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A multicenter, randomized, placebo-controlled, double-blind phase 3 trial with open-arm comparison indicates safety and efficacy of nephroprotective therapy with ramipril in children with Alport's syndrome

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Children with Alport syndrome develop renal failure early in life. Since the safety and efficacy of preemptive nephroprotective therapy are uncertain we conducted a randomized, placebo-controlled, double-blind trial in 14 German sites of pediatric patients with ramipril for three to six years plus six months follow-up to determine these parameters. Pretreated children and those whose parents refused randomization became an open-arm control, which were compared to prospective real-world data from untreated children. The co-primary endpoints were safety (adverse drug reactions) and efficacy (time to progression). Out of 66 oligosymptomatic children, 22 were randomized and 44 joined the open-arm comparison. Ramipril therapy showed no safety issues (total of 216.4 patient-years on ramipril; adverse event rate-ratio 1.00; 95% confidence interval 0.66-1.53). Although not significant, our results cautiously showed that ramipril therapy was effective: in the randomized arm, Ramipril decreased the risk of disease

progression by almost half (hazard ratio 0.51 (0.12–2.20)), diminished the slope of albuminuria progression and the decline in glomerular filtration. In adjusted analysis, indications of efficacy were supported by prospective data from participants treated open label compared with untreated children, in whom ramipril again seemed to reduce progression by almost half (0.53 (0.22-1.29)). Incorporating these results into the randomized data by Bayesian evidence synthesis resulted in a more precise estimate of the hazard-ratio of 0.52 (0.19-1.39). Thus, our study shows the safety of early initiation of therapy and supports the hope to slow renal failure by many years, emphasizing the value of preemptive therapy. Hence, screening programs for glomerular hematuria in children and young adults could benefit from inclusion of genetic testing for Alport-related gene-variants.

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¹⁸EARLY PRO-TECT Alport Investigators are listed in the [Appendix](#).

¹⁹All participating centers are listed in the [Supplementary Material](#).

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Chronic kidney disease (CKD) affects more than 10% of people worldwide.¹ Renal fibrosis is the common endpoint of most CKD.² Therefore, preventing or

delaying the progression of renal fibrosis is one of the most urgent goals in renoprotective medicine.² The type IV collagen disease Alport syndrome (AS) is the second most common monogenic cause of end-stage renal failure (ESRF), responsible for almost 4% of CKD in adults.^{3–6} AS is caused by variants in the *COL4A3*, *COL4A4*, and *COL4A5* genes, which encode for the $\alpha 3$, $\alpha 4$, and $\alpha 5$ chains of type IV collagen.^{4,7} The defective type IV collagen leads to basement membrane defects in the inner ear, eye, and glomerular basement membrane, leading to ESRF early in life (median age: 22 years in Europe).^{6,8}

In a mouse model of AS, time to ESRF can be doubled if therapy with the angiotensin-converting enzyme inhibitor (ACEi) ramipril is started before the onset of proteinuria.⁹ This effect in mice confers only a small benefit if therapy is started after progressive proteinuria has taken hold. Registry data demonstrated that treatment with an ACEi also delays ESRF in humans with AS in a time-dependent manner.⁸ Treatment starting in CKD stage 3 or 4 delays ESRF by a median of 3 years, while treatment starting in CKD stage 2 delays ESRF by a median of 18 years, leaving open the question of whether an even earlier start (CKD stage 0 or 1) is even more effective while remaining safe.

Several features of AS pathogenesis facilitate efforts to address this question. First, the evolutionarily highly conserved type IV collagen in mammals allowed preclinical therapeutic approaches in mice with AS.^{6,10–12} The developmental switch of collagens in children with AS allows a window of opportunity to initiate treatment with an *earlier the better* potential before structural harm to the glomerular basement membrane has been established.^{5,8,11} Second, AS can be diagnosed accurately by genetic testing and has a clearly defined course starting with hematuria, microalbuminuria, and proteinuria progressing to renal fibrosis.⁶ Awareness of family history of renal failure improves adherence to the study protocol. The unmet medical need for effective treatment allowed this trial to have a long period of randomized versus placebo treatment, which is unique in a pediatric trial for a serious disease.¹³ Finally, the pros and cons of renin–angiotensin–aldosterone (RAAS) blockade have been extensively studied in adults.⁸ However, in children with CKD, the Effect of Strict Blood Pressure Control and ACE Inhibition on the Progression of Chronic Renal Failure in Pediatric Patients (ESCAPE) trial, published in 2009, is still the only large clinical trial evaluating the effect of RAAS blockade in conventional versus intensified blood pressure control.¹⁴ Recently, *post hoc* analyses of the ESCAPE trial showed that early proteinuria reduction by ramipril predicted (improved) renal survival in children with CKD.¹⁵ The baseline characteristics in the ESCAPE trial, with very low estimated glomerular filtration rate (eGFR), and the rationale (therapy to delay further kidney damage) are very different than those in the Early Prospective Therapy European Community Trial in Alport syndrome (EARLY PRO-TECT Alport).

Here, we tested the hypothesis that preemptive therapy in children with AS prior to ultrastructural kidney damage is safe and more efficient than later-onset therapy. This question could not be sufficiently answered in registries to justify treatment recommendations in toddlers.¹⁶ As a consequence, this trial is the first randomized and placebo-controlled study to investigate the safety and nephroprotective properties of RAAS blockade in children. Prospectively, an evidence synthesis with observational data was planned, including those patients whose parents refused randomization and who were treated open label, and untreated patients prospectively followed in the US Alport registry. This preemptive approach could benefit most patients in early stages of glomerular kidney diseases.¹⁷

RESULTS

Study objectives, target population, and baseline characteristics

A total of 66 children were eligible for the study at screening (Figure 1). Twenty randomized children and 42 open label-treated children completed the treatment phase. The mean age was 8.8 ± 4.2 years, and many of the children entered the trial before elementary school (18 of 62 [29%] below 6 years of age; Table 1). Baseline albuminuria was comparable in the 2 randomized groups (placebo: 23 [range: 19.1–77.1] versus ramipril: 39.4 [range: 19.8–82.9] mg/g creatinine [gCrea]) (Supplementary Table S2). All patients had normal blood pressure, normal eGFR (Table 1),¹⁸ few comorbidities, and few comedications (Supplementary Tables S8, S9, and S10). Half of our patients (31 of 62; 50%) had relatives with AS who developed ESRF (median age of 35 years, including 4 heterozygous females with X-linked AS; range: 16 to 80 years).

