OPEN

Long-term Follow-up of a Randomized Trial of Tacrolimus or Cyclosporine A Microemulsion in Children Post Liver Transplantation

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Background. The aim of this study was to determine the long-term efficacy and safety of tacrolimus (Tac) and cyclosporine immunosuppression in pediatric liver transplantation (LTx). **Methods.** One hundred fifty-six patients who had taken part in a multicenter, randomized, open, parallel study of Tac and corticosteroids versus cyclosporine A microemulsion (CyA-ME), corticosteroids, and azathioprine. Patients were assessed at regular intervals up to 14 y after LTx. Analysis was conducted descriptively. **Results.** In a long-term follow-up, there was a similar incidence of acute rejection (Tac versus CyA-ME, 5 versus 8) and graft loss (5 versus 10). There were 11 deaths in the cohort, which were from infectious complications/malignancy in the Tac group (n = 2/5) and from chronic rejection/liver failure in the CyA-ME group (n = 3/6). A similar incidence of Epstein-Barr virus and posttransplant lymphoproliferative disease was observed (8 versus 8, 3 versus 3). However, there was a greater incidence of cosmetic adverse events in the CyA-ME cohort, with higher incidences of hypertrichosis (8 versus 27) and gum hyperplasia (20 versus 6). Growth improved equally in both groups. Overall, 81% of patients randomized to Tac remained on Tac therapy at study end, compared with 31% of patients randomized to CyA-ME. Common reasons for switching from CyA-ME included steroid-resistant/acute rejection (n = 12/8) and cosmetic changes (n = 8). **Conclusions.** This study is the first prospective, observational follow-up study of pediatric patients randomized to Tac and CyA-ME to evaluate long-term outcomes. Our analysis was limited by the degree of switchover between the cohorts; however, there were fewer deaths from chronic rejection/liver failure and reduced adverse events with Tac. Long-term use of Tac and Tac combination therapy appears to be safe and effective immunosuppression for pediatric LTx recipients.

(*Transplantation Direct* XXX;00: e00; doi: 10.1097/TXD.0000000000001221. Published online 20 September, 2021.)

INTRODUCTION

Liver transplantation (LTx) is the established treatment for patients with end-stage liver disease. Long-term graft survival in pediatric LTx recipients is approximately 80% at 10 y and is improving because of enhancements in donor procurement, surgical techniques, and posttransplant medical care.[1] However, approximately two-thirds of late deaths in pediatric LTx are caused by immunosuppression-related complications, including infection and malignancy.[2,3]

Received 7 September 2019. Revision received 22 June 2021.

Accepted 4 July 2021.

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We would like to thank Astellas Pharma for funding this project. The authors declare no conflicts of interest.

C.L. and D.K. participated in designing the study. C.L., A.A., P.J., M.B., B.G., J.M., M.C., E.J., R.R., and U.B. participated in data extraction. A.A. and C.L. participated in data analysis. All authors participated in data interpretation and reviewed the article. A.A. and C.L. wrote the the original draft.

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ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001221

Tailored immunosuppression in pediatric LTx is more difficult and complex compared with immunosuppression in adult LTx recipients for many different reasons. Physiological differences in pediatric patients alter the pharmacokinetics of immunosuppressive medications, influencing their absorption, distribution, metabolism, and excretion. These physiological changes are also dynamic, altering as pediatric patients become older.[4] Furthermore, pediatric LTx patients are exposed to immunosuppression medications for a greater period. The influence of this long-term medication exposure on the child's development, infection risk, and carcinogenesis is poorly understood.

Historically, cyclosporine A (CyA) was the predominant prescribed maintenance immunosuppression in pediatric LTx. Cyclosporine A microemulsion (CyA-ME) was formulated for pediatric use because of better absorption and bioavailability than the original formulation Sandimmune.[5] However, because of the cosmetic adverse effects of CyA-ME,[6] Tacrolimus (Tac) has replaced CyA-ME.[7] Several studies have detailed the short-term adverse events of Tac immunosuppression including its effects on renal function, hypertension, dyslipidemia, cardiomyopathy, and food allergies.[2,8,9] However, the long-term influence from cumulative Tac or CyA exposure is poorly understood.

We previously published the 1-y follow-up of a randomized, open, parallel study of Tac and corticosteroids versus CyA-ME, corticosteroids, and azathioprine in 10 European centers.[10] This study reported a statistically significantly higher acute rejection-free rate and corticosteroid rejection-free rate among Tac-treated patients compared with the CyA-ME cohort. Similar findings have been confirmed in other studies.[11-13] However, it is not clear if either Tac or Cya-ME is safe and effective in the long term in pediatric LTx recipients. Therefore, this prospective study aims to follow patients who were randomized in the initial study to identify whether long-term Tac and CyA-ME are safe and effective over a follow-up of 10–14 y. This will help inform clinicians to make evidence-based decisions as to the utility of Tac and CyA-ME in pediatric LTx and to assess the safety of Tac immunosuppression.

