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Everolimus with or without Mycophenolate Mofetil for Graft-versus-Host Disease Prophylaxis after Hematopoietic Stem Cell Transplantation in Children with Acute Kidney Injury: A Single-Center Retrospective Analysis

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A B S T R A C T

Hematopoietic stem cell transplantation (HSCT) serves as a therapeutic intervention for various pediatric diseases. Acute and chronic graft-versus-host disease (GVHD) are decisive determinants of successful allogeneic HSCT. The immunosuppressive agent cyclosporin A (CsA) is most often used to prevent GVHD in pediatric patients, but it is known to be nephrotoxic. Acute kidney injury (AKI) affects 17% to 47% of pediatric HSCT recipients, compromising clinical outcomes. This retrospective single-institution analysis scrutinized the practice of substituting nephrotoxic CsA with an everolimus/mycophenolate mofetil (MMF) combination as GVHD prophylaxis in 57 patients with AKI (n = 53) or central nervous system side effects due to calcineurin inhibitor (CNI) treatment (n = 4) following first allogeneic HSCT. This retrospective cohort study analyzed the clinical courses of 57 children who were switched from CNI-based GVHD prophylaxis (CsA or tacrolimus in single cases) to the everolimus/MMF combination (n = 48) or everolimus alone (n = 9)

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after undergoing their first allogeneic HSCT at the Charité University Medicine Berlin. Serving as a control group were 74 patients undergoing their first allogeneic HSCT during the same period who did not receive everolimus at any time post-transplantation. Patients undergoing mismatched family donor transplantation without subsequent CNI treatment for GVHD prophylaxis were excluded. Study endpoints encompassed the retention parameter course subsequent to the GVHD prophylaxis switch, overall survival (OS), and incidences of underlying disease relapse and acute and chronic GVHD in both treatment groups. Renal function improved significantly after switching from CsA to the everolimus/MMF combination. Crucially, the transition to everolimus did not adversely affect OS following HSCT (hazard ratio [HR], 1.6; 95% confidence interval [CI], 0.74 to 3.5; $P = .23$), especially for patients with nonmalignant diseases (HR, 1.4; 95% CI, 0.34 to 5.9; $P = .64$). The incidences of grade III-IV acute GVHD (HR, 1.82; 95% CI, 0.45 to 7.4; $P = .40$) and severe chronic GVHD (HR, 2.76; 95% CI, 0.69 to 11.0; $P = .15$) were comparable in patients treated with the everolimus/MMF combination and those receiving standard CsA treatment in the control group. OS in patients with malignant underlying diseases was lower in the everolimus group (HR, 2.7; 95% CI, 1.1 to 6.9; $P = .03$), however, event-free survival was similar in patients with an underlying malignant disease treated with either the everolimus/MMF combination or CsA (HR, 0.87; 95% CI, 0.39 to 1.9; $P = .73$). Renal function improved significantly in patients who switched their immunosuppression regimen from CsA to everolimus with or without MMF cotreatment after diagnosis of AKI. Patient outcomes in the everolimus group were comparable to those in the control group. This study provides compelling real-world clinical evidence to support replacing CsA with the everolimus/MMF combination in the management of AKI following HSCT in children.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a therapeutic option for malignant and nonmalignant diseases [1]. Transplantation-related mortality (TRM) has improved substantially over the last several decades, with 5-year overall survival (OS) rising from 41.8% in the years 1984 to 2001 to 79% in 2001 to 2009 [2]. However, patient age, donor type, and disease status can negatively influence TRM [3,4]. Improved management of HSCT complications is crucial to further improve TRM.

Acute kidney injury (AKI) occurs in 17% to 47% of pediatric HSCT cases in the literature and remains a severe problem [5,6]. The reported mortality rate in patients requiring dialysis is 52% to 65% [7]. Higher grade of AKI also leads to significantly worse survival [6]. Thus, preventing progression of AKI remains important.

The success of allogeneic HSCT is strongly influenced by the underlying disease, infection, and occurrence of acute and chronic graft-versus-host disease (GVHD) [8,9]. TRM and OS are poorer in patients experiencing advanced GVHD [10]. Consequently, GVHD prophylaxis is a primary challenge for the clinical management of patients undergoing HSCT, particularly for a benign underlying disease.

A common GVHD prophylaxis strategy involves combining cyclosporin A (CsA) with a short course of methotrexate or mycophenolate mofetil (MMF) [11]. Serotherapy with antithymocyte globulin is applied to further reduce the risk of acute GVHD (aGVHD) and especially chronic GVHD (cGVHD) [12,13]. CsA is known to be nephrotoxic, with the nephrotoxicity attributed to afferent arteriole vasoconstriction [14,15]. A second commonly used calcineurin inhibitor (CNI), tacrolimus, has nephrotoxic properties similar to CsA [16,17]. Consequently, patients with either preexisting or therapy-induced kidney injury require selection of alternative immunosuppressive drug combinations.

T cell antigen recognition leads to strong mammalian target of rapamycin (mTOR) signaling, which drives naïve T cells into effector lineages while inhibiting regulatory T cell induction (reviewed in [18]). Moreover, mTOR activates immune receptor signaling and cellular metabolism and migration and is crucial to generating immune responses [18]. Because mTOR also regulates cell cycling and proliferation, inhibitors also

have anti-proliferative effects beyond the immune system [19]. Sirolimus (rapamycin) is a naturally occurring compound isolated from a soil saprophyte that inhibits mTOR. The hydroxyethylester sirolimus derivative everolimus has a shorter half-life (22 hours versus 72 hours) and has been used successfully as an immunosuppressant after solid organ transplantation in combination with CsA to prevent allograft rejection [20]. Anti-inflammatory effects of everolimus include reduction of CXCL8 (also known as IL-8) and release of vascular endothelial growth factor, as well as sustained IL-1 receptor antagonist (IL1RN) release by neutrophils [21]. Everolimus is metabolized primarily in the gut and liver by cytochrome P450 (CYP) 3A4, 3A5, and 2C8 [22]. Major class effect toxicities observed in cancer patients were stomatitis, infections, noninfectious pneumonitis, fatigue, rash, and diarrhea [23]. Although the natural compound sirolimus appears to enhance CNI nephrotoxicity [24], everolimus lacks nephrotoxicity [25] and even mitigates CNI-induced nephrotoxicity when combined with CsA or tacrolimus [26].

