Effect of everolimus on renal function in patients with tuberous sclerosis complex: evidence from EXIST-1 and EXIST-2

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ABSTRACT

Background. A reduction in renal angiomyolipoma volume observed with everolimus (EVE) treatment in patients with tuberous sclerosis complex (TSC) has been postulated to translate to clinical benefit by reducing the risk of renal hemorrhage and chronic renal failure.

Methods. The long-term effects of EVE on renal function (~4 years of treatment) were examined in patients treated with EVE in the Phase 3 EXIST-1 and EXIST-2 studies. Patients in EXIST-1 had TSC and subependymal giant cell astrocytoma (SEGA), and patients in EXIST-2 had renal angiomyolipoma and a definite diagnosis of TSC or sporadic lymphangioleiomyomatosis. EVE was administered at 4.5 mg/m²/day, with adjustment to achieve target trough levels of 5–15 ng/mL in EXIST-1 and 10 mg/day in EXIST-2. Estimated glomerular filtration rate (eGFR) and creatinine levels were assessed at baseline, at Weeks 2, 4, 6, 8, 12 and 18, then every 3 months thereafter. Proteinuria was graded according to National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

Results. A total of 111 patients from EXIST-1 and 112 patients from EXIST-2 were included in this analysis. Respective mean ages at EVE initiation were 10.5 [standard deviation (SD) 6.45] and 33.2 (SD 10.29) years, and 3.6% and 37.5% of patients had undergone prior renal intervention. Mean baseline eGFR was 115 and 88 mL/min/1.73 m² in EXIST-1 and EXIST-2, respectively. Overall, mean eGFR remained stable over time in both studies, with an decline in renal function mostly confined to some patients with severely compromised renal function before treatment. Patients with prior renal intervention exhibited low eGFR values throughout the study. The incidence of proteinuria increased after initiating treatment with EVE and was mostly Grade 1/2 in severity, with Grade 3 proteinuria reported in only two patients. Measurements of proteinuria were limited by the use of urine dipstick tests.

Conclusions. The use of EVE does not appear to be nephrotoxic in patients with SEGA or renal angiomyolipoma associated with TSC and may preserve renal function in most patients.
INTRODUCTION

Tuberous sclerosis complex (TSC), a hereditary disorder, affects ~1 million people worldwide [1]. TSC1 and TSC2 mutations result in increased activation of mammalian target of rapamycin (mTOR) complex 1 (mTORC1), leading to increased metabolism, proliferation and tumor growth [2]. Up to 80% of patients with TSC develop renal angiomyolipomatoma [1]—tumors comprising blood vessels, smooth muscle-like cells and adipose-like tissue [3–5]. Ultrastructural analysis and immunohistochemical and biochemical expression of pericyte-associated proteins by angiomyolipomatoma suggest that they are derived from vascular pericytes, mesenchymal perivascular cells located on the abluminal surface of capillaries involved in the regulation of microvascular stability, development and function [6]. mTORC1 signaling has been shown to play an important mechanistic role in renal hypertrophy [7, 8]. In preclinical models, deletions in TSC2 result in abnormal renal cell polarity and the development of cysts [9, 10].

Renal angiomyolipomatoma are the most common cause of TSC-related mortality in adults [11, 12]. Compared with sporadic renal angiomyolipomatoma, those associated with TSC usually occur in multiples and are larger, bilateral and more likely to grow [13]. Aneurysms develop frequently from these tumors and may lead to life-threatening spontaneous hemorrhage [14]. Renal angiomyolipoma >3 cm, aneurysm >0.5 cm and renal angiomyolipoma growth increase the risk of renal hemorrhage [14, 15]. Patients with renal angiomyolipomatoma are also at risk for hypertension and renal failure, which leads to increased health care utilization and associated costs [12, 13, 16]. Guidelines recommend first-line treatment with a mammalian target of rapamycin (mTOR) inhibitor for asymptomatic, growing renal angiomyolipoma >3 cm in diameter; however, these guidelines also caution that long-term benefits and safety data are needed [17]. Proteinuria is a known adverse event (AE) associated with mTOR inhibitors, and screening is warranted [18].