Co-primary endpoint safety. The duration of patients' therapy added up to a total of 216.4 patient-years on ramipril. Analysis of 465 adverse events (AEs) before disease progression in the randomized arm showed that ramipril therapy was safe (rate ratio 1.00; 95% confidence interval [CI] 0.66–1.53; Figure 2 and Table 2; see Supplementary Table S11 for a complete list of AEs). For the key secondary safety endpoint AEs over the treatment period, the incidence rate of AEs was very similar in the placebo (0.63) and ramipril (0.60) groups (rate ratio 0.96; 95% CI 0.63–1.45). No malignancies or deaths were reported. In the randomized group, none of the 11 serious AEs were, after second assessment by the data safety and monitoring board, drug related or unsuspected. One of 13 serious AEs in the open arm was related to kidney function: a child with dual RAAS blockade (ramipril plus angiotensin-receptor-blocker) developed acute renal failure and hyperkalemia, which resolved without sequelae after hospital admission. The case was discussed extensively with the data safety and monitoring board, and the parents were instructed to avoid dehydration in their child, who remained in the study. Dual RAAS blockade was restarted under intensified surveillance with 1 additional AE—hyperkalemia—which was not rated as severe, until the end of the study. By the end of the study, 19.0% (8 of 42) of

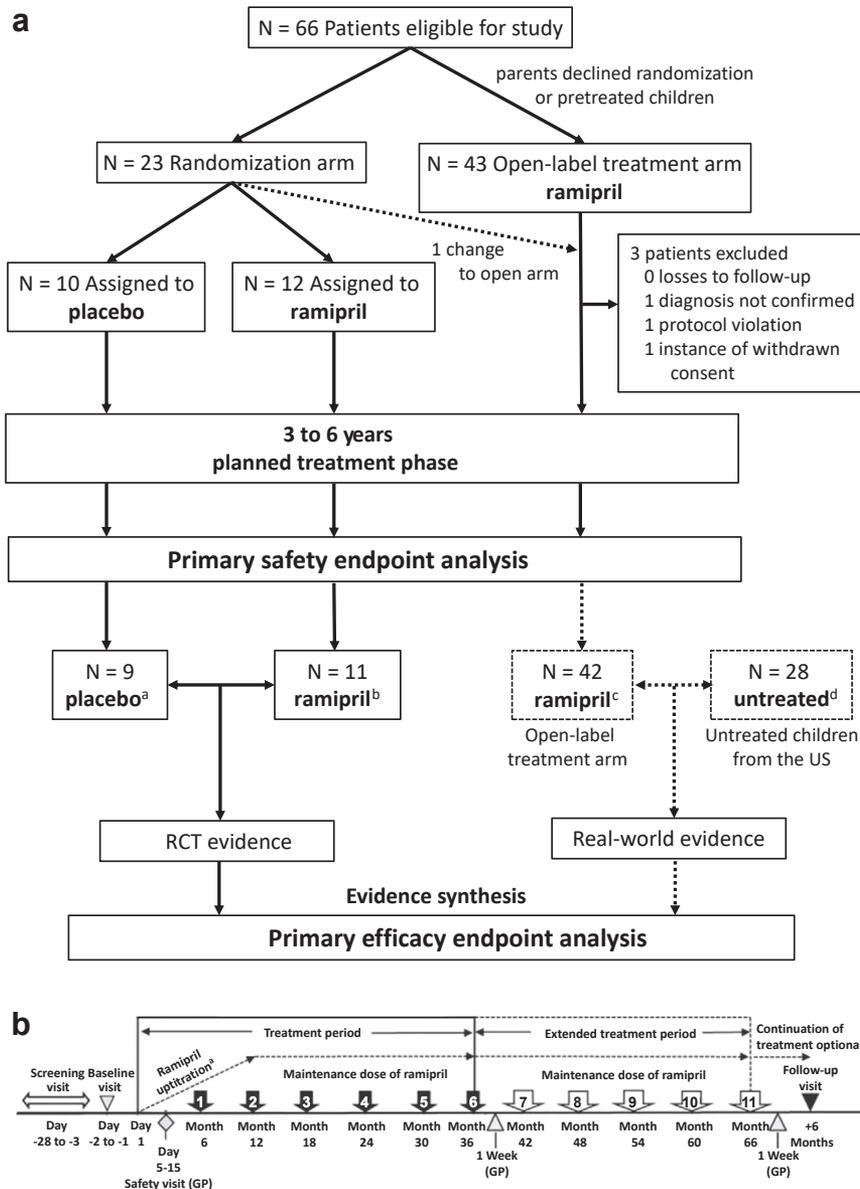


Figure 1 | Eligibility, enrollment, randomization and trial schedule. (a) Flow diagram including the open arm (CONSORT Flow Diagram of randomized patients provided in [Supplementary Material](#)). A total of 20 randomized children completed the trial and were analyzed (RCT evidence). In addition, 42 children in the open-ramipril treatment arm completed the trial, and their data were compared to data from 28 untreated children from the US registry (real-world evidence) in an evidence synthesis approach. (b) Trial schedule with slow up-titration for 12 months, treatment period, and extended treatment period. GP, general practitioner; RCT, randomized controlled trial. ^an = 1 premature study discontinuation; ^bn = 1 protocol violation at baseline; ^cn = 4 premature study discontinuation; however, patient data until study discontinuation included in analysis (plus data from 1 patient until protocol violation); ^duntreated children from US-Alport registry (ASTOR), NCT00481130.

children had received an AT1-antagonist (double RAAS blockade) because of progressive albuminuria (see [Supplementary Table S4](#) for the characteristics of pre-treated children in the open-arm). Four of these children were on double RAAS blockade for more than 1 visit, and they had 61 AEs with a slightly increased event rate of 0.76 (95% CI 0.60–0.97) compared to ramipril monotherapy. Dry cough was reported in 9 patients, in 7 of 9 (77.8%) associated with *common cold* and as *possibly related to study medication* in only 2; the cough led to unblinding in 1 randomized patient

([Figure 3a](#)). The up-titration of ramipril was well tolerated. Dosages were not different between groups (mean 4.4 ± 1.1 mg/m² placebo; 4.5 ± 0.9 mg/m² ramipril; 4.8 ± 1.0 mg/m² in the open arm). Dosages in our normotensive children were very similar to the high dosages reached in the ESCAPE trial with hypertensive children.¹⁴

Co-primary endpoint efficacy. In the randomized controlled trial (RCT) arm, results indicate—although not significant in the classical mathematical definition—that ramipril decreased the risk of progression by almost 50% (adjusted hazard ratio