PATIENTS AND METHODS

The original trial was a 12-mo multicenter, open-label, parallel-group randomized study comparing Tac and corticosteroids with CyA-ME, corticosteroids, and azathioprine in pediatric LTx. The inclusion criteria for the initial study were children aged <16 y, <40 kg in weight who were undergoing a primary LTx between October 1997 and December 2000. Power calculations for this initial recruitment were published in 2004,[10] and the publication only included children who completed the 12-mo study period on their enrollment immunosuppression.

All patients enrolled in the original 1-y study, regardless of whether they were withdrawn from the study, were followed up for an extended period. Patients included within this extended observation were reviewed at years 1, 2, 3, 4, 5, 10, and 14 after LTx (2000–2014). Questionnaires were sent to all centers to record, from routine or clinically indicated visits, patient's medical histories, vital signs, laboratory results, drug concentrations (daily dose and trough), biopsy-proven acute, chronic, steroid-resistant rejection, and side effects (including allergies). Visits by the project manager were also undertaken to participating sites to audit data collection. The last results from these questionnaires were obtained on December 31, 2014, and the analysis was conducted on August 7, 2018.

Assessment

In the initial study recruitment, 185 patients were randomized either to Tac (93) or to CyA-ME (92). Outcomes for this follow-up study include time to first acute or chronic rejection episode, patient survival, graft survival, renal function, Epstein-Barr virus (EBV) infection, posttransplant lymphoproliferative disease (PTLD) incidence, renal function, and adverse events.

Ethical approval at all participating centers was gained for the initial randomized therapeutic trial. Patients were randomized by a centralized telephone randomization system, stratified by age (<3 and ≥3 y), type of donation (deceased or living), and treatment center. The initial study was performed with respect to the revised Declaration of Helsinki 1983.

Statistical Analysis

All patients completing the original 1-y trial were included for extended follow-up. Given the high rate of crossover within the study, we have stated our study results descriptively. Overall, patient survival was defined as the time from transplantation to the death of the patient or the patient's last known follow-up date (at which point the patient was censored). Graft failure was defined as primary nonfunction, patient death, chronic rejection, and cirrhosis. Thus, graft survival was considered as the time to either one of these outcomes or to the censor. All data are quoted as median (interquartile range).

Immunosuppression

The long-term Tac and cyclosporine dosages were administered as per the protocols of each individual center. Overall, the mean Tac daily dose across all 156 patients was 0.143 mg/kg at the end of year 1, reducing to 0.074 mg/kg at year 5 and 0.052 mg/kg at year 10. Mean whole-blood trough concentration was 6.04 mg/L at year 1, 4.90 mg/L at year 5, and 5.67 mg/L at year 10.

Mean cyclosporine daily dose fell from 6.08 mg/kg at year 1 to 4.92 mg/kg at year 5 and 2.54 mg/kg at year 10. Mean whole-blood trough concentration was 136.21 mg/L at year 1, 54.5 mg/L at year 5, and 48.00 mg/L at year 10.

It should be noted that although trough doses decreased with time in both cohorts, we have no guarantee that these were 12-h trough results.

Mean corticosteroid dose fell from 0.159 mg/kg (cyclosporine) and 0.217 mg/kg (Tac) at the end of year 1 to 0.140 mg/kg (cyclosporine) and 0.135 mg/kg (Tac) at year 5 and 0.05 mg/kg (cyclosporine) and 0.0749 mg/kg (Tac) at year 10. All participants started on concomitant corticosteroids at LTx, with optional tapering at month 3. By the last point of follow-up, 76 participants (36 Tac, 36 CyA-ME) were still on corticosteroids.

RESULTS

A total 156 patients from the original study were enrolled in this prospective long-term study. Table 1 details the baseline characteristics at 1 y after transplantation of this cohort. Overall, 79 patients had been originally randomized to

TABLE 1.

Baseline characteristics of the study population at baseline

	Tac (n = 79)	CyA-ME (n = 77)
Median age (IQR), y	1.96 (0.83–6.62)	1.06 (0.69–4.72)
Male, n (%)	41 (51.8)	42 (54.5)
EBV IgG negative, n (%)[a]	30 (37.9)	31 (40.2)
Baseline median eGFR	122	109.6
Primary diagnosis, n (%)		
Biliary atresia	47 (59.4)	40 (51.9)
Cirrhosis	3 (3.8)	7 (9.1)
Alagille's syndrome	6 (7.6)	6 (7.8)
Metabolic syndrome	1 (1.3)	1 (1.3)
Byler's disease	8 (10.1)	4 (5.2)
Other, n(%)	9 (11.4)	13 (16.8)
Deceased graft, n (%)	66 (83.5)	63 (81.8)
Living-related graft, n (%)	13 (16.4)	14 (18.2)
Reduced/split liver graft, n (%)	58 (73.4)	62 (80.5)