Everolimus (EVE), an mTOR inhibitor, has demonstrated efficacy in TSC-associated disorders, including renal angiomyolipoma [19–22]. The effect of EVE on renal function was assessed in patients with TSC in the Phase 3 EXIST-1 and EXIST-2 studies. The primary endpoints for these two studies were subependymal giant cell astrocytoma (SEGA) and renal angiomyolipoma response rate, respectively, and EVE demonstrated significant benefit over placebo (PBO) [20, 21]. In addition, in an exploratory subset analysis of patients in EXIST-1 with renal angiomyolipoma, EVE demonstrated a greater reduction in total renal angiomyolipoma volume versus PBO (angiomyolipoma response rate 53.3% versus 0%) [23]. Following positive results in the double-blind core phases, patients receiving PBO were offered open-label EVE in the extension phases [24, 25].

Some preclinical studies have suggested that mTOR activity that is too high or too low may result in kidney injury [26, 27]. In order to better assess this question in the TSC population, the long-term effects of EVE (~4 years) on renal function in patients from EXIST-1 and EXIST-2 are reported here.

MATERIALS AND METHODS

Study design

EXIST-1 and EXIST-2 were randomized, double-blind, PBO-controlled, Phase 3 studies [20, 21, 24, 25]. EXIST-1 (NCT00789828) examined EVE for treating TSC-associated SEGA [20, 24] and EXIST-2 (NCT00790400) examined EVE for treating renal angiomyolipoma associated with TSC or sporadic lymphangioleiomyomatosis (LAM) [21, 25]. Methods from EXIST-1 and EXIST-2 have been published previously; in brief, both studies comprised double-blind core phases followed by open-label extension phases, with patients being followed for 4–5 years [24, 25]. This retrospective analysis examined long-term renal function data collected prospectively throughout both studies.

Patients

In EXIST-1, eligible patients were 0–65 years of age with a definite diagnosis of TSC according to consensus criteria [28, 29] and growing SEGAs, with one or more target SEGA ≥1 cm in diameter assessed by multiphasic magnetic resonance imaging (MRI) [20, 24, 30]. Patients must have been medically stable and unlikely to require surgery for SEGAs, with no critical hydrocephalus or imminent cerebral herniation [20].

Eligible patients in EXIST-2 had a definite diagnosis of TSC or sporadic LAM per consensus criteria [28, 29, 31], and were ≥18 years old with one or more renal angiomyolipoma ≥3 cm in diameter. Patients requiring angiomyolipoma-related surgery or with renal angiomyolipoma-related bleeding or embolization during the previous 6 months or those with severely impaired lung function were excluded [21, 25, 32].

Local independent ethics committees at each center approved the protocols and the studies were conducted in accordance with the principles of Good Clinical Practice, Declaration of Helsinki and all local regulations. Safety data were reviewed every 6 months by an independent data monitoring committee appointed in each study and all patients (or their legal representatives) provided written informed consent before enrolment [20, 21].

Treatment

Patients were originally randomly assigned 2:1 to receive EVE or PBO in a double-blind phase and then could receive open-label EVE in an extension phase. In EXIST-1, patients received EVE at a starting dose of 4.5 mg/m²/day, with adjustment to achieve target trough levels of 5–15 ng/mL [20, 24]. In EXIST-2, patients received EVE 10 mg/day, with dose modifications allowed based on tolerability [21, 25].

Endpoints and assessments

In EXIST-1, the primary endpoint was SEGA response rate, defined as the proportion of patients with ≥50% reduction from baseline in SEGA volume (in the absence of worsening of ClinicalTrials.gov identifiers NCT00789828 and NCT00790400

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nontarget SEGAs, new lesions $\geq$ 1 cm in diameter and new or worsening hydrocephalus) [20, 24, 30]. Secondary endpoints included time to and duration of SEGAs, in the absence of new target lesions, and red 20% increase from nadir in kidney volume or Grade $\geq$ 2 renal angiomyolipoma-related bleeding). Secondary endpoints included time to and duration of renal angiomyolipoma response, time to progression and safety [21, 25].