Data on the capacity of everolimus to serve as a GVHD prophylaxis are very limited. Until now, only 2 studies have evaluated this question. In the prospective pilot phase 2 EVTAC trial (ClinicalTrials.gov NCT00117702), everolimus in combination with tacrolimus appeared to effectively prevent GVHD in 24 adult patients; however, the trial was terminated prematurely owing to the development of severe sinusoidal obstruction syndrome (SOS) in 25% of the patients [27]. A single-center phase I/II trial (NCT00856505) investigated combining everolimus and MMF as CNI-free GVHD prophylaxis in 24 adult patients with underlying hematologic malignancies but found high rates of acute and chronic GHVD [28]. No published or ongoing studies have tested whether everolimus can provide effective GVHD prophylaxis in pediatric patients undergoing HSCT. The present study aimed to collectively assess clinical information about the immunosuppressive capacity of everolimus alone or in combination with MMF, without the use of CNIs, in children with nephrotoxicity or neurotoxicity issues who underwent their first HSCT. In this retrospective single-center analysis, we evaluated patient courses after substitution of CsA (or tacrolimus in several cases) as CNI-based GVHD prophylaxis with the combination of everolimus and MMF after first allogeneic HSCT in children with severe AKI. We present real-world data supporting the feasibility of replacing CsA as GVHD prophylaxis with the everolimus/MMF combination in children with AKI undergoing HSCT.

METHODS

Setting and Participants

This retrospective cohort study included all patients treated with everolimus as GVHD prophylaxis after their first allogeneic HSCT at the Charité University Medicine Berlin between August 16, 2016, and September 29, 2020. The control group comprised patients who underwent their first HSCT within the same time frame but did not receive everolimus at any point post-transplantation. Patients who underwent mismatched family donor transplantation without subsequent CNI treatment for GVHD prophylaxis were excluded. This work contains only routinely acquired data, presented in an anonymous form. The study was approved by the Charité Ethics Committee under the reference EA2/144/15. This study adheres to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) principles.

Study Design

CsA is standard GVHD prophylaxis at our institution, with administration starting 1 day before HSCT (day -1) at 1.5 mg/kg body weight twice daily for target plasma trough levels between 80 and 100 ng/mL in children with malignant diseases and between 100 and 150 ng/mL in children with benign diseases. Additionally, a short course of methotrexate (MTX) (10 mg/m^2) was administered on days +1, +3, and +6, followed by a folinic acid rescue (15 mg/m^2) on days +2, +4, and +7. As an alternative to the short course of MTX, MMF was administered at 600 mg/m^2 twice daily. Some patients in the malignant disease group (patients 2, 4, 5, 6, 7, 8, 9, 10, 14, 17) received no MTX or only a reduced course of MTX because of an individual history of MTX-related toxicity. In these cases, MMF was added early on.

The decision to switch from standard CNI treatment to everolimus with or without MMF was based on the development and dynamics of acute renal failure and increasing retention parameters (creatinine, cystatin C, urea) in the context of the patient's general condition. The switch to everolimus with or without MMF was preceded by elevated creatinine (>300% of baseline) or cystatin C (>300% of baseline) levels in blood plasma, uremia, severe fluid overload, or a combination of these factors. When feasible, essential comedication was dose-adjusted to the glomerular

filtration rate or substituted with less-nephrotoxic drugs; otherwise, nephrotoxic medication was halted. For instance, acyclovir was paused, amphotericin B was replaced by an azole, and in some cases, vancomycin was replaced by linezolid. If these interventions proved insufficient, CsA was halted and everolimus with or without MMF was initiated.

Everolimus treatment started with 1.6 mg/m^2/day orally in 2 divided doses, with dosing subsequently adjusted to maintain blood trough concentrations between 3 and 8 ng/mL. This trough concentration was maintained irrespective of the comedication. The immunosuppressive potential of everolimus monotherapy was generally considered insufficient, and for that reason MMF was added. However, 8 patients (patients 11, 24, 30, 35, 36, 37, 40, and 53) were switched to everolimus monotherapy without MMF, based on individual treatment decisions. When MMF was coadministered, dosage began with 600 mg/m^2 twice daily. Criteria included high risk for relapse or low risk for GVHD. Although the present study focused on AKI, 4 patients were switched because of CNI neurotoxicity.

Variables

Primary outcomes were the incidences of aGVHD and cGVHD, OS, and underlying malignant disease relapse. Patients were followed for up to 6 years after HSCT. Additional variables included in the analysis were demographic features, transplant type, conditioning type, plasma creatinine level, plasma cystatin C level, and plasma everolimus level.

Data Sources and Measurement

Medical records from all patients were evaluated for the following: demographic features; treatment dates; disease progression; treatment received; HSCT conditioning regimens used; plasma creatinine, cystatin C, and everolimus levels; last follow-up appointment; and the occurrence of acute renal failure, aGVHD, cGVHD, SOS, transplantation-associated thrombotic microangiopathy, and death. Cases of aGVHD or cGVHD occurring after relapse from the underlying disease were not considered in the analysis of GVHD incidence. SOS was defined according to the European Society for Blood and Marrow Transplantation (EBMT) pediatric diagnostic criteria. Transplant-associated thrombotic microangiopathy was defined according to Jodele et al. [29]. Staging and grading of aGVHD were based on EBMT recommendations [30]. Relapse was defined as recurrence of the underlying malignant disease (detected on a morphologic, cytogenetic, or molecular level). Death from any cause was considered an event for OS. The Jaffé method was used to measure plasma creatinine. Everolimus plasma levels were monitored up to 3 times weekly by enzyme-linked immunosorbent assay (ELISA).

Bias

Standard immunosuppression (with CsA) was replaced with the everolimus/MMF combination only in patients with AKI or severe neurotoxicity. This practice represents a selection bias for patients who experienced a significant complication in their HSCT course.

Statistical Methods

Because a patient's everolimus covariate status changes over time, the Simon–Makuch method [31] was used to determine the probabilities of OS and event-free survival (EFS) and the incidences of GVHD and relapse. The Simon–Makuch method generates survival curves for different levels of a time–dependent covariate. It appropriately aligns the number of patients at risk as events (everolimus started, yes/no) develop after HSCT. Cox proportional hazards analysis, incorporating a time-dependent covariate, was applied for OS and EFS. Cumulative incidences for competing events were calculated to evaluate the incidences of relapse and aGVHD and cGVHD, using a cause-specific approach. The effect of the switch to everolimus as GVHD prophylaxis on survival was tested using the Mantel-Byar test. A 2–tailed P value $<.05$ was considered statistically significant. Computations were performed using GraphPad Prism version 8.3.0 (GraphPad Software), Stata IC15 (StataCorp), and Easy R, version 1.60, which is based on R and R Commander [32,33].

RESULTS

Patient Characteristics in the Everolimus and CsA Groups

Everolimus with or without MMF was administered after first allogeneic HSCT as GVHD prophylaxis to 57 patients in total during their clinical courses. Neurotoxicity drove the switch from CsA to everolimus in 4 patients (7.0%; patients 2, 20, 41, and 46), and included posterior reversible encephalopathy syndrome (patients 20, 41, and 46) and leukoencephalopathy (patient 2). Patients 5 and 54 did not receive CsA but rather received the everolimus/MMF combination directly because of preexisting impaired renal function.

