Rapamycin for treatment of type I autosomal dominant polycystic kidney disease (RAPYD-study): a randomized, controlled study

Giovanni Stallone¹, Barbara Infante¹, Giuseppe Grandaliano¹, Christos Bristogiannis², Luca Macarini², Daniela Mezzopane¹, Francesca Bruno¹, Eustacchio Montemurro¹, Annalisa Schirinzi¹, Giovanni Stallone¹, Barbara Infante¹, Giuseppe Grandaliano¹, Christos Bristogiannis², Luca Macarini², Daniela Mezzopane¹, Francesca Bruno¹, Eustacchio Montemurro¹, Annalisa Schirinzi¹, Massimo Sabbatini³, Antonio Pisani³, Tiziana Tataranni⁴, Francesco Paolo Schena⁵ and Loreto Gesualdo⁵

¹Nephrology, Dialysis and Transplantation Unit, Department of Biomedical Sciences, University of Foggia, Foggia, Italy, ²Department of Surgical Sciences, Section of Diagnostic Imaging, University of Foggia, Foggia, Italy, ³Department of Systematic Pathology, University Federico II, Naples, Italy, ⁴Laboratory of Pre-clinical and Translational Research, IRCCS, Centro di Riferimento Oncologico della Basilicata, Rionero in Vulture (Pz), Italy and ⁵Nephrology, Dialysis and Transplantation Unit, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy

Correspondence and offprint requests to: Giovanni Stallone; E-mail: g.stallone@unifg.it; stallonegio63@gmail.com

Abstract

Background. Autosomal dominant polycystic kidney disease (ADPKD) is the most common form of cystic kidney disease. An inappropriate stimulation of mammalian target of rapamycin may represent the converging point in the molecular pathways leading to renal cyst growth.

Methods. The primary objectives of this prospective, open-label, randomized clinical trial were to assess whether rapamycin may reduce the progressive increase in single cyst and total kidney volume in type I ADPKD and the decline in renal function and to identify the optimal rapamycin dose. Fifty-five patients with type I ADPKD were enrolled and randomized to receive ramipril (Group A), ramipril + high-dose rapamycin (Group B, trough level 6–8 ng/mL) and ramipril + low-dose rapamycin (Group C, trough levels 2–4 ng/mL). Rapamycin efficacy was monitored measuring p70 phosphorylation in peripheral blood mononuclear cells.

Results. Both rapamycin doses significantly reduced p70 phosphorylation. Nevertheless, total kidney volume increased in all groups after 24 months, although only in Groups A and B, was the final volume significantly higher compared with the baseline. Single cyst final volume was not significantly different in the three groups, although it was increased in Group A compared with the baseline, whereas in Groups B and C, it was significantly reduced. We did not observe any difference in renal function at 24 months among the three study groups. Group A presented a significant worsening of renal function that remained stable in both Groups B and C.

Conclusions. Our study would suggest that rapamycin does not influence the progression of type I ADPKD, although the higher drug dose tested prevented both the increase in kidney volume and the worsening of renal function (RAPYD-study, EUDRACT No. 2007-006557-25).

Keywords: ADPKD; cyst volume; kidney function; kidney volume; rapamycin

Introduction

Autosomal dominant polycystic kidney disease (ADPKD), the most common form of cystic kidney disease, occurs in 1 out of 800 individuals and affects 500 000 persons in the USA and 4–6 million persons worldwide. This disease accounts for 7–10% of the patients in dialysis, representing the fourth leading cause of end-stage renal disease in most of the Western countries [1, 2]. To date, there is no established medical therapy to slow down or reverse its progression.

There are two forms of ADPKD: type I, caused by mutations in the PKD1 gene and accounting for 85–90% of the cases [3], and type II, due to mutations in the PKD2 gene and accounting for 10–15% of the cases [4, 5]. The protein products of PKD1 and PKD2 genes, polycystin-1 and -2, respectively, are both expressed by renal tubular epithelial cells and have been shown to protect cells from apoptosis under different stress conditions [6–8]. In addition, the polycystin-1/2 complex has been found to inhibit cell proliferation [9]. Abnormal persistent epithelial cell cyst apoptosis and proliferation are two molecular features of ADPKD [10].