In both studies, estimated glomerular filtration rate (eGFR) and creatinine levels were assessed at baseline and Weeks 2, 4, 6, 8, 12 and 18, then every 3 months thereafter. Protein was assessed by urine dipstick by the investigator at baseline and study visits at Weeks 4, 8, 12, 18 and 24, then every 12 weeks thereafter. Results were confirmed by a central laboratory in the event of relevant abnormalities. eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula [33] for patients $\geq$ 18 years of age or the ‘bedside’ Schwartz formula [34] in those $<$ 18 years. Creatinine and proteinuria were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.0 [35].

**Statistical methods**

For both studies, all EVE data from both the double-blind core phase and the open-label extension were combined and analyses were performed on all patients who received one or more dose of EVE. Baseline demographics and patient characteristics were summarized using descriptive statistics {mean [standard deviation (SD)], median (range)}. Baseline was defined as the last available assessment on or before the first dose with EVE. The mean eGFR values and percentage eGFR change from baseline over time as well as median eGFR per baseline chronic kidney disease (CKD) stage were calculated. The proportions of patients with severe renal impairment (GFR $< 30$ mL/min/1.73 m$^2$, CKD Stage 4/5) or with Grade 3 or 4 serum creatinine or proteinuria (per NCI CTCAE version 3.0) at any time during treatment with EVE were determined and urinary protein dipstick results over time were summarized. Statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA).

**RESULTS**

**Patients**

Table 1 shows baseline demographics and clinical characteristics of the 111 and 112 patients in EXIST-1 and EXIST-2, respectively, who received one or more dose of EVE and are included in this analysis. Because of the age at which SEGAs typically appear, all patients who received EVE in EXIST-1 were $< 30$ years of age. Angiomyolipomata more commonly cause symptoms in adulthood, therefore entry criteria for EXIST-2 restricted patients to those $\geq$ 18 years of age. EXIST-1 enrolled more males than females, whereas two-thirds of the patients in EXIST-2 were female. Sixty-four percent of patients in EXIST-1 also had evidence of renal angiomyolipoma, but lesions were smaller than those in patients in EXIST-2. Approximately one-third of patients (38%) in EXIST-2 had undergone renal interventions before recruitment; 23 patients had undergone prior embolization, 20 had prior nephrectomy (total or partial), 4 had surgical removal of a lesion and 1 had thermal ablation. In EXIST-1, four patients had undergone a prior renal intervention (nephrectomy).

At study completion (2 October 2014 and 4 February 2015, for EXIST-1 and EXIST-2, respectively), the median duration of EVE exposure was $\sim 47$ months in both studies. Nearly 75% of the patients completed the studies per the protocol (Table 2) [24, 25].

| Table 1. Baseline demographics and disease characteristics |
|---------------------------------|-----------------|-----------------|
| Characteristics                 | EXIST-1 ($n = 111$) | EXIST-2 ($n = 112$) |
| Age (years)                     | Mean (SD)       | 10.5 (6.4)      | 33.2 (10.3) |
| Age categories, n (%)           | Median (range)  | 9.5 (1.1–27.4) | 32.2 (18.1–61.6) |
| £3                             | 18 (16.2)       | 0               |
| $<$ 10                         | 41 (36.9)       | 0               |
| $\geq$ 18                      | 34 (30.6)       | 0               |
| $<$ 30                         | 18 (16.2)       | 112 (100)       |
| $\geq$ 30                      | 111 (100)       | 49 (43.8)       |
| Male, n (%)                    | 64 (57.7)       | 39 (34.8)       |
| Race, n (%)                    | White 104 (93.7) | 99 (88.4)       |
| Asian 0                        | 11 (9.8)        |                 |
| Other 7                        | 6 (3.6)         | 2 (1.8)         |
| Diagnosis of TSC, n (%)         | 111 (100.0)     | 107 (95.5)      |
| Presence of SEGAs, n (%)        | 111 (100.0)     | 55 (49.1)       |
| Presence of renal angiomyolipoma, n (%) | 71 (64.0) | 111 (99.1)       |
| Prior renal angiomyolipoma-related interventions, n (%) | 4 (3.6) | 42 (37.5) |
| Renal angiomyolipoma lesions $\geq$ 1 cm, n (%) | 0 | 54 (48.6) | 2 (1.8) |
| 1–5                            | 33 (29.7)       | 43 (38.4)       |
| 6–10                           | 8 (7.2)         | 67 (59.8)       |
| Patients with one or more evaluable lesion, n (%) | 38 (34.2) | 110 (98.2)       |
| Sum of volumes of target lesions (cm$^3$) | 10 | 92 |
| Median                         | 0.5–198.1       | 2.8–1611.5      |
| GFR (mL/min/1.73 m$^2$)         | Mean (SD)       | 115 (27.9)$^a$ | 88 (31.9)$^a$ |
| CKD stage at baseline, n (%)    | 1               | 94 (84.7)       | 47 (42.0) |
| 2                              | 13 (11.7)       | 40 (35.7)       |
| 3                              | 3 (2.7)         | 20 (17.9)       |
| 4                              | 0 (0.0)         | 3 (2.7)         |
| Missing                        | 1 (0.9)         | 2 (1.8)         |

