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ORIGINAL ARTICLE

Longitudinal growth on an everolimus- versus an MMF-based steroid-free immunosuppressive regimen in paediatric renal transplant recipients

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Conflicts of interest
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Summary

Concerns have been raised that mammalian target of rapamycin inhibitors in pediatric transplant recipients might interfere with longitudinal bone growth by inhibition of growth factor signaling and growth plate chondrocyte proliferation. We therefore undertook a prospective nested, case-control study on longitudinal growth over 2 years in steroid-free pediatric renal transplant recipients. Fourteen patients on a steroid-free maintenance immunosuppressive regimen consisting of low-dose everolimus (EVR) in conjunction with low-dose cyclosporine (CsA) were compared to a matched cohort of 14 steroid-free patients on a standard dose mycophenolate mofetil (MMF) regimen in conjunction with a standard dose calcineurin inhibitor (CNI). The mean change in height standard deviation (SD) score in the first study year was 0.31 ± 0.71 SD score in the EVR group compared to 0.31 ± 0.64 SD score in the MMF group ($P = 0.20$). For the entire study period of 2 years, the change in height SD score in the EVR group was 0.43 ± 0.81 SDS compared to 0.75 ± 0.85 SDS in the MMF group ($P = 0.32$). The percentage of prepubertal patients experiencing catch-up growth, defined as an increase in height SD score ≥0.5 in 2 years, was similar in the EVR group (5/8, 65%) and the MMF group (6/8, 75%; $P = 1.00$). Longitudinal growth over 2 years in steroid-free pediatric patients on low-dose EVR and CsA is not different to that of a matched steroid-free control group on an immunosuppressive regimen with standard-dose CNI and MMF. Hence, low-dose EVR does not appear to negatively impact short-term growth in pediatric renal transplant recipients.

Introduction

Inhibitors of mammalian target of rapamycin (mTOR) such as sirolimus and everolimus (EVR) are potent immunosuppressants that inhibit the interleukin-2-stimulated T-cell proliferation pathway, in which mTOR is the central component, and the growth factor-driven proliferation of both hematopoietic and nonhematopoietic cells. This dualistic mechanism of mTOR inhibitors (anti-rejection and anti-cellular proliferation) not only prevents rejection episodes, but is also thought to inhibit the proliferation of vascular muscle cells, which then may improve long-term graft outcome [1]. mTOR inhibitors are used as immunosuppressive agents also in pediatric transplant recipients [2,3]. Favorable results have been reported especially for the combination of low-dose EVR with reduced-dose

calcineurin inhibitor (CNI) therapy, which has been shown to be efficacious and safe in prospective trials [4,5].

Because of their antiproliferative properties, concerns have been raised that mTOR inhibitors might interfere with longitudinal bone growth. In animal experiments in fast growing rats sirolimus given in pharmacological dosages impairs longitudinal growth by disrupting angiogenesis in the growth plate and by inhibiting growth plate chondrocyte proliferation through disturbed signaling of growth factors such as insulin-like growth factor I and vascular endothelial growth factor [6,7]. A potential inhibitory effect of mTOR inhibitors on longitudinal growth is a significant concern in children with renal transplants who frequently suffer from short stature because of the underlying disease processes, suboptimal graft function and/or glucocorticoid (steroid) medication. Two published clinical studies [8,9] and one case report [10] have yielded conflicting results, but are difficult to interpret because of the concomitant administration of steroids and inclusion of late pubertal patients. We therefore undertook a case-control study on longitudinal growth over 2 years in steroid-free pediatric renal transplant recipients. Patients on a steroid-free maintenance immunosuppressive regimen consisting of low-dose EVR in conjunction with low-dose CsA were compared to a matched cohort of steroid-free patients on a standard dose mycophenolate mofetil (MMF) regimen in conjunction with standard dose CNI.

Materials and methods

Study design and patients

This was a prospective nested, case–control study over 24 months in pediatric renal transplant recipients. A total of 28 patients were taken from two pediatric nephrology centers in Germany. A selection bias was avoided by including in this analysis all patients receiving therapy consisting of CsA and EVR and who underwent steroid withdrawal during the years 2006–2010. Patient characteristics are depicted in Table 1. Data documentation and descriptive statistics were performed within the platform of the CERTAIN Registry (Cooperative European Pediatric Renal Transplant Initiative, http://certain-registry.eu) [11]. CERTAIN fulfill all regulatory and ethical requirements of the European Union and Germany in particular regarding patients' data privacy and security and was approved by the ethics committees of each contributing center. Written informed consent of patients' parents or guardians was obtained before documentation of patient's data in the registry.