Table 1 | Baseline characteristics of EARLY PRO-TECT Alport

Characteristic	EARLY PRO-TECT Alport trial						US registry	
	Placebo (n = 9)		Ramipril (n = 11)		Open (n = 42)		Placebo (n = 28)	
Male sex, n (%)	9	(100.0)	11	(100.0)	40	(95.2)	28	(100)
Ethnicity, n (%)								
Caucasian	8	(88.9)	10	(90.9)	42	(100.0)		
Turkish	1	(11.1)	1	(9.1)	0	–		
x-chromosomal, n (%) ^a	7	(77.8)	11	(100.0)	33 ^a	(78.6)		
Autosomal, n (%)	2	(22.2)	0	–	8	(19.0)		
Disease stadium at baseline, n (%)								
0	5	(55.6)	5	(45.5)	11	(26.2)	19	(67.9)
I	4	(44.4)	6	(54.5)	19	(45.2)	9	(32.1)
II	0	–	0	–	12	(28.6)	–	–
Mean age, yr (SD)	7.7	4.2	8.3	4.9	9.2	4.1		
Median age, yr (SD)	7.0	(5.0–8.0)	6.0	(3.0–14.0)	10.0	(5.0–13.0)	7.0	(5.0–10.0)
Mean height, cm (SD)	125.3	30.0	133.8	32.6	133.9	26.7		
Median height, cm (IQR)	115.0	(111.4–134.0)	122.5	(105.4–169.4)	135.7	(108.8–153.0)		
Mean z score, height, % (SD)	–0.5	1.6	0.5	1.1	–0.4	1.2		
Median z score, height, % (IQR)	–0.5	(–1.5 to 0.8)	0.5	(–0.5 to 1.0)	–0.6	(–1.1 to 0.4)		
Mean weight, kg (SD)	32.7	22.5	34.8	21.9	34.3	19.9		
Median weight, kg (IQR)	25.0	(17.3–31.0)	26.2	(16.7–54.3)	29.4	(19.4–43.0)		
Mean z score, weight, % (SD)	0.4	2.1	0.3	1.0	–0.2	1.3		
Median z score, weight, % (IQR)	0.2	(–1.7 to 1.7)	–0.0	(–0.6 to 1.2)	–0.4	(–0.9 to 0.2)		
Mean body mass index, kg/cm ² (SD)	18.6	4.8	17.5	3.5	17.5	3.4		
Median body mass index, kg/cm ² (IQR)	17.8	(15.5–20.1)	16.7	(15.1–17.7)	16.5	(15.7–17.9)		
Mean systolic heart pressure, mm Hg (SD)	111.8	10.5	109.2	14.4	105.1	9.5		
Median systolic heart pressure, mm Hg (IQR)	108.0	(105.0–117.0)	110.0	(97.0–116.0)	103.0	(98.0–112.0)		
Mean z score systolic blood pressure, % (SD)	1.3	1.2	0.6	1.1	0.2	1.0		
Median z score systolic blood pressure, % (IQR)	1.2	(0.9–2.3)	0.8	(–0.4 to 1.4)	0.1	(–0.3 to 0.9)		
Mean diastolic blood pressure, mm Hg (SD)	63.4	8.7	61.6	6.7	61.2	8.3		
Median diastolic blood pressure, mm Hg (IQR)	61.0	(61.0–63.0)	59.0	(56.0–67.0)	60.0	(57.0–66.0)		
Mean z score diastolic blood pressure, mm Hg (SD)	0.3	1.3	–0.1	0.9	–0.2	1.3		
Median z score diastolic blood pressure, mm Hg (IQR)	0.2	(–0.7 to 0.6)	–0.2	(–0.9 to 0.6)	–0.3	(–1.0 to 0.3)		
Mean albumin in urine at screening, mg/gCrea (SD)	69.9	72.4	49.6	59.7	290.6	407.2		
Median albumin in urine at screening, mg/gCrea (IQR)	31.3	(24.3–99.9)	18.1	(15.4–61.3)	135.0	(22.1–272.5)		
Mean albumin in urine at baseline, mg/gCrea (SD)	61.1	81.3	76.4	101.5	284.5	395.1	25.3	24.3
Median albumin in urine at baseline, mg/gCrea (IQR)	23.0	19.1–77.1	39.4	19.8–82.9	94.0	23.3–364.7	15.7	(9.8–38.8)
Mean eGFR at screening, ml/min (SD)	126.5	15.8	131.2	23.6	128.1	29.2		
Median eGFR at screening, ml/min (IQR)	121.5	(115.7–134.8)	130.1	(107.6–151.5)	125.0	(108.9–152.8)		

EARLY PRO-TECT Alport, Early Prospective Therapy European Community Trial Alport; IQR, interquartile range.

^aPlus 1 boy in the open arm with probably x-chromosomal inheritance (based on the genealogical tree), but not examined in terms of molecular genetics.

Patients' characteristics at baseline indicated early-phase disease still in the oligosymptomatic stage, very young age, accurate diagnosis (confirmed by genetic testing or kidney biopsy in all patients or their close relatives; see [Supplementary Table S1](#)), normal blood pressure, and (for the time being) normal renal function.

(HR) 0.51; 95% CI 0.12–2.20; [Figure 2](#)). Only 27.3% (3 of 11) in the ramipril group but 55.6% (5 of 9) in the placebo group progressed during follow-up ([Figure 3](#)). Differences between groups favor ramipril, but they are limited in their meaningfulness due to the low number of children whose parents consented to randomization. Therefore, we enriched and confirmed our efficacy data by supplementing the RCT with data comparing the open arm of our trial with untreated children from the US ([Figures 2 and 3](#)).¹⁹

The beneficial ramipril effect was sustained in comparison to the considerably healthier untreated children from the US with a less-severe genotype, less-severe disease, younger age, and much less albuminuria (see [Supplementary Table S3](#)) with an unadjusted HR of 0.86 (95% CI 0.41–1.81). In the open-arm group, 57.1% (24 of 42) of children were pretreated with an ACEi, reflecting a sicker population. However, a high number (12 of 28; 42.9%) of the healthier untreated US patients progressed, and 17 of 42 (40.5%) of the sicker open-

arm treatment group progressed ([Figure 3](#)). Adjusted for age and disease status at baseline, ramipril again reduced progression by almost 50% (adjusted HR 0.53; 0.22–1.29), although the reduction was not significant according to the classical mathematical definition ([Figure 2](#)).^{19–21}

Using a Bayesian evidence synthesis approach, the findings of the nonrandomized comparison were incorporated into the RCT. This resulted in an HR of 0.52 in virtually the same treatment effect, but a more precise estimate, indicated by a much shorter 95% CI of 0.19–1.39 (compared to 0.12–2.20; [Figure 2](#)).

