statistical Analysis

Absolute and relative volumetric changes during the two treatment periods with or without sirolimus were compared by means of ANOVA for crossover design performed using the PROC MIXED procedure of SAS 9.1 (SAS Institute, Cary, NC), which adjusted for the period and carryover effects.[43] Within-group comparisons were assessed by paired t test or McNemar test as appropriate. Correlations between changes in volumes and changes in GFR were evaluated using Pearson test. The best cutoff sirolimus dosage or trough blood level segregating patients with relentless cyst growth from those with stable or decreasing cyst volumes was identified by ROC curve analysis. Sensitivity and specificity of ROC were computed. The sensitivity represents the percentage probability that the reduction or reversal in cyst volume growth is detected by sirolimus dosage or trough blood level higher than a given value. The specificity represents the percentage probability that sirolimus dosage or trough blood level lower than a given value does not result in reduction or reversal in cyst volume growth. The ROC analysis provides the threshold value of sirolimus dosage or trough levels with the optimal tradeoff between sensitivity and specificity (highest accuracy). All calculations were made using MedCalc 11.1.0.0 (MedCalc Software, Mariakerke, Belgium).

Data are given as means ± SD and 95% confidence intervals, unless otherwise stated. $P < 0.05$ was considered as statistically significant.

ACKNOWLEDGMENTS

DISCLOSURES

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