In the EVR group, the immunosuppressive protocol has been published previously [3,4]. In brief, all patients enrolled in this trial received induction therapy with basiliximab (Simulect, Novartis Pharma AG, Basel, Switzerland) on day 0 and 4 post-transplant. CsA microemulsion (Neoral, Novartis Pharma, Basel, Switzerland) was administered as published previously [3,4]. EVR was started at 2 weeks post-transplant with a dose of 0.8 mg/m² two times per day and target trough levels of 4–6 μg/l. Target trough levels were reduced to 3–5 μg/l at 6 months post-transplant. Blood levels of EVR and CsA were determined by liquid chromatography/mass spectrometry. Prednisolone (300 mg/m²) was administered during transplant surgery and in the first week post-transplant (60 mg/m²/day), then decreased every 7 days [3,4]. Six months post-transplant, a protocol biopsy was performed. In the absence of subclinical rejection, prednisolone was set to an alternate day scheme and then discontinued 3 months later.

In the MMF group for patients receiving CsA as the CNI, immunosuppression was composed of standard-dose CsA (5–10 mg/kg/day) divided into two or three single doses, target trough level of 70–140 μg/l (EMIT immunoassay, Dade-Behring, Germany) and MMF [1200 mg/m² body surface area (BSA) per day, divided into two single doses]. For patients in the MMF group on tacrolimus (TAC) as the CNI, TAC was administered at an initial daily dose of 0.3 mg/kg given in two divided doses postoperatively. Subsequent doses were adjusted based on clinical evidence of efficacy and occurrence of adverse events and guided by the following trough level ranges: 10–12 μg/l for days 0–21, 8–10 μg/l for days 22–183 and 5–10 μg/l from day 183 onwards. The daily MMF dose was 1200 mg/m² given in two doses for the first 2 weeks. Thereafter, the daily dose was 600 mg/m² given in two doses (adjusted if medically indicated). Before steroid withdrawal, patients received 5 mg/m² BSA prednisone per day (or the equivalent of 4 mg/m² BSA methylprednisolone per day). The corticosteroid dose was slowly tapered over a 12-week period (i.e. 0.35 mg/m² BSA/week or 0.7 mg/m² BSA/2 weeks) until cessation.

Table 1. Patient characteristics at baseline.

Parameter	EVR group (N = 14)	MMF group (N = 14)	P-value
Gender, male	11 (78%)	11 (78%)	1.00
Age at transplantation (years)	6.9 ± 5.1	7.8 ± 4.2	0.62
Age at study entry (years)	7.9 ± 5.1	9.1 ± 4.2	0.52
Bone age at study entry (years)	5.9 ± 4.5	7.0 ± 3.4	0.48
Time point post-transplant at steroid withdrawal (years)	1.0 ± 0.2	1.2 ± 0.8	0.25
BMI SDS	1.10 ± 0.12	0.94 ± 0.13	0.29
Donor type			
Living-related, n (%)	4 (28%)	4 (28%)	1.00
Deceased donor, n (%)	10 (72%)	10 (72%)	1.00

Data are mean ± SD or number (%).
BMI SDS, body mass index standard deviation score; EVR, everolimus; MMF, mycophenolate mofetil.

Patients who remained continuously off steroids for at least 2 years were considered for inclusion in this case–control study. The time point of completed steroid withdrawal was defined as the baseline visit. The mean time point of steroid withdrawal (EVR group, 1.0 ± 0.2 years; MMF group, 1.2 ± 0.8 years) was comparable between the two groups (Table 1). For each patient in the EVR group, a case-control counterpart was identified by means of the following seven matching criteria: (i) age at renal transplantation, (ii) age at study entry, (iii) gender, (iv) transplant function as estimated glomerular filtration rate (eGFR according to Schwartz) [12] at study entry, (v) graft source, (vi) height SD score at study entry and (vii) pubertal status (Table 1). Both groups were also comparable regarding bone age, gender and graft source (living vs. deceased donors). These factors are important, because they are known to influence growth outcome of transplanted children [13,14]. Exclusion criteria were (i) primary diseases that may severely interfere with growth such as syndromic diseases or infantile nephropathic cystinosis, (ii) bone age ≥13 years in girls and ≥15 years in boys, (iii) unstable clinical condition during follow-up because of severe intercurrent diseases, poor metabolic control, poor or dubious adherence to treatment, (iv) multiorgan transplant, and (v) treatment with recombinant human growth hormone.