$^a$Baseline is defined as the last available assessment on or before the start date of EVE treatment.

$^b$Includes embolization and partial nephrectomy.

$^c$Evaluable target renal angiomyolipoma lesions $\geq$ 1 cm.

$^d$n$$_0$ = 100.

$^e$n = 98.
Renal function

Overall, mean eGFR remained stable over time in both studies (Figure 1). Mean eGFR values were lower in EXIST-2 than in EXIST-1. One patient in EXIST-1 and eight in EXIST-2 experienced severe renal impairment (GFR < 30 mL/min/1.73 m²) at least once postbaseline. All of these patients had considerably compromised renal function before starting EVE (i.e. Stage 3/4 CKD) and eight exhibited continued renal function decline (Table 3). In other patients, including 27 with eGFR <60 mL/

Table 2. Patient disposition

| Duration of EVE exposure (months), (median (range)) | EXIST-1 (n = 111) | EXIST-2 (n = 112) |
|-----------------------------------------------|------------------|------------------|
| Completed per protocol, n (%)                  | 82 (73.9)        | 83 (74.1)        |
| Discontinued, n (%)                            | 29 (26.1)        | 29 (25.9)        |
| AE                                            | 10 (9.0)         | 9 (8.0)          |
| Administrative problems                        | 7 (6.3)          | 2 (1.8)          |
| Lost to follow-up                              | 3 (2.7)          | 1 (0.9)          |
| Patient withdrawal of consent                  | 6 (5.4)          | 7 (6.3)          |
| Disease progression                            | 1 (0.9)          | 5 (4.5)          |
| New treatment for indication under study       | 1 (0.9)          | 2 (1.8)          |
| Abnormal laboratory value                      | 0                | 1 (0.9)          |
| Death                                         | 1 (0.9)          | 1 (0.9)          |
| Protocol deviation                             | 0                | 1 (0.9)          |

aAEs leading to discontinuation in EXIST-1 were Acinetobacter bacteremia, aggression, anemia, zoosperinia, blood alkaline phosphatase level increase, focal segmental glomerulosclerosis, need for neurosurgery, neutropenia, pneumonia, pneumothorax, sinusitis, stomatitis and viral infection (one patient each, 0.9%).

bAEs leading to discontinuation in EXIST-2 were angioedema, bronchosperm, convulsions, diarrhea, hypersensitivity, localized edema, malaise, pancreatic carcinoma, nasal sinus cancer, proteinuria, rhabdomyolysis and skin toxicity (one patient each, 0.9%).

cSEGA progression defined as one or more of the following: increase from nadir of 20% in SEGA volume to a value greater than baseline, unequivocal worsening of non-target SEGA lesions, appearance of a new SEGA lesion >1.0 cm in longest diameter and new or worsening hydrocephalus.

dRenal angiomyolipoma progression defined as one or more of the following: ≥25% increase from nadir in angiomyolipoma volume, ≥20% increase from nadir in the volume of either kidney with a value greater than baseline, appearance of new angiomyolipoma ≥1 cm and Grade ≥2 angiomyolipoma-related bleeding.

ePatient sought other treatment for disease under study.