Secondary efficacy endpoints. The key secondary efficacy endpoint—*albuminuria while on study drug*—is shown in [Figure 3e](#). The slope of progression of albuminuria is lower in the ramipril group than in the placebo arm, supporting our co-primary efficacy endpoint. Our study population was normotensive at baseline ([Table 1](#)) and remained normotensive in all groups throughout the study without severe

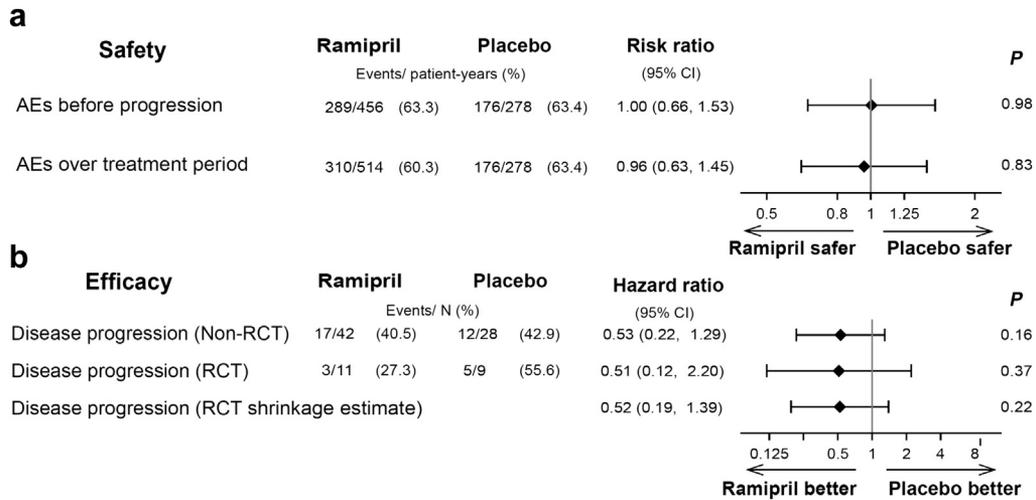


Figure 2 | Co-primary safety and efficacy endpoints. (a) Co-primary safety endpoint *incidence of adverse drug reactions before disease progression*: Compared to placebo, ramipril therapy was safe, as well as the secondary endpoint *incidence of adverse drug reactions over treatment period* (see Table 2; complete list of adverse events [AEs] provided in Supplementary Table S11). **(b)** Co-primary efficacy endpoint *time to disease progression*: Ramipril therapy decreased the risk of progression by almost 50%. This effect size is very similar in the open-arm comparison versus the younger and healthier untreated US patients. Incorporating these results into the randomized data by Bayesian evidence synthesis resulted in a more precise estimate indicated by a much shorter 95% interval estimate. The *P* value of the shrinkage estimators is a Bayesian predictive *P* value. CI, confidence/credible interval; RCT, randomized controlled trial.

episodes of symptomatic hypotension (Table 1; Figure 3c). Weight and longitudinal growth did not differ between groups (Supplementary Figures S1 and S2). Exploratory endpoints such as hearing loss and ocular changes reflect extrarenal symptoms of AS and therefore will be published as a separate work.

The exploratory endpoint *speed of change in GFR* is most relevant for long-term kidney function. All children had a normal GFR at screening (median eGFR 125.3 (111.1–149) ml/min for all patients), which limits the usability of formulas.¹⁸ Nonetheless, if anything, the eGFR loss from screening to year 3 (36 months) was different in favor of ramipril (Supplementary Table S5).

DISCUSSION

The prevalence of CKD is increasing, with more than 10% of people worldwide affected.¹ AS is a model disease of progressive CKD; its distinct features of pathogenesis gave rise to the advantages that we have made use of in this trial.^{6,18} The unique study objectives are to determine the safety and efficacy of ramipril therapy in children, before they develop kidney damage.^{13,22}

The EARLY PRO-TECT Alport trial still is one of very few double-blind placebo-controlled randomized trials in children worldwide. It is the first prospective interventional clinical trial ever in AS. Patients' characteristics emphasize the preemptive character of our trial, but they also make the statistical challenges evident in our small but well-defined study population. Our RCT meets the challenges by changing to a flexible follow-up with up to 6 years on therapy. Implementation of prospective registry data in data analysis

and interpretation takes the common problems of trials in rare kidney diseases into account.

Treatment recommendations for AS are limited to patients with overt proteinuria¹⁶ and advise physicians to wait for the results of this trial to assess safety of early intervention. The dedication of parents, children, and pediatricians to this excessive time on (possible) placebo therapy to improve the evidence cannot be overemphasized in a disease with early ESRF running in families. The RCT design, in addition to our Bayesian evidence synthesis with *real-world* US-registry data, is the best possible attempt under difficult circumstances in a severe rare disease to maximize evidence for the safety and efficacy of ramipril.¹⁹ The HR of 0.51 (although ramipril's efficacy was not significant in the classical mathematical definition) translates to 5.4 children who need to be treated with ramipril for 3 years to prevent one progression of the disease in one child (number-needed-to-treat for time-to-progress: 5.4).²³

Given the inherent difficulties of recruitment into RCT in rare diseases in general and in pediatrics in particular, we adopted a novel Bayesian evidence synthesis approach to maximize the information from the RCT. The idea was to consider the RCT result in light of other data that would allow the assessment of the treatment effect, an approach sometimes referred to as *borrowing*, as the RCT borrows evidence from external sources. Having included an open-label arm, it was natural to compare this to untreated controls, of course with appropriate adjustments to account for the nonrandomized comparison. The adopted approach gives less weight to the nonrandomized data in comparison to the randomized data, as we regard the evidence arising from the RCT as stronger evidence. The actual weight depends on the similarity of the effect

Table 2 | Numbers of patients with disease progression (co-primary efficacy endpoint) and adverse events, serious adverse events (AEs), and events of special interest