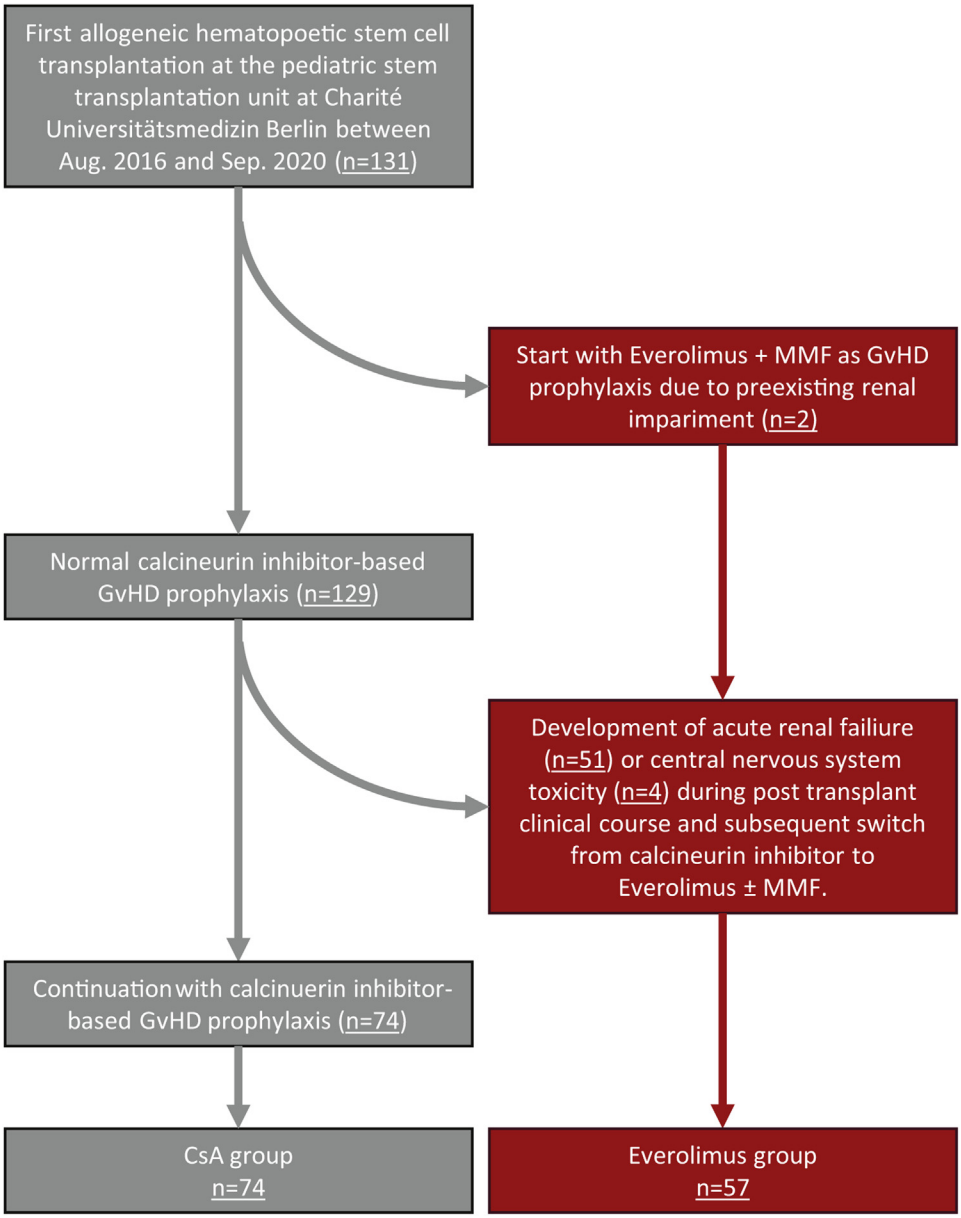

Figure 1. Study flow chart.

The remaining 51 patients (89.5%) received everolimus with or without MMF because of escalating retention parameters and subsequent acute renal failure (Figure 1).

The everolimus group was significantly older than the CsA group ($P < .001$; Table 1). The 2 groups are composed of patients with different malignant and benign underlying diseases. The most frequent diagnoses in both group (everolimus or control) were acute lymphoblastic leukemia and sickle cell disease (Table 1). One patient (14) in the everolimus group with acute lymphoblastic leukemia also had trisomy 21. Most patients in both groups underwent HSCT from a matched unrelated donor and received either bone marrow or peripheral blood stem cells after T cell receptor α/β depletion with T cell addback. The main conditioning regimen was total body irradiation- or treosulfan-based (Table 1). The median duration of follow-up was 36.9 months (range, 0.6 to 71.7 months). One patient in the everolimus group (patient 42) was lost to follow-up from day 201 but was still alive at the time of this report. One patient in the CsA group with a malignant underlying disease was lost to follow-up on day 211, without further information.

Descriptive Data for the Everolimus Treatment Group

Patients were switched from either tacrolimus or CsA treatment to everolimus with or without MMF at varying times relative to HSCT (Figure 2).

Table 1
Patient Characteristics

Characteristic	Everolimus Group (N = 57)		CsA Group (N = 74)	
Age at HSCT, yr, median (range)	11.4 (0.2-19.6)		6.3 (0.2-26.3) ($P < .001$)	
Biological sex, n (%)				
Female	23	(40.4)	32	(41,6)
Male	34	(59.6)	45	(58,4)
Ethnicity, n (%)				
Caucasian	31	(54.4)	50	(67.6)
Arabic	16	(28.1)	20	(27.0)
Sub-Saharan African	10	(17.5)	3	(4.1)
Other	0	(0)	1	(1.4)
Underlying disease, n (%)				
Acute myeloid leukemia	2	(3.5)	9	(12.2)
Fanconi anemia	4	(7.0)	1	(1.4)
Acute lymphoblastic leukemia	15	(26.3)	30	(40.5)
Myelodysplastic syndrome	6	(10.5)	3	(4.1)
Severe aplastic anemia	3	(5.3)	0	(0.0)
Non-Hodgkin lymphoma	1	(1.8)	4	(5.4)
Sickle cell disease	13	(22.8)	10	(13.5)
Thalassemia	0	(0.0)	5	(6.8)
Severe combined immunodeficiency	2	(3.5)	2	(2.7)
Chronic granulomatous disease	1	(1.8)	5	(6.8)
Diamond-Blackfan anemia	2	(3.5)	0	(0.0)
X-linked adrenoleukodystrophy	1	(1.8)	0	(0.0)
Other	7	(12.3)	5	(6.8)
Donor, n (%)				
Matched unrelated donor	29	(50.9)	50	(67.6)
Matched sibling donor	23	(40.4)	23	(27.0)
Mismatched donor	5	(8.8)	1	(1.4)
Graft source, n (%)				
Bone marrow	23	(40.4)	47	(63.5)
Peripheral blood stem cells*	33	(57.9)	27	(36.5)
Cord blood + bone marrow	1	(1.8)	0	(0.0)
Conditioning regimen, n (%)				
TBI/VP16	10	(17.5)	14	(18.9)
Flu/VP16	1	(1.8)	0	(0)
Treosulfan-based	29	(50.9)	33	(44.6)
Flu/Cy-based	5	(8.8)	2	(2.7)
Flu/TT-based	4	(7.0)	10	(13.5)
Bu/Cy-based	1	(1.8)	5	(6.8)
Bu/Flu-based	7	(12.3)	10	(13.5)