Recently, polycystin-1 has been shown to regulate mammalian target of rapamycin (mTOR) and its downstream effectors [11]. mTOR is a serine–threonine kinase that plays a central role in the regulation of cell proliferation, growth, differentiation and survival [12]. Shillingford et al. [13] suggested that an inappropriate stimulation of this kinase can induce the development of renal cyst. Indeed, specific mTOR inhibition by rapamycin was shown to reduce dramatically cyst growth in polycystin-1-null mice and in several other experimental models of...
Rapamycin and type I ADPKD

Cystic kidney disease [14]. In addition, a small retrospective analysis demonstrated that the use of rapamycin in ADPKD patients receiving a kidney transplant reduced liver volume, suggesting the feasibility of a clinical intervention [15].

On the basis of these observations, we designed the Rapamycin for treatment of Autosomal dominant Polycystic Kidney Disease (RAPYD-study, EUDRACT No. 2007-006557-25; http://eudract.emea.eu.int/), a prospective, randomized clinical study in type I ADPKD patients with mild–moderate renal failure, to evaluate the efficacy and safety of different rapamycin doses in the treatment of this common genetic disease.

Materials and methods

Study population

In this prospective, open-label randomized clinical trial, 55 ADPKD patients followed in the outpatient clinic of any of the two participating centres (University of Foggia and Bari, Italy) were enrolled between November 2007 and November 2008. The duration of the planned follow-up was 24 months. The Ethical Committees of both participating centres approved the study protocol. The study was carried out according to the Declaration of Helsinki (IV Adaptation). All consecutive in- or outpatients of both genders with clinical, genetic and ultrasonographic diagnosis of type I ADPKD who satisfied the eligibility criteria were asked to participate in the study. All patients who gave their informed consent were included in the study. The inclusion criteria were clinical, genetic and ultrasonographic diagnosis of type I ADPKD, age between 18 and 65 years and an estimated glomerular filtration rate (eGFR) between 40 and 90 mL/min/1.73 m², evaluated by the abbreviated Modification of Diet in Renal Disease (MDRD) formula [16]. The exclusion criteria included evidence of active infection; evidence of infection on chest X-ray; use of any investigational drug or treatment of a common genetic disease.

Patients were randomized to treatment with different rapamycin doses. Both antihypertensive agents were withdrawn up to 4 weeks prior to the enrolment; known hypersensitivity to rapamycin; patients on dialysis; contraceptive failure; creatinine >1 g/24 h; psychiatric disorders and any condition preventing full comprehension of the purposes and risks of the study; clinical evidence of any malignancy within 3 years before enrolment, with the exception of adequately treated basal and squamous cell carcinomas of the skin; and HIV-positive test. A negative serum pregnancy test before rapamycin administration and the use of a medically acceptable method of contraception throughout the treatment period and for 3 months following discontinuation of rapamycin were mandatory for women with childbearing potential.

The dropout criteria were pregnancy, death and withdrawal of consent.

Design and study objectives

The primary objectives were to assess whether rapamycin may reduce the progressive increase in single cyst and total kidney volume in type I ADPKD and the decline in renal function and to identify the optimal rapamycin dose to achieve the beneficial results without incurring in major side effects.

After a screening assessment, all patients entered a run-in phase of 2 months in which eGFR and 24 h proteinuria were evaluated every 2 weeks. At the beginning of this period, in patients already treated with angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin II receptor blockers (ARBs), these drugs were withdrawn for at least 4 weeks before performing the laboratory tests. Other antihypertensive agents were allowed for blood pressure control in any phase of the study.