Safety/tolerability

One patient in EXIST-1 and one patient in EXIST-2 experienced Grade 3 elevations in serum creatinine [>3.0 × upper limit of normal (ULN)]. Grade 1 or 2 serum creatinine elevations (>ULN to 3.0 × ULN) occurred in 3.6 and 15.2% of patients in EXIST-1 and EXIST-2, respectively. Only two of the

FIGURE 1: Mean eGFR over time in the EXIST-1 and EXIST-2 studies. The MDRD formula [33] was used to estimate GFR for patients ≥18 years of age and the ‘bedside’ Schwartz formula [34] was used to estimate GFR in patients <18 years of age.
serum creatinine elevation events in EXIST-1 and seven in EXIST-2 were reported as AEs. Proteinuria was classified as an AE by the investigator in 3.6% of EXIST-1 and 17.9% of EXIST-2 patients during the course of the studies; this was mostly Grade 1 or 2 (1+ or 2+ to 3+ on a urine dipstick test) in severity. Grade 3 proteinuria (4+ on a urine dipstick test) was reported as an AE in only two patients in EXIST-2; both were suspected to be related to treatment and one led to study discontinuation.

Table 3. Individual trends over time in patients with eGFR <30 mL/min/1.73 m² at any time throughout the EXIST-1 and EXIST-2 studies

| Patient | Treatment group | eGFR, mL/min/1.73 m² | Baseline<sup>a</sup> | Month<sup>b</sup> 1 | Month<sup>b</sup> 3 | Month<sup>b</sup> 6 | Month<sup>b</sup> 12 | Month<sup>b</sup> 18 | Month<sup>b</sup> 24 | Month<sup>b</sup> 30 | Month<sup>b</sup> 36 | Month<sup>b</sup> 42 | Month<sup>b</sup> 48 | Month<sup>b</sup> 54 |
|---------|-----------------|----------------------|-----------------------|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| EXIST-1  | EVE             | 53.4                 | 61.7                 | 51.6                | 53.1              | 43.1              | 41.5              | 36.9              | 27.1              | 32.7              | 24.0              | –                 | –                 |
| 2       | EVE             | 48.8                 | 42.7                 | 37.6                | 35.7              | 37.5              | 33.1              | 39.7              | 40.2              | 34.3              | 28.5              | 25.2              | 26.3              |
| 3       | EVE             | 51.2                 | 48.8                 | 31.3                | 42.2              | 41.2              | 46.7              | 37.7              | 21.6              | 30.7              | 27.9              | 28.3              | 24.5              |
| 4       | EVE             | 50.2                 | 50.7                 | 53.5                | 48.6              | 47.0              | 45.1              | 43.8              | 40.6              | 34.5              | 29.4              | 28.9              | 30.3              |
| 5       | EVE             | 35.8                 | 33.5                 | 33.5                | 33.4              | 33.3              | 29.4              | 27.7              | 24.9              | 26.2              | 27.5              | 27.5              | 27.4              |
| 6       | EVE             | 29.2                 | 29.2                 | 21.3                | 26.0              | 25.9              | 22.2              | 17.1              | 17.8              | 19.3              | 19.2              | –                 | –                 |
| 7       | Placebo         | 23.0                 | 24.3                 | 23.2                | 23.3              | 20.6              | 17.9              | 18.2              | 15.0              | 11.9              | –                 | –                 | –                 |
| 8       | Placebo         | 24.3                 | 20.2                 | 30.5                | 21.9              | 24.7              | 25.7              | 22.8              | 24.8              | 21.7              | –                 | –                 | –                 |
| 9       | Placebo         | 42.8                 | 42.7                 | 39.6                | 36.9              | 36.8              | 36.7              | 23.4              | 20.5              | 17.5              | –                 | –                 | –                 |

All eGFR measurements were done centrally and timings of measurements are approximate.

<sup>a</sup>Refers to the last measurement of eGFR before starting EVE.

<sup>b</sup>One month = 30.4 days. Values come from the measurement on the day closest to the end of the month for each patient.