Bu indicates busulfan; Cy, cyclophosphamide; Flu, fludarabine; Mel, melphalan; TBI, total body irradiation; Treo, treosulfan; TT, thiotepa.

* With T cell receptor α/β depletion and 10×10^6/kg body weight T cell addback.

The median time after HSCT when immunosuppression was changed to everolimus was 22 days (range, 1 to 98 days). Patients with signs of aGVHD grade II-IV (benign underlying diseases) and grade III-IV (malignant underlying diseases) received systemic corticosteroids (prednisolone) (Figure 2). Everolimus treatment was initiated between 2 days before and 98 days after HSCT (median, post-transplantation day 22) and administered for a median duration of 47 days (range, 11 to 128 days) in patients with underlying malignant disease (patients 1 to 18) or 128 days (range, 11 to 355 days) in patients with underlying benign disease (patients 19 to 57) (Figure 2). MMF

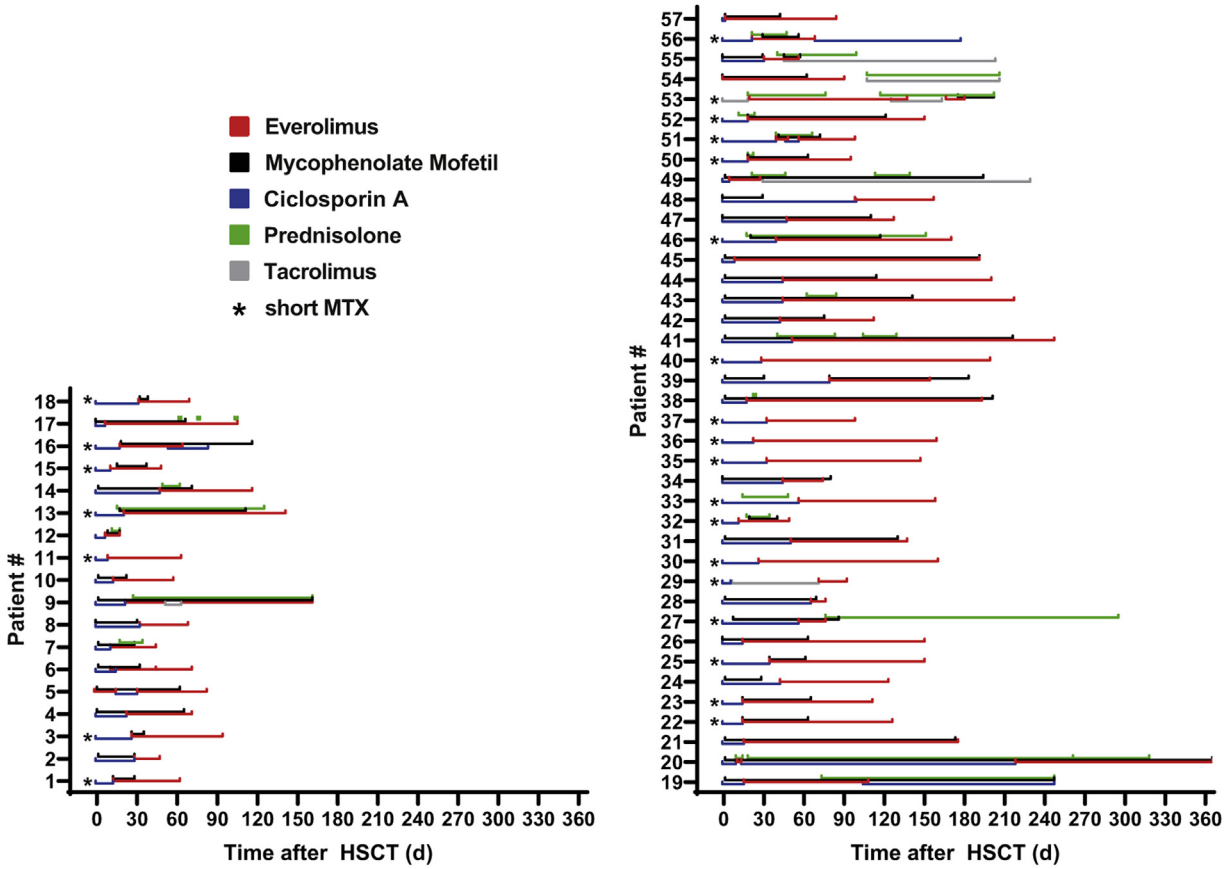

Figure 2. Timeline of immunosuppressive drug use in patients who received everolimus with or without MMF. Each of the 57 children who received everolimus at any time during their clinical course after HSCT is shown individually. Different colors indicate different immunosuppressive drugs. Patients 1 to 18 had an underlying malignancy and patients 19 to 57 had a benign underlying disease.

was administered for a median of 29 days (range, 0 to 364 days) in patients with an underlying malignant disease (patients 1 to 18) and for a median of 64 days (range, 0 to 364 days) in patients with an underlying benign disease (patients 19 to 57) (Figure 2). Nine patients (11, 29, 30, 35, 36, 37, 40, 48, and 53 in Figure 2) received only everolimus without the addition of MMF, representing 14% of the patients in the everolimus group. Reasons for not adding MMF to the immunosuppressive regimen depended on individual risk factors. For patient 11, it was the high risk of relapse. Patients 35 and 37 had a low individual risk for GVHD development, and the switch to everolimus was already later than 30 days after HSCT. Patient 29 had severe virus reactivation and was switched to everolimus after day 60 post-HSCT. Information on the exact reason for omitting MMF is missing for patients 30, 36, 40, and 53.