At the end of the run-in phase, all eligible patients were randomly assigned to ramipril alone (Group A) or ramipril plus high-dose rapamycin (Group B) or ramipril plus low-dose rapamycin (Group C). An allocation sequence was generated at the coordinating centre by random number tables; a list divided into blocks of six was adequately concealed for the whole study period to prevent attempts to subvert randomization. The investigators did not have any information regarding the blocks. The Scientific Secretariat performed central telephone randomization for every eligible patient.

In Group B, rapamycin administration was given with a loading dose of 3 mg. The maintenance dose was 1 mg/day aiming at a blood trough level between 6 and 8 ng/mL. In Group C, the patients did not receive a loading dose and the maintenance dose of rapamycin was 1 mg/day to reach a blood trough level between 2 and 4 ng/mL. In all groups, treatment with ramipril started at a dose of 2.5 mg/day that was then increased by 1.25 mg/day every month to achieve and maintain a systolic and diastolic blood pressure of <120-80 mmHg. Hypokalaemia was monitored and did not exceed the value of 5.5 mEq/L.

The investigators examined each patient at randomization, every month during the first 6 months and every 3 months thereafter. Blood pressure and heart rate were measured with the patient in a sitting position at each visit. Blood and 24 h urinary samples were obtained for the assessment of rapamycin trough levels, creatinine, urea, sodium, potassium, albumin, total proteins, glucose, cholesterol, triglycerides, liver enzymes, bilirubin, complete blood count and protein urinary excretion. Hyperlipidaemia was defined as serum cholesterol and/or triglycerides >200 mg/dL.

Magnetic resonance imaging technique

All patients recruited for our study underwent a standardized magnetic resonance imaging (MRI) examination at the baseline (T=0) and after 24 months of initiation of drug therapy (T=24) with the same MRI scanner. MRI was performed using a 1.5 T (ACHIEVA®, Philips Medical System, Eindhoven, the Netherlands) with a maximum gradient strength of 30 mT/m, a slew rate of 150 mT/m/s and a four-channel phased-array coil, centred over the inferior costal margin. Patients were positioned supine on the magnet bed, head first oriented, with arms over the shoulders.

Patients were informed about the mode of execution of the examination and were accurately explained about the importance of remaining motionless and holding their breath during the acquisition of sequences in breath-hold, to avoid motion artefacts that could compromise the quality of the images and thereby create difficulties for the observers in obtaining the kidney and cyst volumes. No specific preparatory measures were taken before the examination. The hydration state was not monitored, and no clinical signs of over hydration (e.g. peripheral oedema, jugular venous distension, high blood pressure) were observed in the patients.

We avoided the use of gadolinium contrast-enhanced sequences with these patients, with compromised renal function, since it does not imply any advantage in kidney and cyst volume measurements [17].

The imaging protocol of our study provides:

(i) Localizer sequence.

(ii) Axial DUAL fast field echo (FFE) T1-weighted sequences (TR = 147 ms; double TE = 4.6/2.3 ms in phase and out of phase; slice thickness = 5 mm; gap = 0).

(iii) Axial single-shot T2-weighted sequences (TR = 338 ms; TE = 80 ms; matrix = 256 × 256; slice thickness = 5 mm; gap = 0).

(iv) Coronal BALANCE FFE T2-weighted sequences (TR = 3.8 ms; TE = 1.9 ms; flip angle = 60°; matrix = 256 × 256; slice thickness = 5 mm; gap = 0).

Both axial and coronal images were obtained in breath-hold (slice thickness 5 mm, gap 0) to minimize movement artefacts. The field of view was suitable to the patient’s stature (350–500 mm).

To measure the kidney and the cyst volumes, we used the disc-summation method [17].