Physical assessments

Standard anthropometry was performed at 12-month intervals. To obtain age-independent estimates of body size and mass, height and body mass index (BMI) (after logarithmic transformation to obtain normally distributed data) were converted to SD score (SDS) values, related to age- and gender-specific means and SD of European reference populations [15,16]. The stage of puberty was assessed by Tanner's method [17]. In all patients pubertal status was documented. To account for growth retardation, height age rather than chronologic age was used in the calculation of BMI SDS to assign each individual to an age class in the reference population [18]. Bone age was determined according to the method of Greulich and Pyle [19].

Statistical analysis

Results are expressed as mean ± SD. Normal distribution of the data was evaluated using the Shapiro–Wilks test. Univariate comparisons of continuous variables between two groups were performed using paired *t*-test for normally distributed data. To test the hypotheses that EVR is noninferior to MMF regarding longitudinal growth with a margin of −0.2 the differences in delta height SDS were compared using a one-sided *t*-test with $\alpha = 0.025$. Corresponding confidence intervals were given. Analysis of variance on repeated measurements was used to detect any significant changes of clinical or laboratory data over time within each study group. Linear correlations between parameters were tested by Pearson's correlation analysis. Differences in means with a two-tailed $P < 0.05$ were considered as statistically significant. All statistical analyses were performed using SPSS software, version 19 (SPSS, Chicago, IL, USA).

Results

Immunosuppressive therapy

Through the matched study design, the EVR and MMF group were comparable regarding gender distribution, age at transplantation, age at study entry, time point at initiation of steroid withdrawal, BMI, degree of initial growth retardation, renal function, and donor source (Table 1 and 2). The immunosuppressive regimen, the respective drug dosage, and predose plasma or blood concentrations over the 2-year study period are shown in Table 3.

Rejection episodes and graft function

Renal allograft biopsies because of deterioration in kidney function were performed in five patients in the EVR group and four patients in the MMF group during the study period. In the EVR group two patients had borderline changes according to Banff 09 [20], treated by an increase in the maintenance CsA dose; 3 patients showed mild interstitial fibrosis. In the MMF group 2 patients showed findings consistent with chronic CNI-induced nephrotoxicity, 1 patient had mild nephrocalcinosis and 1 patient no pathologic findings. No patient in either group received steroid pulse

Table 2. Standardized height and eGFR at baseline and during 2 years of study.

Parameter	EVR group ($N = 14$)	MMF group ($N = 14$)	*P*-value
Height SDS			
Baseline	−0.82 ± 1.01	−1.14 ± 0.89	0.38
1 year	−0.50 ± 0.96	−0.90 ± 1.23	0.34
2 years	−0.40 ± 0.93	−0.50 ± 1.21	0.77
Bone age (years)			
Baseline	5.9 ± 4.5	7.0 ± 3.4	0.48
1	7.3 ± 5.0	8.5 ± 4.2	0.26
2	8.2 ± 5.3	9.7 ± 3.6	0.18
eGFR (ml/min/1.73 m^2)			
Baseline	69.3 ± 23.4	66.9 ± 17.1	0.76
1	62.7 ± 21.7	64.8 ± 16.2	0.76
2	55.5 ± 18.8	60.6 ± 19.6	0.70

Data are given as mean ± SD.
eGFR, estimated glomerular filtration rate; EVR, everolimus; MMF, mycophenolate mofetil.

Table 3. Immunosuppressive therapy.

Immunosuppressive drug dosage and predose concentration	Baseline	1 year	2 years
EVR group (n = 14)			
EVR (mg/m^2 per day)	1.92 ± 1.19	1.47 ± 0.90*	1.54 ± 0.79
EVR C_0 (μg/l)	4.81 ± 1.32	4.83 ± 1.31	3.94 ± 0.83
CsA (mg/m^2/day)	160 ± 77.0	131 ± 63.0	105 ± 53.0**
CsA C_0 (μg/l)	56.7 ± 20.1	43.0 ± 26.1	42.5 ± 26.5
MMF group (n = 14)			
MMF (mg/m^2/day)	618 ± 225	500 ± 204	477 ± 243
MPA C_0 (μg/l)	3.22 ± 1.92	3.32 ± 3.54	3.12 ± 2.24
CsA (mg/m^2/day) (n = 4)	231 ± 119	188 ± 100	145 ± 83.0**
CsA C_0 (μg/l) (n = 4)	81.8 ± 21.0	99.2 ± 58.9	61.5 ± 19.0
TAC (mg/m^2/day) (n = 10)	6.39 ± 3.21	3.20 ± 2.49	2.88 ± 1.17*
TAC C_0 (μg/l) (n = 10)	7.68 ± 3.12	5.94 ± 1.74	6.12 ± 1.82