Everolimus trough levels in blood plasma remained within the targeted range in most cases and ranged from 1.01 to 23.97 ng/mL (median, 5.46 ng/mL) (Figure 3). Everolimus had to be discontinued in 1 patient who developed interstitial pneumonitis (patient 27; Common Terminology Criteria for Adverse Events [CTCAE] grade 3) and in 2 patients who developed painful oral ulcers (16 and 56; both CTCAE grade 2). In the everolimus group, we found an incidence of hepatic SOS of 21.5% (12 cases), which was comparable to the literature [34]. Of these patients, 8 had onset of SOS before their immunosuppression

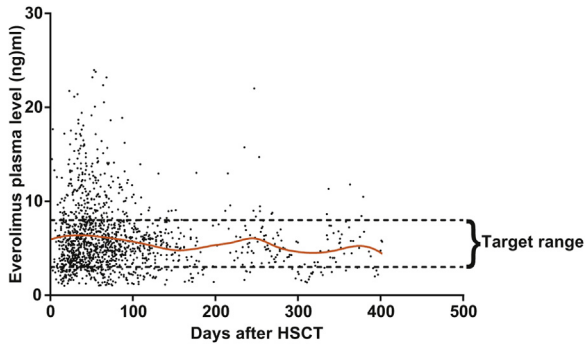

Figure 3. Everolimus plasma levels. The everolimus target ranges were 3 to 8 ng/mL (dashed lines). The solid line is a spline-smoothing curve.

regimen was switched to everolimus. One of those patients (patient 34) had CTCAE grade 4 SOS, and all others had CTCAE grade 3. This means that no association between this adverse event and the everolimus treatment can be made. Transplantation-associated thrombotic microangiopathy occurred in only 2 cases (3.5%) (Supplementary Table S1). Overall, everolimus treatment was tolerated well, and no extensive toxicities occurred necessitating treatment discontinuation, although very high everolimus plasma levels (>25 ng/mL) were measured in some cases (Figure 3).

Renal Function Recovered after Switch to Everolimus-Based GVHD Prophylaxis

Renal function was evaluated by creatinine and plasma cystatin C levels in blood plasma relative to the baseline on the day of HSCT (day 0). Among the 57 patients who received everolimus with or without MMF for GVHD prophylaxis, the impact of switching to everolimus or the everolimus/MMF combination could be analyzed in 55 patients based on creatinine analysis and in 48 patients based on cystatin C analysis. Patients were excluded from these analyses because of preexisting renal impairment in 2 patients who were directly started on everolimus treatment (patients 5 and 54) and because of missing cystatin C data collection points in 7 patients (patients 2, 14, 34, 40, 41, 42, and 46). From the day that everolimus treatment was started, it significantly reduced plasma creatinine from the mean of 294% ± 158% relative to baseline (measured on HSCT day 0) to 158% ± 67% measured 14 days later ($P < .0001$) (Figure 4A). Relative to this baseline on HSCT day 0, plasma cystatin C decreased from 210% ± 68% of baseline to 135% ± 50% ($P < .0001$) at 14 days after initiating everolimus treatment (Figure 4B). The normal range of cystatin C is age-independent. This allows us to show a median absolute baseline value of 1.07 mg/dL (range, 0.5 to 2.4 mg/dL) on HSCT day 0. After 1 year, surviving patients in the everolimus group had a median cystatin C level of 1.05 mg/dL (range, 0.5 to 2.3 mg/dL), which was not significantly different from baseline at day 0 (Supplementary Figure S1A). Measured relative to baseline, the median creatinine level decreased to 128% (range, 34% to 627%), and the median cystatin C level decreased to 113% (range, 47% to 355%) at 1 year post-HSCT, remaining slightly elevated for patients receiving everolimus (Supplementary Figure S1B). Only 2 patients in the everolimus group had to undergo renal replacement therapy (patients 12 and 28), both of whom died from uncontrollable infection. Switching the immunosuppression regimen from CsA to everolimus with or without MMF cotreatment, led to significant recovery of renal function after HSCT in these pediatric patients.

The Everolimus/MMF Combination Effectively Prevents GVHD

Changing the immunosuppressive strategy following HSCT may result in the undesirable complication of aGVHD or cGVHD. The cumulative incidence of grade II-IV aGVHD shows similar results for both groups analyzed in a competing events model (Figure 5A). Of the patients who received everolimus, 10.0% developed grade II-IV aGVHD, compared to 18.2% of the control group (hazard ratio [HR], 0.88; 95% confidence interval [CI], 0.29 to 2.7; $P = .82$) (Figure 5A). The incidence of grade III-IV aGVHD did not differ significantly between the everolimus and control groups (everolimus: 7.5%; control: 7.0%; HR, 1.82; 95% CI, 0.45 to 7.4; $P = .40$) (Figure 5A). Signs of grade III-IV

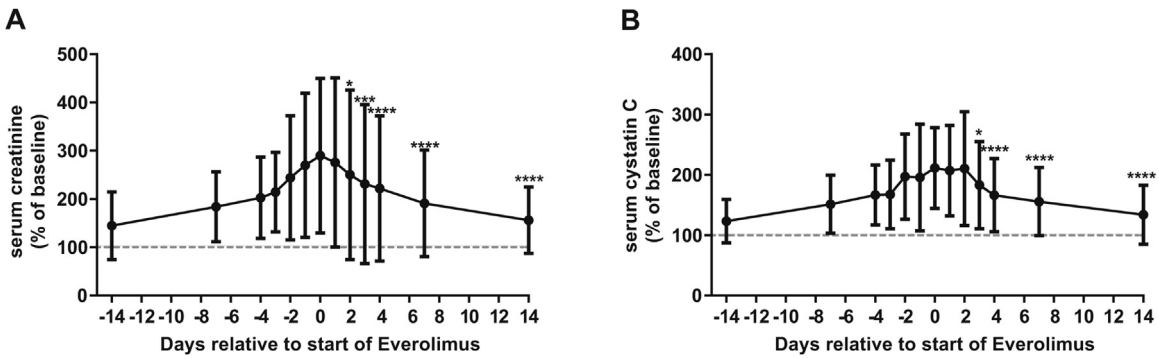

Figure 4. Development of retention parameters after the switch to everolimus with or without MMF. The grouped blood levels of creatinine (A) and cystatin C (B) of children who developed acute renal failure during their post-HSCT course is shown at the time of their switched to everolimus with or without MMF. Data are normalized to the creatinine and cystatin C levels on the day of HSCT, respectively, which are considered the baseline levels. $^{*}P < .05$; $^{***}P < .001$; $^{****}P < .0001$ using the mixed-effects model with Dunnet's multiple comparisons test.