Events	EARLY PRO-TECT Alport trial					
	Placebo (n = 9)		Ramipril (n = 11)		Open (n = 42)	
Patients with disease progression, n (%)	5	(55.6)	3	(27.3)	17	(40.5)
Median time before progression, yr (IQR)	3.5	(2.1–4.0)	3.6	(0.5–4.4)		
Total time on Ramipril, yr		11.1 ^a		41.7		163.6
Median time on initial treatment, yr (IQR)	3.5	(2.1–4.0)	4.0	(3.5–4.5)	4.0	(3.5–4.6)
Median follow-up, yr (IQR)	4.1	(4.0–5.1)	4.1	(3.6–4.5)	4.0	(3.5–4.6)
Patients with ≥1 AE, n (%)	9	(100.0)	11	(100.0)	42	(100.0)
AEs before progression, n (patient-years)	176	(277.5)	289	(456.2)		
AE event rate before progression, no. events/patient-years (95% CI)	0.631	(0.45–0.88)	0.63	(0.49–0.82)		
AE rate ratio for ramipril vs. placebo before progression (95% CI)		1.00 (0.66–1.53)				
AEs over treatment period, n (patient-years)	176	(277.5)	310	(514.1)	835	(1372.1)
AE event rate during treatment period, no. events/patient-years	0.631	(0.45–0.88)	0.603	(0.47–0.78)		
AE rate ratio for ramipril vs. placebo during treatment period (95% CI)		0.96 (0.63–1.45)				
Total no. of serious AEs	4	(44.4)	7	(63.6)	13	(31)
Other (planned hospital admission, idiopathic paresis, fatigue)	2	(22.2)	1	(9.1)	3	(7.1)
Infection (causing hospital admission)	0	(0)	3	(27.3)	4	(9.5)
Epilepsy	2	(22.2)	1	(9.1)	0	(0)
Trauma, fracture, or accident (causing hospital admission)	0	(0)	1	(9.1)	4	(9.5)
Low blood glucose (asymptomatic)	0	(0)	1	(9.1)	0	(0)
Acute renal failure	0	(0)	0	(0)	1	(2.4)
Dry cough	0	(0)	0	(0)	1	(2.4)
Other events of special interest						
Hyperkalemia	0	(0)	0	(0)	2	(4.8)
Macrohematuria	2	(22.2)	4	(36.4)	3	(7.1)
AEs of severe intensity (without hospital admission): neuroborreliosis, tonsillitis, sinusitis, gastric pain	0	(0)	1	(9.1)	3	(7.1)
Smoker, n (%)	0	(0)	0	(0)	0	(0)
Deaths, n (%)	0	(0)	0	(0)	0	(0)
Malignancies, n (%)	0	(0)	0	(0)	0	(0)

CI, confidence interval; EARLY PRO-TECT Alport, Early Prospective Therapy European Community Trial Alport; IQR, interquartile range.

^aRandomized patients receiving placebo and showing disease progression were unblinded and started on open-label ramipril.

Data summarize the number of patients with disease progression in the EARLY PRO-TECT Alport trial. In addition, time before disease progression for efficacy analysis, long-term follow-up, the high number of AEs for safety analysis, and serious AEs are listed.

observed in the nonrandomized data in comparison to the effect in the RCT. This is often referred to as dynamic borrowing. It means that the weight would be higher the more similar the effects from the RCT and the nonrandomized treatment are, which is a natural, intuitive approach. The effects from the randomized and nonrandomized treatment comparison turned out to be quite similar, which meant that the non-randomized comparison lent some support to the RCT, resulting in a much narrower interval for the treatment effect. In fact, the length could be reduced by 42% (32% on the scale of the log HR), whereas the HR remained virtually the same. This is equivalent to an increase in sample size of the RCT from n = 20 to n = 43, which means that the n = 70 patients included in the open-label arm and the untreated US cohort are *worth* (in terms of weight of evidence) n = 23 randomized patients in this analysis.

The strict preemptive inclusion criteria hindered recruitment. However, although the urine dipstick test was negative for albuminuria for most patients at baseline, the dipstick test was positive for microhematuria even in our youngest patient. Therefore, glomerular microhematuria must be valued as the first warning sign of a possible devastating outcome leading to early renal failure in any CKD.²⁴ The preemptive character of our study explains why we had to use albuminuria as a

surrogate parameter of the progression of renal disease. Albuminuria in Alport mice,²⁵ in humans,⁹ in children (ESCAPE trial), and in adults is a well-accepted risk factor.^{15,16,24,26} In a genetic progressive renal disease such as AS, there is a strong scientific rationale to not wait for the onset of albuminuria before starting an effective therapy. Microalbuminuria in AS reflects delayable renal damage, when the upper limit of tubular reabsorption is reached and glomerular damage can already be documented.^{6,24}

Early proteinuria reduction by ramipril predicted improved renal survival in children in the ESCAPE trial.¹⁵ The baseline eGFR in the ESCAPE trial was one-third of the eGFR in our trial, and the median proteinuria was about 10 times higher than that in our trial. Still, both studies show nephroprotective effects in favor of ramipril. This is particularly important because, despite the preemptive start of ramipril in our trial, patients continue to progress (albeit more slowly). Therefore, with the help of registry data, the further positive effect of ramipril already can be predicted with great certainty in children with early stages of AS.^{8,14,15,27}

Baseline albuminuria was almost 2 times lower in the placebo (23 mg/gCrea) versus the ramipril (39.4 mg/gCrea) group. Therefore, more children in the placebo group needed a tripling of albuminuria to reach the primary efficacy