**FIGURE 2**: Boxplots of the change in eGFR based on treatment and baseline CKD stage during the double-blind phases of (A) EXIST-1 and (B) EXIST-2. The MDRD formula [33] was used to estimate GFR for patients ≥18 years of age and the ‘bedside’ Schwartz formula [34] was used to estimate GFR in patients <18 years of age.
discontinuation but resolved after dose reduction/interruption. This patient was the only patient between the two studies that permanently discontinued treatment because of proteinuria.

**DISCUSSION**

Previously published reports from the EXIST-1 and EXIST-2 studies [20, 21, 23–25, 32] have demonstrated that the use of EVE was associated with >40–50% reduction in renal angiomyolipoma volume with continued volume reduction over time. The current data add to our knowledge, demonstrating that renal function was generally stable over ~4 years of EVE treatment. A decline in renal function, with progression to Stage 4 CKD, was confined to only those patients with compromised renal function at baseline (Table 3). As a result, there is the need for ongoing close monitoring of renal function in these patients as with any patient with advanced CKD. On average, the lower eGFR values reported in EXIST-2 as compared with EXIST-1 likely reflect differences in disease severities and trajectories of renal angiomyolipoma and patient age in these two patient populations.

The natural course of kidney function decline in TSC is not well known, although some existing data in larger populations of TSC patients suggest that kidney function declines more rapidly than in the general population. A retrospective database analysis of renal involvement in patients with TSC in the UK found that the prevalence of Stage 3 or higher CKD in the TSC population was more than five times greater than in the UK general population.
population [relative risk 5.4 (95% confidence interval 3.7–8.0); P < 0.001]. The estimated prevalence of CKD increased from 3.1% in 18- to 24-year-olds to 41.2% in 45- to 54-year-olds in the TSC population, compared with 0.1–2.9%, respectively, in the UK general population [36].

As would be expected based on the differing enrolment criteria of the studies, renal disease was more severe in patients in EXIST-2 than in EXIST-1. Renal angiomyolipoma burden at baseline was greater in EXIST-2; 7% of those in EXIST-1 and 60% of those in EXIST-2 had six or more renal angiomyolipoma lesions measuring ≥1 cm and 37.5% of the patients in EXIST-2 had undergone prior renal angiomyolipoma-related interventions (Table 1), with nephrectomy (full or partial) in 20 patients (18%). By contrast, in EXIST-1, 4 of 111 patients (3.6%) had undergone a prior renal intervention. In patients who undergo nephrectomy, renal function is typically halved [37], which could have contributed to the lower eGFR in the patients in EXIST-2 compared with EXIST-1 (Figure 1), and is one of the reasons why clinical guidelines recommend avoidance of surgical and embolic or ablative therapies where possible in patients with renal angiomyolipoma [17]. Kidneys with a large renal angiomyolipoma burden can still contribute significantly to renal function [38], therefore the decision to undergo nephrectomy should be balanced against loss of function. Our data indeed show the difference that previous nephrectomy has on eGFR in patients in EXIST-2, but no significant decline in eGFR over time was observed during treatment in these patients. The effects of age likely contributed to the differences between the observed eGFRs in each study (Figure 3). With regards to the increase in percentage change of eGFR seen in EXIST-1, it is possible we may be observing that predominantly younger patients with TSC renal disease and early impairment of GFR improve on EVE. However, later time points have fewer patients due to discontinuations or not reaching that length of treatment by the cutoff date, so caution should be exercised in interpreting the results of the latest time points of the figures showing eGFR. It should also be noted that although both studies estimated GFR in adults using the MDRD equation, because the MDRD formula is believed to overestimate GFR in children, the Schwartz formula was used to estimate GFR in patients <18 years of age (the majority of the population in EXIST-1). Among other differences with the MDRD equation, the Schwartz formula includes patient height as a variable instead of age [33, 34].