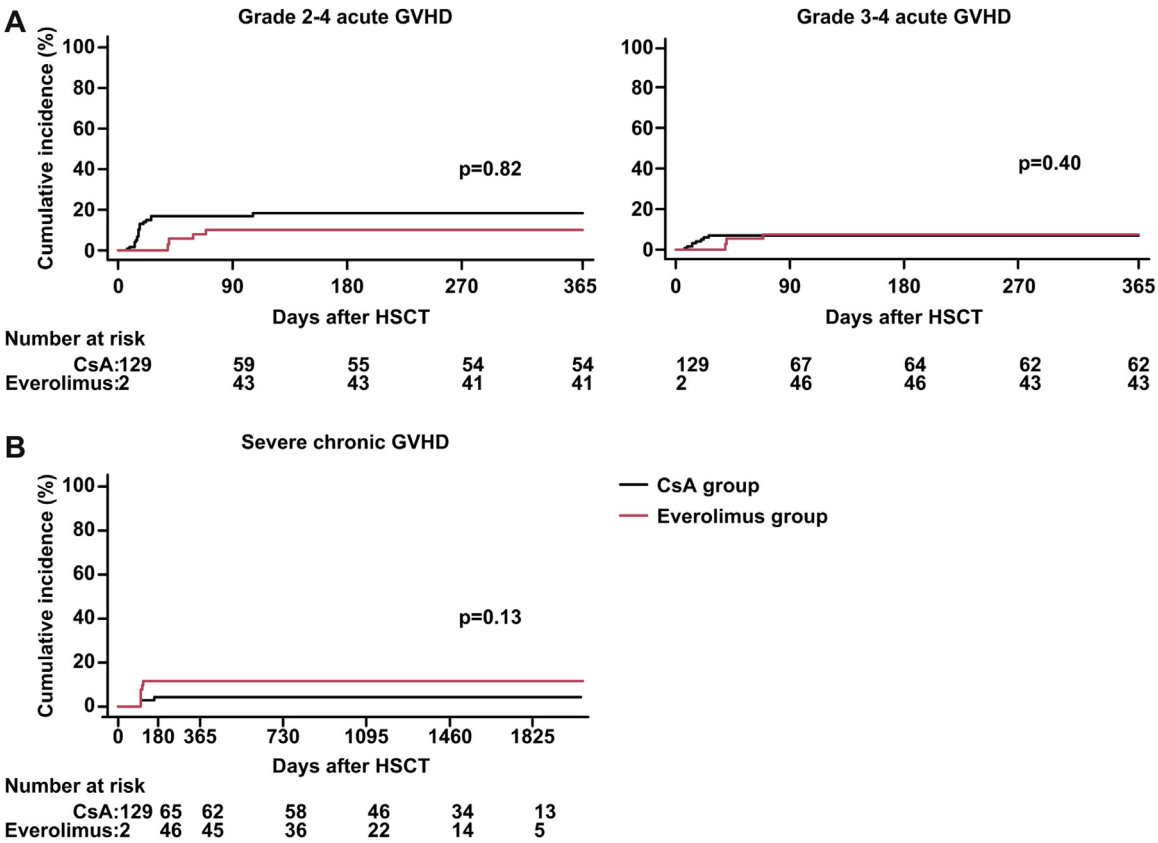

Figure 5. Cumulative incidence of grade II-IV aGVHD and grade III-IV aGVHD (A) and of severe cGVHD (B) for all patients. Incidence curves were generated using a competing events model to reflect the time dependent covariate of switch to everolimus. *P* values were determined using the Mantel-Byar test.

aGVHD developed in 6 patients who received everolimus, with GVHD symptoms developing before everolimus initiation in 3 (50%) of these patients. Only 1 patient (patient 14) experienced relapse of mild GVHD symptoms after everolimus was started.

Irrespective of the onset of GVHD symptoms before or after the switch to everolimus, we compared the rates of aGVHD grade 0/I, II, III, and IV between the everolimus and CsA groups. We found slight trending differences in grade II aGVHD (3 of 57 cases in the everolimus group versus 10 of 74 cases in the CsA group) and in grade III aGVHD (4 of 57 cases in the everolimus group versus 2 of 74 cases in the CsA group) (Supplementary Figure S2). These additional results confirm comparable aGVHD incidence in the 2 study groups. Notably, of the patients who received only everolimus without MMF, only patient 53 developed a higher-grade aGVHD (grade III). Among patients receiving everolimus, 6 (11.5%) developed severe cGVHD (intestinal in all cases), compared to only 3 patients (4.3%) in the control group (Figure 5B). However, this difference was not statistically significant (HR, 2.76; 95% CI, 0.69 to 11.0; $P = .15$).

Of the 6 patients with severe cGVHD in the everolimus group, 1 patient had de novo cGVHD and in the other 5 patients cGVHD resulted from previous severe aGVHD (grade II-IV). Of those 5 patients with preceding aGVHD, 2 had the onset of first aGVHD symptoms before the switch to everolimus. Mild to moderate cGVHD occurred in 3 patients in the everolimus group and in 5 patients in the CsA group.

Sickle cell disease and acute lymphoblastic leukemia were the most prevalent underlying diseases in the study cohort, and we assessed their potential to act as confounders in the analysis of GVHD incidence. However, removing these patients from the analyses did not change the risk for developing aGVHD or cGVHD. Patients who received everolimus more frequently underwent transplantation with a manipulated peripheral blood stem cell graft (as opposed to bone marrow grafts) containing a defined amount of T cells. However, the incidence of aGVHD or cGHVD was not affected by the graft source (Table 2). In

Table 2
Adjusted HRs for Graft Source and Underlying Disease Status

Characteristic	aGVHD grade II-IV			aGVHD grade III-IV			Severe cGVHD		
	HR*	95% CI	*P* value	HR*	95% CI	*P* value	HR*	95% CI	*P* value
Stem cell source: bone marrow vs manipulated peripheral blood stem cells	0.96	0.43-2.1	.92	1.88	0.60-5.9	.28	3.37	0.82-13.9	.09
Diagnosis									
Acute lymphoblastic leukemia	1.0	0.43-2.5	.92	0.96	0.30-3.06	.9	0.57	0.14-2.36	.43
Sickle cell disease	0.65	1.86-2.3	.50				0.39	0.05-2.85	.36

* Fine-Gray subdistribution hazard model.

summary, our data show that everolimus combined with MMF is effective GVHD prophylaxis in children with AKI after first allogeneic HSCT.