All measurements were carried out on Coronal BALANCE FFE T2-weighted images by two observers experienced in abdominal MRI. The observers were blinded to group assignment and dates of examinations. The patient data were transferred for analysis to our post-processing workstation where kidney and cyst volumes were measured by the use of a semi-automated contour tracing software (ViewForum 8.5, V11.1 2006). We manually traced the contours of the kidney and the selected cysts, so we obtained the area of each section. The kidney volumes were calculated by multiplying all outlined areas by the section thickness and summing the volume of each section. Non-kidney...
parenchyma was identified by checking adjacent slides if needed and excluded from the measurement. We measured the cyst volumes in the same way. We chose to measure a cyst that we considered most representative for each kidney and could provide as much as possible the requirements of reproducibility. Genetic analysis DNA isolation and linkage analysis. Genomic DNA was derived from whole blood using PureLink Genomic DNA Mini kit (Invitrogen), according to the manufacturer’s protocol. Informed consent was obtained from each participant. Family members of the probands were recruited to participate after receiving his or her permission. The majority of families were too small to establish linkage to either PKD1 or PKD2 markers, but in the largest pedigrees, it was possible to perform the microsatellite analysis with the following markers: SM7, CW2, 16AC2.5 and KG8 for the analysis of PKD1 [18]. The markers D4S1534, D4S1563, D4S423 and D4S414 were employed for PKD2 linkage [19]. The PCR products for the different markers were mixed in order to obtain a single sample per patient. A total of 0.3 μl of the marker GeneScan-500 LIZ (Applied Biosystems) and 10 μl of deionized formamide were added to 1 μl of each one of these samples. The samples were analysed by capillary electrophoresis in an ABI PRISM™ 3130 sequencer (Applied Biosystems).

PCR amplification, denaturing high-performance liquid chromatography analysis and DNA sequencing. The duplicated region of PKD1 was amplified as five PKD1-specific fragments by long-range PCR (LR-PCR), and exons were amplified from these fragments after dilution 1:1000 to avoid genomic DNA carryover. LR-PCR to amplify Gen 2–12, Gen 13–15, Gen 15–21 and Gen 22–34 was performed using the Takara LA Taq (Takara BIO Inc.) in brief, each reaction containing 100 ng genomic DNA, 5 pmol each primer, 400 μM each dNTP, 2.5 mM MgCl2, 2.5 U Takara LA Taq and the supplied buffer was heated to 98°C for 1 min. Subsequently, the reaction was incubated for 30 cycles of 98°C, 10 s; annealing T, 20 s; and 72°C, 3 min. Because of the extreme GC richness of exon 1, a different protocol was employed: the reaction containing DNA (100 ng), primers (6 pmol each), dNTPs (200 μM each), MgCl2 (1.5 mM), 0.5 U KAPA2G Robust HotStart DNA Polymerase (KAPA Biosystems) and the supplied GC buffer was heated to 95°C for 3 min and then was incubated for 35 cycles of 95°C, 10 s; annealing T, 20 s; and 72°C, 3 min.

The LR-PCR products served as a template for 51 nested PCRs, while the unique region of the PKD1 gene was amplified from genomic DNA in 14 additional gene segments. Each amplification for denaturing high-performance liquid chromatography (DHPLC) analysis was amplified as a fragment of 150–450 bp and then analysed using the Wave system HT (Trangenomic). The ampiclon length, annealing temperature for PCR amplification and resolution temperature for DHPLC analysis have been reported previously [20]. Samples showing an aberrant elution profile were re-amplified and subjected to direct sequencing by using the Big-Dye Terminator kit (Applied Biosystems) and an ABI-PRISM 3130 Genetic Analyzer (Applied Biosystems), according to the manufacturer’s instructions. Missense mutations were confirmed by a segregation analysis with the disease when samples from other family members were available and by DHPLC screening on 300 normal chromosomes.

P70S6 kinase phosphorylation Peripheral blood mononuclear cell (PBMC) were isolated by density separation over a Ficoll-Paque™ (GE Healthcare, Sweden) gradient [460 units of gravity (g) for 30 min]. PBMCs were washed three times with phosphate-buffered saline, pH 7.4/1 mM EDTA (Sigma, Milan, Italy). Cells were then counted and their viability was assessed by Trypan Blue exclusion. PBMCs were then lysed in Triton X-100 in the presence of a 1% (vol/vol) protease-inhibitor cocktail and a 1% (vol/vol) phosphatase-inhibitor cocktail. An enzyme-linked immunosorbent assay (ELISA) kit specific for phospho-p70S6kinase² was used, according to the manufacturer’s protocol, and the results were normalized to the total p70S6 kinase content (p70S6 kinase total and phospho-T389-p70S6 kinase ELISA kit; BioSource, Camarillo, CA).