Reduction of the renal angiomyolipoma burden without evidence of renal function impairment may slow or halt the progression of CKD and reduce the risk of hemorrhage. The mechanism of EVE-associated amelioration of premature GFR decline in TSC is unknown. In particular, it is unknown whether it is due to a direct effect on renal angiomyolipomas. mTOR has been implicated in podocyte maintenance and glomerular disease associated with diabetic nephropathy, with activation of mTORC1 resulting in effaced foot processes and proteinuria [26, 27, 39]. mTORC1 haplotype insufficiency (i.e. independent of renal angiomyolipomas) may provide a possible alternate mechanism for progression of CKD in TSC. Preclinical studies have demonstrated that mTORC1 appears to contribute to the development of normal-size glomeruli and podocytes, whereas decreased mTORC1 activity results in glomerular injury [26, 27]. Thus too much or too little mTOR activation may cause renal injury, but our results show that careful dose titration preserved eGFR in most patients. Only nine patients developed Stage 4 CKD (eGFR <30 mL/min/1.73 m²), and it is unclear whether this was despite EVE treatment or if EVE exacerbated their underlying renal impairment. Furthermore, the timing and effects of mTOR inhibition appear to be important, with early inhibition with rapamycin preventing renal injury caused by mTORC1 activation in podocytes [26, 39]. This suggests that continued surveillance and earlier treatment may lead to improved longer-term renal function.

Proteinuria is a known mTORC1 inhibitor–associated AE [18]. Proteinuria has previously been reported in small
single-arm studies with the mTOR inhibitor sirolimus for the treatment of TSC-associated renal angiomyolipomas [40, 41], with an incidence of 27.8% reported in one study [40]. However, the majority of cases in that study were mild (trace or 1+ on a urine dipstick) in severity, with few patients (7.1%) experiencing more severe proteinuria (3+ or 4+ on a urine dipstick) [40]. The presence of proteinuria in baseline laboratory analyses in EXIST-1 and EXIST-2 suggest that proteinuria may be a feature of TSC renal disease. This may be further supported by findings in the double-blind phase of EXIST-2, in that proteinuria was more common in patients receiving PBO than in those receiving EVE (8% versus 4%) [21]. In both studies, most patients had negative or trace protein values over time; however, the proportion of patients with negative results did decrease shortly after EVE initiation and the proportion with 1+ and 2+ values was higher after initiation of EVE. The proportion of patients with higher values remained at ~6% or less after 1 year and through the end of the study.

Proteinuria is often mild, transient or variable in patients taking EVE for TSC. There is no evidence from this study or the TSC literature that it is a marker for hyperfiltration nephropathy in this situation, and the etiology may be distinct from that which occurs in renal transplantation. Not uncommonly, there is an element of tubular proteinuria in patients with TSC on mTORC1 inhibitors, and the mTORC1 pathway clearly regulates proximal tubule endocytosis [42]. However, it would seem prudent that if proteinuria is >1 g/day and rising progressively, the dose of EVE should be adjusted (reduced or temporarily suspended until proteinuria is <1 g/day). It is probable that proteinuria in this situation is partly or mainly a functional effect (perhaps mediated by inhibiting the mTOR pathway in podocytes or renal tubular cells) because, in practice, it almost always decreases or resolves on dose reduction. In patients who are likely to have hyperfiltration nephropathy for other reasons (e.g. GFR <60 mL/min/1.73 m² and/or hypertension) it seems reasonable to use an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker.

Limitations to our analyses include limited numbers in this subanalysis, use of open-label data (no PBO comparison), the use of two different EVE dosing methods between the two studies, assessment of renal function only by serum creatinine (which is influenced by factors other than renal function, such as muscle mass) and calculation of GFR by two different equations. Measurement of other biomarkers of kidney function such as cystatin C or creatinine clearance would provide useful additional information regarding kidney function in these patients. Also, the proteinuria analyses were limited in that urine creatinine was characterized by dipstick with local laboratory testing and central measurements of proteinuria were not performed. Finally, patients with severe kidney function impairment (defined as creatinine >1.5 times ULN) were excluded from study enrolment in both EXIST-1 and EXIST-2.

In conclusion, data from EXIST-1 and EXIST-2 suggest preservation of renal function as assessed by eGFR and serum creatinine elevations, except in some patients whose renal function is already severely compromised. Overall, EVE does not appear to be nephrotoxic in patients with SEGK or renal angiomyolipoma associated with TSC. However, long-term monitoring of such patients and further studies are warranted.

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CONFLICT OF INTEREST STATEMENT
None declared. The results presented in this article have not been published previously in whole or part, except in abstract format.

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