Switching to Everolimus-Based GVHD Prophylaxis Does Not Impact OS

OS did not differ significantly between patients treated with everolimus and those receiving CsA (HR, 1.6; 95% CI, 0.74 to 3.5; *P* = .23), with 92.3% of the everolimus group and 95.3% of the CsA group surviving for 100 days and 79.8% of the everolimus group and 84.1% of the CsA group surviving for 2 years (Figure 6A). OS in patients with a nonmalignant disease was similar in the 2 groups, with 94.7% of the everolimus group and 97.1% of the CsA group surviving for 100 days and 89.6% of the everolimus group and 90.6% of the control group surviving for 2 years (HR, 1.4; 95% CI, 0.34 to 5.9; *P* = .64) (Figure 6B). OS was significantly lower in patients with underlying malignant disease who were treated with everolimus (HR, 2.7; 95% CI, 1.1 to 6.9; *P* = .03). For patients with malignant diseases, the 2-year OS rate was 58.6% in the everolimus group compared with 83.7% in the CsA group (Figure 7A); however, at 2 years, only 28.2% (4 of 18) of the patients treated with everolimus experienced cancer relapse, compared with 52.8% (21 of 43 patients) of patients treated with CsA (HR, 0.55; 95% CI, 0.19 to 1.6; *P* = .27) (Figure 7B). Although this difference did not reach statistical significance, it may suggest a trend indicating that everolimus-based GVHD prophylaxis following HSCT could be beneficial for relapse-free survival in children with a malignant disease. EFS in patients with underlying malignant disease was equivalent in the 2 study groups (HR, 0.84; 95% CI, 0.43 to 2.0; *P* = .88) (Figure 7C). Taken together, these data demonstrate that OS is not adversely affected by the switch from CsA to everolimus-based GVHD prophylaxis for children who undergo HSCT.

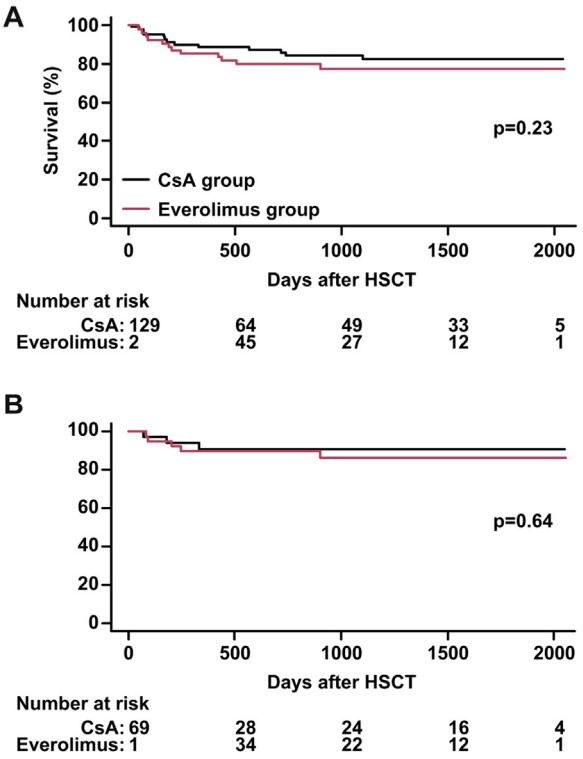

Figure 6. OS shown in a Simon and Makuch plot to reflect the time-dependent covariate of switching to everolimus for all patients (A) and for all patients with a benign underlying disease (B). *P* values were determined using the Mantel-Byar test.

DISCUSSION

The aim of this retrospective study was to evaluate the feasibility of switching GVHD prophylaxis in pediatric patients following first allogeneic HSCT from a CNI to everolimus, with or without MMF, when CNI treatment had to be discontinued because of nephrotoxicity or neurotoxicity. We show that retention parameters decreased significantly following the switch from CsA to everolimus with or without MMF. The use of everolimus was not associated with a significantly higher

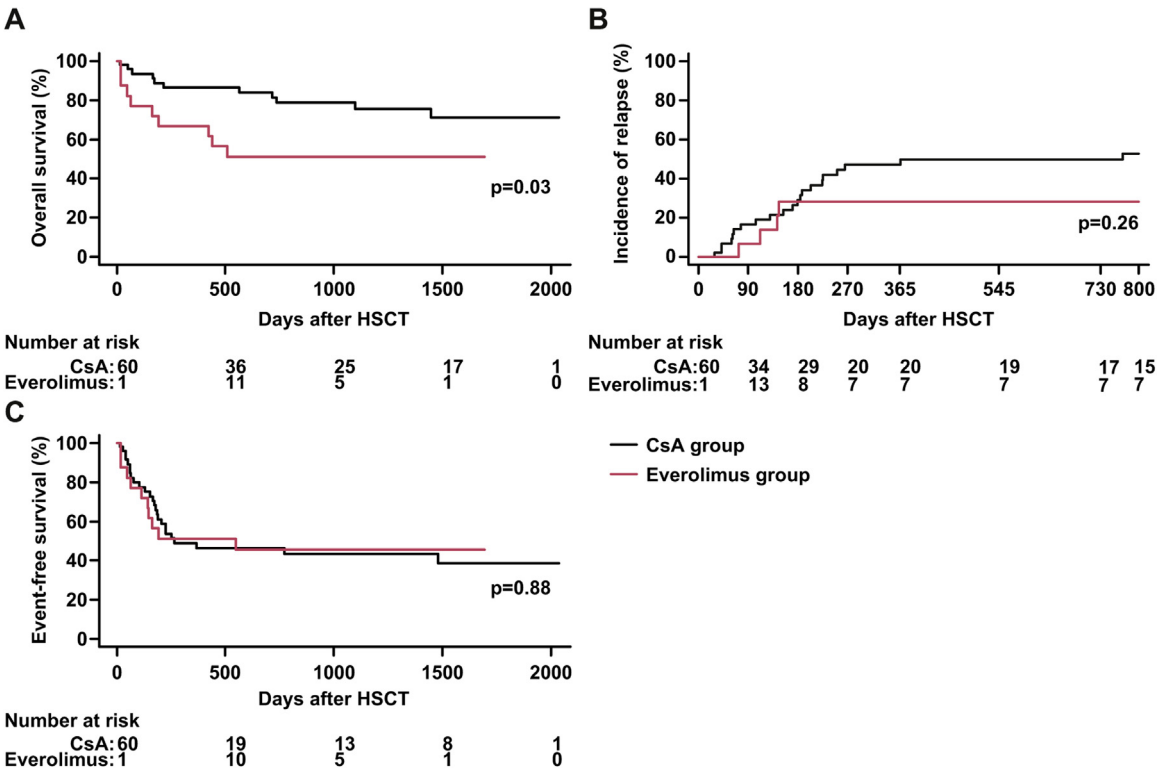

Figure 7. OS (A), relapse incidence (B), and EFS (C) of patients with malignant underlying diseases. The patients of our cohort with malignant underlying diseases were selected and analyzed for their OS, incidence of relapse, and EFS with the time dependent covariate of switch to everolimus with or without MMF. A and C show Simon-Makuch plots, and B shows cumulative incidence using a competing events model. P values were determined using the Mantel-Byar test.

overall incidence of aGVHD or cGVHD. Even though AKI was a serious complication during the post-transplantation course, patients who received everolimus had similar OS as the control group. In fact, OS was especially similar in patients with benign underlying disease in the 2 groups. Switching to everolimus-based GVHD prophylaxis led to worse OS in patients with a malignant underlying disease but did not impact EFS.