Data are mean ± SD or number.
TAC, tacrolimus; CsA, cyclosporine; EVR, everolimus; MMF, mycophenolate mofetil. One year and 2 year data were compared to baseline by ANOVA on repeated measurements.
*P < 0.05; **P < 0.01.

therapy during the study period. eGFR at baseline was similar in both groups (Table 2). The mean loss of eGFR during 2 years of study in the EVR group was numerically higher (13.8 ml/min/1.73 m²) than in the MMF group (6.3 ml/min/1.73 m²), but did not achieve statistical difference [95% confidence interval (CI) −19.58 to +6.93; P = 0.097].

Longitudinal growth

Figure 1 depicts the corresponding growth data for the first and second year of study. During both observation periods, the growth rates between the EVR and MMF group were comparable. For the entire study period of 2 years, the change in height SD score in the EVR group was 0.43 ± 0.81 SDS compared to 0.75 ± 0.85 SDS in the MMF group (95% CI −0.34 to +0.98; P = 0.65). The mean change in height SD score in the first study year was 0.31 ± 0.71 SD score in the EVR group compared to 0.31 ± 0.64 SD score (95% CI −0.49 to +0.49; P = 0.20) in the MMF group. In the second year of study, the mean change in height in the EVR group was 0.11 ± 0.29 SD score compared to 0.40 ± 0.58 SD score in the MMF group (95% CI −0.12 to +0.70; P = 0.68). Growth velocity at 1 year in the EVR group was 7.3 ± 2.1 cm/year compared to 7.8 ± 3.6 cm/year in the MMF group (95% CI −1.77–2.86; P = 0.63). Growth velocity during the 2nd year of study was also comparable between the EVR group (6.7 ± 2.7 cm/year) and the MMF group (7.9 ± 2.4 cm/year; 95% CI −0.07–3.34; P = 0.19). The numerically lower

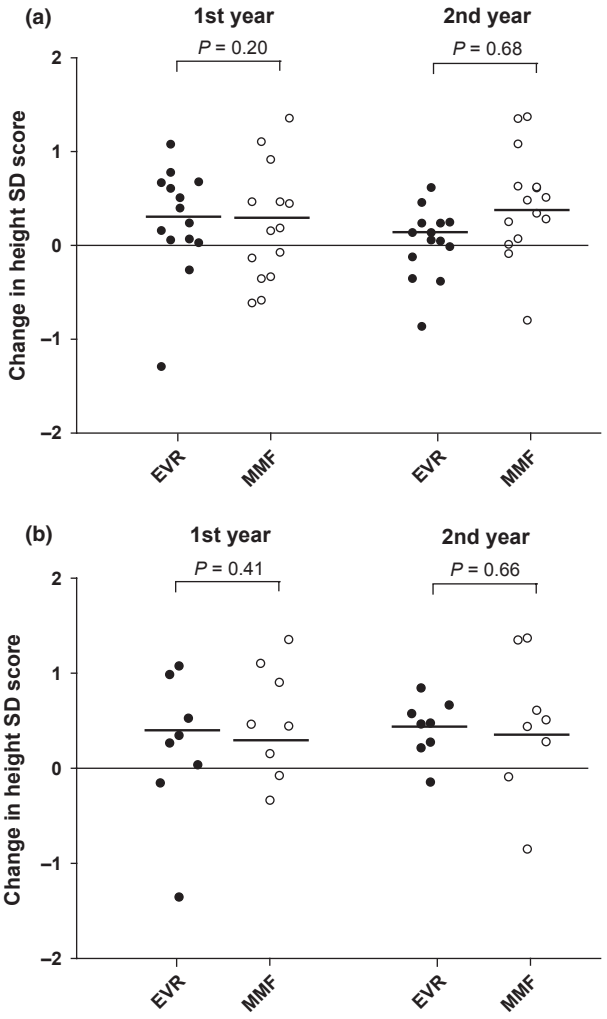

Figure 1 Growth expressed as change in height SD score during the first and second year after steroid withdrawal in the everolimus (EVR)-treated group and the mycophenolate mofetil (MMF)-treated group. Panel A, all patients (n = 14 per group); panel B, patients who remained prepubertal during the study period of 2 years (n = 8 per group).