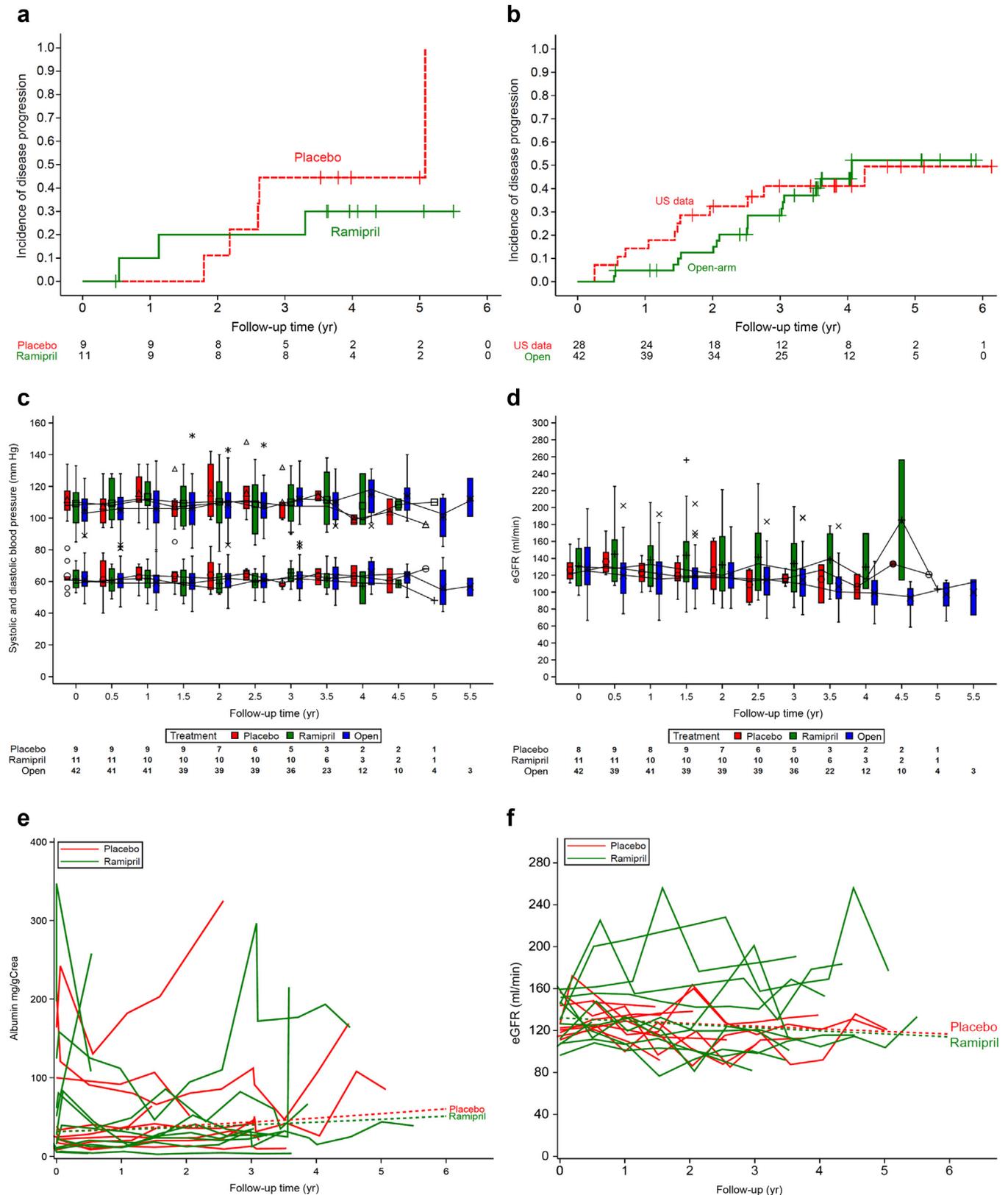


Figure 3 | Co-primary and key secondary efficacy endpoints: time to disease progression and albuminuria while on study drug. (a) Progression of renal disease in the randomized arm. Only 27.3% (3 of 11) in the ramipril group, but 55.6% (5 of 9) in the placebo group, progressed to the next disease level. Two of the 3 children who progressed in the ramipril arm did so in the first 12 months during uptitration before the maximum tolerated dose was reached. These 2 premature progression events have a negative effect on our actual data. However, this slow uptitration regimen was used for safety and tolerability reasons. Note that all censors are beyond the 3-year (continued)

endpoint, so progress of disease in the placebo group may have been even more difficult to achieve compared to the ramipril group. Still, although not a significant effect, ramipril therapy decreased the risk of progression by almost 50% in the RCT arm, which is in line with the HR (even if unadjusted) of open-treated children compared with untreated children from the US. Nevertheless, other confounding factors, such as different medical systems and different genetic variants, need to be acknowledged. Evidence synthesis of both RCT data and *real-world data* is considered to provide the best evidence achievable in a rare disease in children. Even though the US patients were younger, healthier (in terms of more missense variants), and in earlier stages of AS, a higher number of them progressed compared to our older and sicker open-arm treatment group. Our secondary endpoints *increase in albuminuria* (Figure 3e) and *speed of loss of eGFR* (Figure 3f) support the conclusion that ramipril treatment is effective.

One event of acute renal failure in a child with dual RAAS blockade in the open arm of our trial resolved without sequelae after rehydration. Dual RAAS blockade was restarted under intensified surveillance with 1 further adverse event until the end of the study. During the course of our trial, dual RAAS blockade was started in 8 children, 4 of whom were on double RAAS blockade for more than 1 visit, showing a slightly increased event rate of 0.76 compared to ramipril monotherapy (0.60). This single, serious kidney-related AE indicates that children with AS who progress from stage I to stage II of disease are sicker, have worse kidney function, and face a higher risk of side effects. Again, these implications highlight the clinical evidence of our efficacy endpoint. In conclusion, dual RAAS blockade should be used with extreme caution in children with AS.

If therapy is safe, these strong indicators for clinically significant efficacy (although not significant in the classical mathematical definition) would justify preemptive initiation of therapy, with AS being a disease with a predictable 100% certain unfavorable outcome of early renal failure. The beneficial effect of ACEis in proteinuric stage 2 (delay of ESRF

by 18 years) has well been established previously.⁸ ACEis are standard off-label therapy in all treatment recommendations.¹⁶ Thus, our trial is the first to address safety in an oligosymptomatic pediatric clientele with progressive CKD. More than 200 patient-years on ramipril therapy and numerous AEs for analysis allow careful conclusions that ramipril may be safe and has no observable negative effects on daily living in children (Supplementary Table S10). Still, our trial cannot completely exclude all potential risks of early RAAS blockade, which must be weighed by the child's parents (and their individual history regarding renal failure running in their family) and the attending physician against the individual risk of an even earlier kidney failure.

In a recent population cohort study, the use of ACEis was associated with an increased risk of lung cancer.²⁸ The cohort study adult population is completely different than children with AS. Considerations by the European Medicines Agency (EMA) to add their own safety data regarding ramipril to improve safety evidence have already been debated. Safety data from our trial contribute to a possible approval extension for ramipril for the indication AS. Extension of our study for the capture of long-term safety data has already been discussed with the determination to extend it on an international basis as EARLY PRO-TECT ALport XXL.

AS can be diagnosed early in life and is treatable. The low costs of ramipril make therapy affordable for all health systems. The sooner ACE-inhibition is initiated, the more effectively renal failure can be delayed. Therefore, we suggest that the assessment of hematuria and microalbuminuria in children and young adults should include molecular genetic screening for Alport variants. Genetic testing is available in most countries,²⁹ but will it be cost effective?