Epidermal growth factor urinary concentration At each visit, a nine-hour urine sample was collected. The urine was centrifuged and the supernatant was harvested, aliquoted and kept at −20°C until used. The epidermal growth factor (EGF) urine concentration was evaluated by ELISA and the results were normalized to urine creatinine excretion.

Statistical analysis The primary outcomes were the reduction in total kidney and cyst volume and the rate of renal function decline by means of the change in eGFR over time. Adverse events and side effects of the drugs were monitored and recorded in the report form. Results were evaluated on an intention-to-treat analysis in all randomized patients, irrespective of adherence to the assigned treatment. Dichotomous and polychotomous baseline characteristics were compared by the χ² or Fisher’s exact test. Continuous characteristics were compared by ANOVA, ANCOVA or paired Student’s t-test, as appropriate. The slope of eGFR over time was obtained by linear regression analysis. The predictive value of rapamycin blood trough levels in predicting the per cent change in total kidney volume, investigated by the area under the receiver operating characteristic (ROC) curve, using the mean of per cent changes of Groups B and C (8%) as the cut-off value, was calculated. All analyses were performed using the SPSS statistical software (release 15.0) and a P-value of <0.05 was considered significant.

Results Out of 105 ADPKD patients assessed for eligibility, 50 were excluded since they did not fulfill the inclusion/exclusion criteria (Figure 1). The remaining 55 patients were randomized and assigned to Group A (18 patients), Group B (19 patients) or Group C (18 patients). During the run-in phase, ACE-I and/or ARBs were withdrawn in 22 of 55 patients (40%). Fifty-three patients were analysed for the primary outcome; 2 patients withdrew from the study, both randomized in Group A (Figure 1).

Baseline characteristics All of the patients presented a family history as well as a clinical and genetic diagnosis of type I ADPKD. No differences among the groups were observed for the main demographic variables (mean age at the baseline was 45.3 ± 7.3, 42.8 ± 6.3 and 42.3 ± 10.6 years for Groups A, B and C, respectively; the gender ratio M/F was 9/7, 6/13 and 6/12 for Groups A, B and C, respectively). During the study period, a calcium channel blocker was added to the anti-hypertensive therapy in 7 patients of Group A, 10 of Group B and 9 of Group C. A statin was introduced in 9 patients of Group B and in 2 patients of Group C. The median values of basal total kidney and single cyst volumes, as determined by MRI and digital image processing, are reported in Table 1. No statistically significant differences in kidney and cyst volumes were observed among the three study groups at the baseline, although Group B presented lower basal cyst and kidney volumes compared with Group A and C.

Pharmacokinetic and pharmacodynamic data The drug blood levels in Group B and C patients were monitored throughout the 24 months of observation (Figure 2A). The mean rapamycin trough levels were 6.4 ± 0.3 ng/mL for Group B and 3.2 ± 0.4 ng/mL for Group C. The differences between the two groups were statistically significant at each time point (Figure 2A).

We also investigated the phosphorylation of p70S6 kinase, a downstream target of mTOR, in PBMCs of...
the patients enrolled to confirm the ability of the drug doses used to inhibit mTOR in the clinical setting. We did not observe any difference among the three groups in the basal level of phosphorylation of p70S6 kinase. As shown in Figure 2B, Group A patients did not present any significant change in the levels of phospho-p70S6 kinase, whereas both Group B and C patients showed a significant reduction in p70S6 kinase-specific phosphorylation, compared with T0 and Group A. Moreover, Group B patients showed a significant reduction in p70S6 kinase phosphorylation compared with Group C patients at the end of observation.