growth rate in the EVR group during the 2nd year of study may be because of the numerically higher loss of transplant function (Table 2). The corresponding height SD score data at 2 years after steroid withdrawal were comparable between the two groups comparable (95% CI −0.93 to +0.80; P = 0.77) (Table 2). The mean bone age at 1 year and at 2 years after study entry was not significantly different between the groups (Table 2).

Because the analysis of growth in pubertal patients is hampered by uncertainties about the onset and duration of the pubertal growth spurt, we performed a subgroup analysis of longitudinal growth in those patients who remained prepubertal during the study period of 2 years. Sixteen patients were available for this analysis, 8 in the EVR group

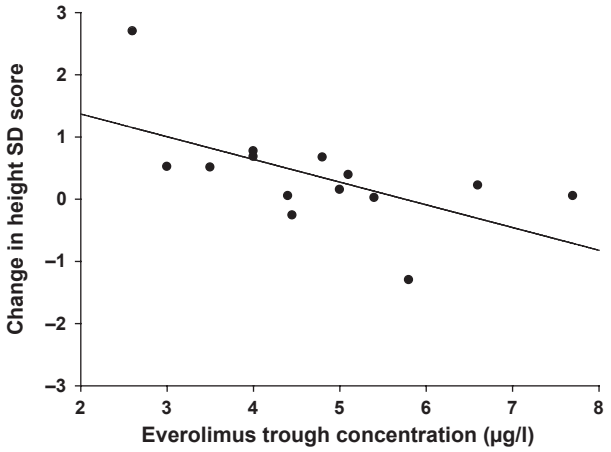

Figure 2 Growth expressed as change in height SD score as a function of everolimus trough levels one year after steroid withdrawal. There was a statistically significant correlation ($r = 0.582$, $P = 0.034$).

and 8 in the MMF group. The baseline characteristics (gender, age at transplantation, age at study entry, time point post-transplant of steroid withdrawal, height SD score at study entry, BMI and donor source) were comparable between the two groups. Mean standardized height at baseline was -0.93 ± 1.24 SD score in the EVR group and -0.96 ± 1.70 SD score in the MMF group ($P = 0.96$). For the entire study period of 2 years, the change in height SD score in the EVR group was 0.63 ± 1.04 SDS compared to 1.14 ± 0.80 SDS in the MMF group (95% CI -1.67 to $+0.65$; $P = 0.73$). Figure 1 b depicts the corresponding growth data for prepubertal patients for the first and second year of study. During both observation periods, the growth rates between the EVR and the MMF group were comparable. Mean change in height SD score of prepubertal patients at 1 year was 0.46 ± 0.90 SDS in the EVR group and 0.38 ± 0.67 SDS in the MMF group (95% CI -0.96 to $+0.74$; $P = 0.41$). During the 2nd year of study the mean change in height SD score in the EVR group was 0.51 ± 0.75 SDS and 0.43 ± 0.71 SDS in the MMF group (95% CI -1.12 to $+0.43$; $P = 0.66$). The percentage of prepubertal patients experiencing catch-up growth, defined as an increase in height SD score ≥ 0.5 in 2 years, was similar in the EVR group (5/8, 65%) and the MMF group (6/8, 75%; $P = 1.00$).

In order to assess whether there is a potential relationship between EVR exposure and growth, we analyzed the correlation between the change in height SD score during the 1st year of study and the EVR trough levels at 1 year after steroid withdrawal. There was a statistically significant inverse correlation ($r = -0.582$, $P = 0.034$) (Fig. 2). This correlation tended to be significant also for the subgroup of prepubertal patients ($n = 8$; $r = -0.676$, $P = 0.065$). There was no such correlation between the EVR trough levels at 2 years and the change in height SD score during the 2nd year of study ($r = -0.054$, $P = 0.42$).