The genetic diagnosis of AS costs approximately \$3000. The annual costs of ramipril are below \$100, and ramipril delays ESRF by a median of 18 years.⁸ The costs of renal replacement therapy for 18 years are \$540,000 (\$30,000 per year). Thus, the early diagnosis of any child with AS would save the healthcare system more than half a million dollars. The gain in quality of life and hope (and opportunities for

Figure 3 | (continued) minimum time on therapy (except for 1 child, who was unblinded before month 6 because of dry cough). **(b)** Progression of renal disease in the open-arm versus untreated boys from the US registry. Even though the US boys were younger, healthier, and in earlier stages of disease, 12 of 28 (42.9%) of them progressed, compared to only 17 of 42 (40.5%) of the sicker patients in the open-arm group. The *threshold* for progression was harder to reach for most US boys (in level 0, only a tripling of albuminuria defines progression) and easier to reach for our open-treatment arm patients (in level 1, a doubling of albuminuria or albuminuria above 300 mg/gCrea [grams creatinine] defines progression). **(c)** Systolic and diastolic blood pressure over the treatment period. Note that blood pressure does not drop significantly in the (previous normotensive) randomized ramipril group during the uptitration of ramipril and is not different from the placebo group. **(d)** Course of estimated glomerular filtration rate (eGFR) over the treatment period as an exploratory endpoint. The decrease of eGFR during the trial is highest in the placebo group and lowest in the ramipril group, which again supports the results of our primary efficacy endpoint. **(e)** Individual course of albuminuria in the randomized arm, supporting the efficacy data in favor of ramipril. The slope of progression of albuminuria is lower in the ramipril group than in the placebo arm. Note that the co-primary efficacy endpoint is a doubling or tripling of albuminuria in randomized patients. Therefore, every significant increase in albuminuria led to unblinding and a premature end to the individual patient curve (because the patient switched to open-label ramipril). Therefore, the fact that the progress rate is twice as high in the placebo arm makes it impossible to show greater differences in the slopes of albuminuria in this figure (see Supplementary Table S6). **(f)** Individual course of eGFR in the randomized arm supporting the efficacy data in favor of ramipril. Note that, similar to albuminuria in panel (e), the fact that the progress rate is twice as high in the placebo arm makes it impossible to show greater differences in slopes of eGFR in this figure (see Supplementary Table S7). Three years after screening, the placebo group lost 9.8 ml/min of their eGFR, in contrast to the ramipril group, which had no loss in eGFR over the 3-year period. Loss of eGFR in the placebo arm is similar to that in the sicker open-arm group, with a loss of 13.5 ml/min after 3 years.

additional future treatment options)³⁰ for the affected families cannot be calculated in monetary terms.

In conclusion, our study reaches the most important co-primary endpoint—safety. Preemptive ramipril therapy prior to kidney damage in as-yet-oligosymptomatic children with AS aged 2 years and older is safe. Future treatment recommendations should take into careful consideration whether nephroprotective therapy should be started in children with AS as early as the stage of micro-hematuria, even prior to the onset of micro-albuminuria in most cases. Our trial provides very important evidence for making this decision. However, initiation of therapy remains an individual risk–benefit challenge for parents and caregivers and must take family history of early renal failure into account. Our study aims to achieve a conceptual shift toward preemptive organoprotective therapy with ACEis in the early CKD stages. This might apply for most glomerular CKDs, as identification of heterozygous variants in Alport genes with a range of glomerular pathologies, including focal segmental glomerulosclerosis and diabetic kidney disease, increases.³ Thus, our study fills a very important gap in the assessment of glomerular hematuria and microalbuminuria in daily clinical practice and supports a possible approval extension for ramipril for the indications of AS and proteinuric glomerular kidney diseases in the field of pediatrics.

METHODS

Study population

CONSORT reporting guidelines were followed (see CONSORT Checklist in [Supplementary Material](#)). Starting in 2012, all children aged between 24 months and 18 years undergoing care at 14 German trial sites were screened, and eligible individuals were approached for participation. Key inclusion criteria were diagnosis of AS by kidney biopsy and/or genetic testing (by patient or close relative; see [Supplementary Table S1](#)), and normal renal function (eGFR above 90 ml/min). To qualify for randomization, children needed to be untreated (with an ACEi) and to be in stages 0 or I of disease. ACEi-pretreated children, children in stage II of disease, or those whose legal representatives refused randomization could be included in the open-label treatment arm. Stages of AS were defined as follows⁸:

- (i) stage 0: microhematuria without microalbuminuria,
- (ii) stage I: microalbuminuria: 30–300 mg albumin/ gCrea, and
- (iii) stage II: proteinuria: >300 mg albumin/gCrea.

Written informed consent was obtained from all legal representatives and from all patients aged 6 years and older. According to the German Medicines Act, the study was approved by all ethics committees and the Federal Institute for Drugs and Medical Devices (BfArM). The trial was registered at www.ClinicalTrials.gov (NCT01485978); the EudraCT number is 2010-024300-10.

Study design

The treatment phase was 3 to 6 years ([Figure 1](#)). The rationale and design were published and presented at a meeting at the European Medicines Agency in 2014.¹³ The oral, once-daily dose of ramipril was uptitrated from 1 mg/m² to the target maximum dose of 6 mg/m² body surface area in 2-month intervals.¹³ The dosing, missing doses, and AEs were recorded in a patient diary. Parents were advised to withhold treatment during intercurrent illnesses such as diarrhea.

The eGFR was calculated using the revised Schwartz equation (no formula has ever been validated on children with GFR above 90 ml/min).¹⁸ AS progression of disease was defined as follows:

- (i) progression in stage 0: albuminuria >30 mg albumin/gCrea in combination with a 3-fold increase from baseline in albuminuria, confirmed within 2 weeks;
- (ii) progression in stage I: two-fold increase from baseline albuminuria, confirmed within 2 weeks or albuminuria >300 mg albumin/gCrea in a single assessment; and
- (iii) progression in stage II: two-fold increase from baseline albuminuria, confirmed within 2 weeks.

Definition of progression in stage 0 (3-fold increase of albuminuria) and stage I (2-fold increase) differs, however, reflecting the nature of the disease and the expected greater fluctuations in laboratory values in the single-digit microgram range. Possible progress of albuminuria was evaluated monthly in a blinded way by the blinded coordinating principal investigator. Albuminuria was assessed in 24-hour urine in most patients (or, if not available in toddlers, in spontaneous office urine). If progression was confirmed in an additional visit, randomized patients were unblinded and treated open-label with ramipril.

Study endpoints and randomization

The 2 co-primary endpoints were (i) *safety—incidence of adverse drug reactions before disease progression* and (ii) *efficacy—time to disease progression*. Key secondary safety endpoints were (i) *safety—adverse drug events over treatment period* and (ii) *efficacy—albuminuria while on study drug*. Exploratory safety and efficacy endpoints are listed in the [Supplementary Statistical Analysis Plan](#).¹⁸ Assessment and classification of all AEs were made before unblinding the data. The randomization used permuted blocks of variable sizes and was stratified by study site. Initially, the allocation ratio was 1:1. With study protocol version 4.0, however, the ratio was changed to 2:1 for ramipril to placebo.

Sample size calculation and power considerations

As described in the trial design paper,¹³ the original design aimed at randomizing 80 patients in a 1:1 ratio, to ramipril or placebo. Assuming progression probabilities of 20% and 50% at 3 years in the ramipril and the placebo groups, respectively, 28 progression events could be expected across the 2 groups. This number of events provides a power in excess of 80% at a 2-sided significance level of 5% given an HR of 0.322. The risk of dropouts was considered minimal in this population.