**Primary outcomes**

*Kidney volumetric analysis.* The mean values of kidney and cyst volumes at the baseline and after 24 months are shown in Table 1. In all three groups of patients, we observed an increased total kidney volume. Specifically, in Groups A and B, there was a statistically significant increase in kidney volume after 24 months of treatment.

### Table 1. Efficacy and safety profiles of the patient’s population

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After 24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Efficacy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney volume (mean) (mL)</td>
<td>1869 ± 668</td>
<td>1493 ± 672</td>
</tr>
<tr>
<td>Cyst volume (mean) (mL)</td>
<td>166 ± 49</td>
<td>140 ± 95</td>
</tr>
<tr>
<td>Kidney function (MDRD) (mL/min)</td>
<td>62.7 ± 13.6</td>
<td>61.4 ± 17.4</td>
</tr>
<tr>
<td>Safety</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>0.12 ± 0.1</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Oral ulcers (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hyperlipidaemia (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean cholesterol (mg/dL)</td>
<td>146 ± 39</td>
<td>138 ± 59</td>
</tr>
<tr>
<td>Mean triglyceride (mg/dL)</td>
<td>161 ± 28</td>
<td>156 ± 41</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>126 ± 11</td>
<td>130 ± 0.8</td>
</tr>
<tr>
<td>Anaemia (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infection (%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*P = 0.003 Group A compared with baseline.

**P = 0.02 Group B compared with baseline.

***P < 0.0001 Group A versus T0.

†P < 0.0001 Group B versus T0.

‡P < 0.0001 Group C versus T0.

*P = 0.01 Group A versus baseline.

‡P = 0.01 Group B compared with Group A.

#P = 0.01 Group B compared with Group C.

‖P < 0.001 Group B compared with Group C.
(P = 0.003 and 0.02, respectively), while in Group C, the increase failed to reach the statistical significance (Table 1 and Figure 3A). However, we did not observe any significant difference in change of kidney volume among the three study groups at the end of the observational period. On the contrary, the cyst volume was increased in Group A patients after 24 months of treatment (P < 0.0001 versus basal), whereas it was significantly reduced in Group B (P < 0.0001 versus basal) and Group C (P < 0.0001 versus basal) (Table 1 and Figure 3B). Also, in the case of single cyst volume, we did not observe any significant difference among the three study groups at the end of the observational period.

Finally, the ability of the rapamycin trough level to predict a significant reduction in kidney volume was investigated applying an ROC curve analysis. The area under the curve was 0.771 [95% confidence interval (CI): 0.611–0.888, P = 0.001] and the cut-off blood trough level was 3.2 ng/mL (sensitivity 96% and specificity 60.6%) (Figure 3C).

Renal function analysis. The renal function at the baseline and at the end of 2-year observation is shown in Table 1. We observed a decline in estimated creatinine clearance in Group A patients (P = 0.01, T24 versus T0), whereas in Groups B and C after 24 months, we observed a slight increase in GFR, although the differences did not reach statistical significance (Figure 3D). The changes in the estimated creatinine clearance in the three groups at the end were not significantly different.

In a post hoc analysis, we pooled the two rapamycin treatment groups in the attempt to increase the statistical power. We did not observe any significant differences at T0 between the control and treatment groups in renal...
A significant increase in urine protein excretion at 2-year follow-up compared with Group A patients after 24 months of observation was observed in renal transplant recipients. In our study population, we did not observe any case of oedema and pneumonia. Baseline proteinuria was similar in the three groups. Both Group B and C patients presented an increase in urine protein excretion at 2-year follow-up compared with the baseline. However, only Group B patients presented a significantly higher proteinuria compared with Group A patients after 24 months of observation (Table 1). Oral ulcers were observed in 6 of 19 patients of Group B and in 2 of 18 patients of Group C. Cumulative incidence of hyperlipidaemia was significantly higher in Group B (9 of 19 patients) compared with Group A (0 of 16) and Group C patients (2 of 18) at the end of follow-up (P < 0.001). We observed a mild and self-limiting anaemia in 2 of 19 patients in Group B. Two patients (one in Group B and one in Group C) developed an infection (urinary tract infection in both cases) (Table 1).