Discussion

This is the first study that investigated longitudinal growth in steroid-free pediatric renal transplant recipients on an EVR-based regimen compared to an adequately matched control group. We observed that growth rates were comparable between the two groups, independently whether growth was calculated as change in height SD score or as cm per year. Also the percentage of prepubertal patients experiencing catch-up growth was comparable between the two groups. This finding is consistent with our previous observation that growth velocity in pediatric renal transplant recipients under a regimen of EVR with reduced CsA was similar to that reported in trials which did not include an mTOR inhibitor in the immunosuppressive regimen [3,4]. The strength of our study is that the analysis was restricted to patients on a steroid-free immunosuppressive regimen. Thereby, we were able to avoid a potential interference of steroids with the interpretation of longitudinal growth data. It has been shown previously that the impact of corticosteroids on longitudinal growth is highly variable, even when patients with the same BSA-adjusted prednisone equivalent dosage are compared [21]. This result appears to be because of between-patient variability in steroid metabolism resulting in variable exposure despite similar body weight-adjusted dosages [21].

Other investigators have analyzed the potential impact of mTOR inhibitors on longitudinal growth. Rangel and Ariceta have described the case of an 11-year old girl who developed linear growth failure at 5 years after kidney transplantation when she was switched from CsA to sirolimus in response to CNI-related hemolytic uremic syndrome [10]. Her height normalized when recombinant human growth hormone (rhGH) was initiated and steroid therapy was withdrawn. In a cohort study of 34 children (11 of whom were pre-pubertal), treatment with sirolimus was associated with reduced growth over a 2-year period compared to matched controls who did not receive sirolimus [8]. A second cohort study did not confirm this finding: Hymes *et al.* reported that 25 children receiving sirolimus did not exhibit growth impairment versus a control group receiving TAC over a 2-year follow-up period [9].

There are various explanations for these conflicting results. As mentioned above the variable effect of steroids on longitudinal growth is difficult to control for. Second, there are certain pharmacologic differences between EVR and sirolimus. EVR is the 40-O-(2-hydroxyethyl) derivative of sirolimus, a modification that results in some important pharmacokinetic differences between the two drugs. EVR is more hydrophilic than sirolimus, and is absorbed more

rapidly from the gut with more systemic clearance than sirolimus [22]. As a result, the elimination half-life of EVR is shorter than for sirolimus (mean 28 h vs. 62 h) [23,24]. We cannot exclude that diffusion of the more hydrophilic EVR from the supplying capillaries into the growth plate and the resulting local concentration is lower than that of sirolimus. However, the most likely explanation for the differences in the impact of these two mTOR inhibitors on longitudinal growth is the degree of systemic exposure. Because we combined EVR therapy with low-dose CsA, the degree of EVR exposure could be kept much lower than in protocols which use sirolimus in a CNI-free regimen. In our study, the mean EVR predose blood concentration at baseline was 4.81 μg/l, at 1 year 4.83 μg/l and at 2 years 3.94 μg/l, while for example in the study of Gonzalez *et al.* [8] the mean corresponding blood concentrations of sirolimus were 7.82 ± 3.92 μg/l at baseline, 7.43 ± 3.38 μg/l at 1 year and 6.31 ± 1.98 μg/l at 2 years of study, hence approximately 63% higher. A dose- or exposure-dependent impact of mTOR inhibitors on longitudinal growth can therefore not be excluded. This line of reasoning is supported by our observation of an inverse correlation between EVR exposure and longitudinal growth (Fig. 2).

We acknowledge some limitations of this observational study. Small patient numbers and the exploratory study design make it difficult to draw definite conclusions. The central question is how non-inferiority of growth on two immunosuppressive regimens is defined. If one considers a difference in change of height SD score of ≤0.2 SDS after 2 years of study to be noninferior between groups proceeding on a SD of 0.5 based on values from previous studies on growth [25], at least 99 patients per treatment arm would be necessary to provide 80% power for between-group comparisons made at an adjusted significance level of 2.5%. Given the small number of pediatric renal transplant patients available, such patient numbers are difficult to obtain.

In conclusion, longitudinal growth over 2 years in steroid-free pediatric patients on low-dose EVR and CsA is not different to that of a matched steroid-free control group on an immunosuppressive regimen with standard-dose CNI and MMF. Hence, low dose EVR does not appear to negatively impact short-term growth in pediatric renal transplant recipients.

Authorship

HB, LuP, BH, TA, AS, BH, BT and LP: Participated in the research design. BT, HB and LP: Participated in the writing of the first draft of the article. All authors participated in the performance of the research, in data analysis and interpretation and approved the final version of the article.

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