Our study provides real-world evidence as first proof of concept for the feasibility of using everolimus in combination with MMF as an immunosuppressive strategy for GVHD prophylaxis in pediatric patients with preexisting kidney injury or AKI after allogeneic HSCT.

Given that patients are less physically active and lose muscle mass during the course of HSCT, creatinine clearly has limitations as a marker of renal insufficiency. For this reason, we added cystatin C plasma levels and other clinical factors, including fluid overload, to our analysis. Because patient evaluation was not standardized during their clinical courses, we limited our analysis to the validated laboratory parameters creatinine and cystatin C. A standardized clinical evaluation for pediatric patients experiencing kidney injury during HSCT and follow-up that includes evaluation of more precise glomerular filtration rate combining creatinine and cystatin C, as proposed by Schwartz et al. [35] or Zappitelli et al. [36], should be implemented to benefit future retrospective studies of patient course.

Although we were able to analyze a considerably large pediatric HSCT cohort of 131 patients, the study's retrospective nature diminishes the impact of our findings. The underlying diseases and conditioning regimens used also varied substantially in our cohort, making it hard to identify specific subgroups that might benefit or be adversely affected by a switch to everolimus/MMF GVHD prophylaxis. Prospective, randomized analyses in larger cohorts of pediatric patients undergoing HSCT for underlying malignant diseases would help determine whether the everolimus/MMF combination leads to higher TRM or a lower relapse rate in this subgroup. The everolimus group was significantly older than the CsA group and consequently more susceptible to AKI necessitating the switch of immunosuppression regimens [37]. A prospective, randomized validation of these results in ongoing and future trials would be beneficial to address this selection bias in our study.

Our study significantly extends the data on the use of everolimus or mTOR inhibition in allogeneic HSCT. Adding sirolimus to the standard CsA/MTX combination for GVHD prophylaxis in 91 adult patients following HSCT improved relapse-free and OS in a 2-arm randomized phase 3 trial (NCT01231412) [38]. Pidala et al. [39] demonstrated a lower incidence of aGVHD and cGVHD in 74 adult patients randomized for treatment with a sirolimus/tacrolimus combination compared to treatment with MTX/tacrolimus (NCT00803010). In the EVTAC trial, everolimus in combination with tacrolimus appeared to effectively prevent GVHD while causing severe SOS in 25% of patients [27]. In contrast to these results in adult patients, only 1 patient developed a severe CTCAE grade 4 SOS in the everolimus group. Most cases of SOS occurred before everolimus was started. Otherwise, no life-threatening toxicity attributable to everolimus was observed in the children in the study cohort treated at our institution. However, in 3 patients, everolimus treatment had to be discontinued because of mild to moderate adverse events (stomatitis and interstitial pneumonitis). The ameliorated renal function in all 3 patients also allowed the switch back to CNI treatment for continued GVHD prophylaxis.

The everolimus/MMF combination failed to show effective GVHD prevention in a single-center phase I/II trial in adults (NCT00856505) [28]. However, there are 2 main differences between the NCT00856505 trial and our study. First, we treated exclusively children and individual young adults, and GVHD rates in pediatric transplantation patients are known to be lower than those in adults [40]. Second, all but 2 of our patients were switched from CNI treatment to everolimus only after a median of 22 days following HSCT. Of the 2 patients in our study who received everolimus/MMF from the start as GVHD prophylaxis, patient 54 experienced severe aGVHD beginning on day 40 post-HSCT and ultimately died from severe cGVHD involving the gut and lungs, and patient 5 is alive at 3 years post-HSCT and shows no signs of cGVHD.

Putting our treatment into perspective, it is important to consider studies examining the connection between GVHD incidence and immunosuppression in the first days following HSCT. Bianchi et al. [41] showed that high serum CsA level ($>$195 μg/L) at 10 days after transplantation was correlated with a significantly lower incidence of grade II-IV aGVHD. A higher starting dose of CsA also was shown to reduce the risk of aGVHD [42]. Considering the literature and patient 5 in our everolimus group, it appears that sufficient immunosuppression at a very early stage following HSCT is critical to prevent the development of aGVHD and cGVHD. Whether GVHD prophylaxis that starts with everolimus and MMF treatment is more effective in children receiving HSCT could be pursued in randomized clinical trials.

Interestingly, switching GVHD prophylaxis from CsA to everolimus with or without MMF did not significantly alter OS in our cohort, despite the increased mortality in pediatric patients with AKI described in the literature [5,7,43,44]. This observation might suggest that switching to everolimus/MMF prophylaxis may have a beneficial effect in children, counteracting the negative impact of AKI. One possible explanation for this might be a favorable effect on immune reconstitution. How everolimus and MMF treatment impacts immune reconstitution is not yet fully understood, however. Higher circulating regulatory T cell (Treg) numbers were measured in stable renal transplant recipients treated with an mTOR inhibitor but not in those treated with a CNI [45]. Rapamycin treatment reduced the activity of alloreactive conventional T cell activity in a murine GVHD model, but Tregs still provided GVHD protection [46]. In contrast to these results, Schaefer et al. [28] reported that everolimus/MMF treatment in adult patients with hematologic malignancies led to protracted overall, Treg, and naïve $CD4^+$ T cell reconstitution after HSCT in a single-center phase I/II trial (NCT00856505) compared with historical controls. A direct antitumor effect of everolimus also may contribute to or cause a potential beneficial effect [47,48]. Phosphatidylinositol-3-kinase (PI3K)/AKT and mTOR signaling are hyperactivated in 50% to 80% of acute myeloid leukemia cases [49]. When combined with the hypomethylating agent azacitidine, everolimus positively influenced OS and overall response rates in patients with advanced acute myeloid leukemia [50]. Everolimus has been associated with favorable rates of complete remission in combination with chemotherapy for relapsed acute lymphoblastic leukemia in pediatric patients [51]; however, whether this effect also contributes to the graft-versus-leukemia effect after HSCT remains to be determined.

Our present study represents the first clinical evidence that a conversion from CNI-based GVHD prophylaxis to everolimus/MMF during the clinical course after HSCT appears safe and feasible in pediatric patients with various underlying diseases. The earliest appropriate starting time of

everolimus/MMF remains to be defined but it probably should not be during the first week post-HSCT. The duration of immunosuppressive treatment with everolimus/MMF depends strongly on the patient's specific clinical course. Based on our data and in-house experience, we generally recommend that everolimus treatment be provided until day +180 post-HSCT for children with underlying benign diseases and until day +80 for children with underlying malignant diseases in the absence of clinical evidence of aGVHD or cGVHD.

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Data availability: All data that support the findings of this study are available from the corresponding author on reasonable request.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jtct.2025.01.002.

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