All the adverse events observed were mild or moderate according to the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) version 2.0.

Ancillary study

EGF urine excretion. The EGF has an important role in the expansion of renal cysts [21]. We observed an increased EGF urinary excretion in Group A patients (P = 0.001 versus T0), whereas the treatment groups were characterized by a significant reduction after 24 months (Group B: P = 0.0001 versus T0; Group C: P = 0.0001 versus T0) (Figure 4).

Discussion

The results of our pilot study suggest that rapamycin treatment at a dose effectively inhibiting p70S6 kinase in circulating PBMCs does not significantly slow down cyst growth and renal function decline featuring the natural history of ADPKD, although only in the control and in the low rapamycin dose groups, did we observe a significant worsening of renal function and a significant increase in total kidney volume. The current guidelines for the treatment of ADPKD include only the optimal blood pressure control [10, 22], although Schrier et al. [23] did not observe any advantage of a rigorous control of hypertension in preserving renal function in ADPKD patients. In addition, even though ADPKD is a condition characterized by a well-documented hyperactivity of the renin–angiotensin–aldosterone system [24], the use of ACE-I and/or ARB did not show any specific effect in delaying the progressive decline in renal function towards end-stage renal disease [24, 25].

The increasing knowledge on the molecular mechanisms linking the genetic defect underlying ADPKD and cystogenesis has opened a brand new scenario in the therapeutic options of this genetic disease [25]. Based on this knowledge, several studies demonstrated the effectiveness of different therapeutic approaches, including targeting of Src and EGF receptor kinase, in experimental models of the disease [26, 27]. The modulation of cAMP intracellular production, through somatostatin administration, was the first of the new therapies to be introduced in the clinical setting [28].

In the last 5 years, a specific role for mTOR in the pathogenesis of ADPKD was suggested by several experimental studies [14]. Different reports that evaluated the efficacy of rapamycin administration in experimental models of ADPKD demonstrated a significant reduction in kidney cyst burden [14, 29, 30]. Although toxicity was noted, a significant benefit was still seen when circulating rapamycin levels were appropriate for clinical use. Importantly, in a retrospective review of kidney volume in transplant recipients with ADPKD, significant regression in kidney size was seen in those who received rapamycin when compared with those who did not [15]. Recently, three prospective randomized clinical trials [31–33] evaluated the efficacy of the mTOR inhibitors in patients affected by ADPKD with conflicting results. Walz et al. [31] suggested that although everolimus, a further mTOR inhibitor, appeared to slow down the increase in total kidney volume, this change did not correlate with an improvement in the estimated GFR. Serra et al. [32] observed that treatment with rapamycin for 18 months did not slow kidney growth and did not modify the GFR. Finally, Perico et al. [33] did not observe any difference in the increase in total renal volume or change in GFR between rapamycin and conventional treatment. However,
in their experience, the absolute cyst volume was virtually stable in the rapamycin arm, whereas it increased with conventional treatment; the parenchymal volume increased significantly with rapamycin and was stable with conventional therapy. Our results showed that the treatment with mTOR inhibitor may slow down the renal function decline. Moreover, we observed, like Serra et al. [32] and Perico et al. [33], an increase in total kidney volume in all three groups, but in contrast with the study of Serra et al. [32] and in agreement with the observation of Perico et al. [33], we showed a reduction in cyst volume.

In this scenario, our study presents two significant differences with the above-mentioned clinical trials: the use of different doses of rapamycin and the genetic characterization of ADPKD.