With regard to the safety analyses, the 40 patients randomized to ramipril and followed up by 3 years would contribute a total of 120 patient-years on ramipril. Additionally, 40 patients in the open-label ramipril arm would add another 120 patient-years. Patients randomized to placebo and switching to active treatment following progression were expected to contribute another 30 patient-years. This would result in a total of 270 patient-years on treatment. Assuming a Poisson distribution, this total length of follow-up is sufficient to estimate an AE rate of 1 in 10 person-years with a precision of 20%.

In the first year, challenges to recruitment became obvious: (i) children whose urine dipstick had been negative for albuminuria had albuminuria in the range of 300 mg/gCrea as revealed in the more-thorough laboratory examination, excluding them from randomization; (ii) randomization versus placebo was rejected by parents because of the severity of their child's disease; (iii) observational evidence for effectiveness of ACEi-therapy published in 2012 reduced the willingness of investigators to participate in such a long, tightly

financed academic study; and (iv) our efforts for expansion of the trial outside of Germany (to Paris and London) failed due to very low numbers of oligosymptomatic patients eligible for randomization in these countries (at that time, Germany intended to genetically diagnose children with AS earlier than other European countries). To make randomization more attractive, we changed to a 2:1 randomization regime in 2014. All changes in the trial protocol were approved by the relevant parties including all ethics committees and the Federal Institute for Drugs and Medical Devices (BfArM) (see [Supplementary Statistical Analysis Plan](#)). To compensate for the lower number of randomized children, we switched to a flexible follow-up design, expanding the treatment phase to up to 6 years. Prospective *real-world* data from untreated children from the US registry (NCT00622544) were used for evidence synthesis.

Statistical methods

Treatment differences were characterized by rate ratios and reported with 95% CIs and *P* values testing the null hypothesis that the rate ratio is equal to 1. The co-primary efficacy endpoint was modeled using a Weibull regression. The model included effects for treatment, age at baseline, and disease status at baseline. Following logarithmic transformation, the albuminuria and eGFR were analyzed using a Gaussian linear model with random intercepts and random slopes. The subject-specific random effects were assumed to follow a bivariate normal distribution with unstructured covariance. Treatment differences were expressed in terms of differences in the mean group-specific slopes and reported with 95% CIs and *P* values testing the null hypothesis that the difference is 0.

The data from the 2 randomized treatment arms were complemented by data from the open-label treatment arm and a cohort of *n* = 28 untreated Alport children ([ClinicalTrials.gov](#) NCT00622544). In a first step, randomized and nonrandomized data were analyzed separately, and then, in a second step, the data were combined in a Bayesian evidence synthesis approach following the procedure detailed in Röver *et al.* (2019).²⁰ Co-primary interest was in the shrinkage estimate of the RCT treatment effect, which is reported with 95% credible interval and posterior predictive *P* value.²⁰ The application of the Bayesian evidence synthesis approach was deemed necessary and appropriate, as AS is a rare condition.¹⁹ The nonrandomized comparison was adjusted by important prognostic factors that were available in the registry, that is, age at baseline and disease status at baseline. As a sensitivity analysis, propensity score adjusted analyses were performed confirming the primary analysis.

The reported *P* values are all 2-sided; *P* values less than 0.05 are considered statistically significant. The data regarding time to progression and occurrence of AEs were complete; the mixed-effects models used for the repeated measures are robust to some extent to missing data. The analyses were carried out using SAS version 9.4, except for the Bayesian evidence synthesis approach, which was implemented using the R package *bayesmeta* available from Comprehensive R Archive Network.²¹ For details, see the trial statistical analysis plan ([Supplementary Data Statistical Analysis Plan EARLY PRO-TECT Alport](#)).

APPENDIX

List of EARLY PRO-TECT Alport investigators (Investigators who are coauthors are not listed.)

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DISCLOSURE

All the authors declared no competing interests.

DATA STATEMENT

The complete data set, including the complete list of adverse events, intensity, hospitalization and adverse event category, and primary and secondary endpoints, is available to researchers for research projects (that have to be proven by the Institutional Review Board of the University Medicine Goettingen) on request to the corresponding author.

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The German Federal Ministry of Education and Research funded the trial (01KG1104) after an international peer-reviewed 2-step application process, which required a 2-arm RCT design, but did not have any further influence on the trial design or analyses. Sanofi-Aventis Germany, which supplied the trial medication free of cost, did not have any influence on the trial design or analyses. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

The trial was registered at [www.ClinicalTrials.gov](#) (NCT01485978); the EudraCT-number is 2010-024300-10. The full trial protocol and the Statistical Analysis Plan can be accessed in the [Supplementary Material](#). The full data set can be assessed upon request. The trial results were presented as an abstract at the Late-Breaking Clinical

Trials Session of the Congress of the American Society of Nephrology in Washington, DC, on November 7, 2019.

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AUTHOR CONTRIBUTIONS

BT, LTW, LP, KL, HF, BL-S, H Z, PH, HS, SK, UJ, JG, BH, M G, BH, RE, and CL were principal investigators of the local trial sites and contributed to trial planning, study protocol, data collection, data interpretation, and discussion of the results and the manuscript. CEK was principal investigator for the US-Alport registry (NCT00622544) and contributed to data collection, data interpretation, and discussion of the results and manuscript. MH contributed to all data analysis, data interpretation, content and design of all figures, and discussion of the results and manuscript. JB contributed to final validation of all clinical and genetic data, data analysis, data interpretation, content and design of all figures, and discussion of the results and manuscript. TF contributed as head statistician responsible for the statistical analysis plan, power calculation and data analysis, data interpretation, and discussion of the results and manuscript. OG contributed as initiator and head principal investigator of the trial, who wrote the trial concept, applied for funding, had access to and assessed the final data, and wrote the final manuscript.

SUPPLEMENTARY MATERIAL

[Supplementary File \(PDF\)](#)

CONSORT Checklist.

CONSORT Flow Diagram.

List of Investigators.

Figure S1. Weight.

Figure S2. Height.

Table S1. Patient's genetic variant and kidney biopsy.

Table S2. Patient characteristics of full patient cohort.

Table S3. Baseline characteristics of open-arm versus untreated patients.

Table S4. Characteristics of pretreated children in the open arm.

Table S5. Mean eGFR by visit.

Table S6. Mixed-effects model results for log albuminuria until disease progression.

Table S7. Mixed-effects model results for log eGFR while on initial treatment.

Table S8. List of co-medication.

Table S9. List of premedication.

Table S10. List of preexisting diseases.

Table S11. List of adverse events (AEs), intensity, hospitalization, and AEcategory.

Statistical Analysis Plan.

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