Indeed, the key strength of the present study lies in the attempt to identify a target rapamycin trough level in order to obtain a significant reduction in cyst volume. In our experience, although none of the rapamycin doses tested significantly influenced kidney volume and renal function, the group of patients with the higher drug trough levels was the only one in which we did not observe either a worsening in renal function or an increase in cyst volume. In addition, our ROC curve analysis would suggest a cut-off in rapamycin blood trough level higher than 3 ng/mL to obtain a significant effect on the progression of cyst growth. This concentration is significantly lower than the levels used in the kidney transplantation settings [34] and would guarantee a low immunosuppressive effect of rapamycin, potentially allowing long-term treatment without a significant incidence of infections. Moreover, our patient population was screened for type I ADPKD through a genetic approach. Thus, we were able to exclude type II ADPKD patients characterized by a slower progression of cyst volume. In addition, two further advantages of our study are the observation on urine EGF excretion and the measurement of phospho-p70 levels in PBMCs. We showed that EGF urinary excretion was dramatically reduced by rapamycin treatment and it is well known that this growth factor has an important role in the expansion of renal cysts [21]. Our observation would only indirectly suggest that rapamycin may reduce the expression and excretion of this growth factor, although there are no data supporting a correlation between EGF urinary excretion and the concentration of the growth factor within the cyst. Finally, we demonstrated, investigating the phosphorylation of p70S6 kinase, a direct downstream target of mTOR, that also in the group of patients receiving the lower dose of rapamycin, was able to efficiently inhibit mTOR activity.

The main limitation of the present study is represented by the lack of what would be considered a ‘hard’ endpoint. Indeed, our primary outcomes are represented by two surrogate endpoints: the changes in total kidney and cyst volume. The decision to consider these two clinical features as primary outcomes is mainly due to the natural history of ADPKD. Indeed, as shown in several studies, the decline in renal function in this genetic disease is quite slow in its early phase, and including patients with a mild reduction in renal function would not allow us to see any influence of the proposed therapy in a short-term study like the present one [35]. On the other hand, there is an increasing body of evidence suggesting that total kidney and cyst volume may consistently predict the decline in renal function towards end-stage renal disease [35, 36].

The second weakness may be considered the number of patients included in the study. We did not perform a formal calculation since the present randomized trial was designed as a dose-finding study. Our results may, then, represent a new point to start for the next clinical trials to confirm the feasibility to use mTOR inhibitors as a reliable treatment for ADPKD.

In addition, the potential identification of a relatively low target dose is meaningful since the rapamycin-induced adverse events are dose-dependent and represent the main limit for the use of the drug in the immunosuppressive regimen of kidney transplantation [37]. The main clinical trials evaluating the effects of the drug in kidney transplant recipients are characterized by a very high dropout rate due to drug-related adverse events [37]. Thus, it is of particular interest to note the lack of any dropout in the present study in the two groups of treatment and the relatively low incidence of adverse events.

In conclusion, our results would suggest that although rapamycin treatment is relatively safe, it does not significantly affect the progression of ADPKD. However, the higher rapamycin dose used reduced both the increase in total kidney volume and the worsening in renal functions observed in the other two study groups. Thus, our data may still suggest that there is a window of opportunity to treat this genetic disease and strongly support the opportunity of a large randomized controlled trial to better define the role of mTOR inhibition in the treatment of type I ADPKD.

Acknowledgements. The authors wish to acknowledge Wyeth and Pfizer, which supplied the study drug at free of cost.

Authors’ contributions
G.S. and B.I. designed the research and wrote the manuscript. G.G. analysed the data and revised the manuscript. C.B. and L.M. performed the MRI and analysed the data. D.M. and F.B. performed the research. E.M. and A.S. performed the genetic analysis. M.S., T.T. and A.P. performed the research. F.P.S. and L.G. supervised the manuscript.

Conflict of interest statement. G.S., G.G. and F.P.S. received honorary fees for lectures and advisory boards from Wyeth, Novartis and Roche. F.P.S. received unrestricted research grants from Wyeth, Roche and Novartis. L.G. received honorary fees for lectures and advisory boards from Baxter, Amgen-Dompé, Sandoz, Malesci, Genzyme and Roche and has unrestricted research grants from GE Healthcare, Wyeth and Sandoz.

References
Rapamycin and type I ADPKD


Received for publication: 27.2.2012; Accepted in revised form: 19.4.2012