Comparisons between the Tac and CyA-ME cohorts by Mann-Whitney U analysis reported *P* value >0.05. eGFR units are (mL/min/1.73 m^2).
[a]Data not recorded for 14 patients randomized to Tac and 17 patients on CyA-ME.
CyA-ME, cyclosporine A microemulsion; EBV, Epstein-Barr virus; eGFR, estimated glomerular filtration rate; IgG, immunoglobulin G; IQR, interquartile range; Tac, tacrolimus.

open-label dual Tac regimen (Tac/corticosteroids), and 77 were randomized to CyA-ME therapy (CyA-ME/corticosteroids/azathioprine). All children were aged ≤16 y and of ≤40 kg in bodyweight. Median (interquartile range [IQR]) of follow-up of this cohort was 11.0 y (3.3–11.2) posttransplant.

Time to First Biopsy-proven Acute or Chronic Rejection

A total of 13 patients, who had not had acute or chronic rejection before the first year after LTx, experienced acute or chronic rejection after 1 y posttransplantation (8 CyA-ME, 5 Tac). For the Tac cohort, this included 5 episodes of biopsy-proven acute rejection and 2 episodes of biopsy-proven chronic rejection. For the CyA-ME cohort, there were 9 episodes of biopsy-proven acute rejection and 4 episodes of biopsy-proven chronic rejection.

All patients were receiving their randomized medication at the time of rejection. Median time to rejection was 2.9 y (IQR, 1.13–5.29) in the CyA-ME cohort versus 6.75 y(2.48–9.17) in the Tac cohort post-LTx.

Treatment for rejection was as per center protocol but consisted of either increasing/reducing CyA-ME dose, increasing/decreasing Tac dose, adding further medication, or switching medications.

Patient Survival

A total of 11 patients died in this long-term review (Tac n = 5, CyA-ME n = 6). Causes of death among the Tac group included recurrence of disease, gastrointestinal bleed, T-cell lymphoma, hemolytic anemia, and unknown. In the CyA-ME group, the 6 deaths were caused by intractable seizures, chronic liver failure, PTLD, gastrointestinal bleed, intraoperative death, and multiorgan failure. All deaths were spread across the 14-y follow-up. Table 2 details the characteristics of the 11 patient deaths.

Median time to patient death in the Tac cohort was 1.8 y (IQR, 1.75–2.3) versus CyA-ME 2.85 y (IQR, 1.58–6.08) post-LTx. Four patients had switched immunosuppression by the time of death. Three had switched from CyA-ME to Tac before death. One of these patients had switched because of acute rejection (233 d post-LTx). This patient died from chronic liver failure after recurrence of portal vein thrombosis and hypertrophic obstructive cardiomyopathy (576 d post-LTx).

The second patient switched because of chronic rejection (520 d post-LTx). This patient died from multiple organ failure (hypertension, bradycardia, conjugated hyperbilirubinemia, sepsis, and low urinary output) 4188 d post-LTx. The third patient switched immunosuppression at 25 d post-LTx because of steroid-resistant rejection. This patient died at 2749 d because of intraoperative complications during retransplantation.

One of the patients had switched from CyA to Tac. This patient switched immunosuppression because of renal dysfunction (162 d post-LTx). This patient died from an unknown cause (853 d).

Graft Loss

A total of 15 patients had primary graft loss 1 y after LTx (CyA-ME n = 10, Tac n = 5). Causes of primary graft loss included death (3 versus 5), chronic rejection (4 versus 0), and cirrhosis (3 versus 0). Median time to primary graft loss was 3.51 y (IQR, 1.75–6.95) after LTx. Table 3 describes the causes of 15 graft losses over the follow-up period.

Epstein-Barr Virus and Posttransplant Lymphoproliferative Disease

Epstein-Barr Virus

Sixteen patients were reported to have de novo EBV infection or de novo EBV-related problems >12 mo post-LTx, with equal incidence in both cohorts (Tac n = 8; CyA-ME n = 8). Median (IQR) time to EBV diagnosis in years was 2.87 (2.10–3.38) post-LTx.

Posttransplant Lymphoproliferative Disease

Six patients developed PTLD 12 mo post-LTx (Tac n = 3; CyA-ME n = 3). Three patients had previously switched immunosuppression. Median (IQR) time to the development of PTLD in years was 2.60 (1.38–3.67). This gave an overall PTLD rate of 3.8%. A total of 2 patients died from PTLD, giving a death rate of 33.3%.

Last Known Immunosuppression

Eleven patients died >12 mo after LTx (Tac n = 5, CyA-ME n = 6), so we determined the last known immunosuppression in the surviving 145 patients (Tac = 74; CyA-ME n = 71).

Cyclosporine A Microemulsion

Twenty-two of 71 (31%) patients enrolled to CyA-ME remained on CyA-ME either as monotherapy or combination therapy at their last point of follow-up. A total of 38 of 71 (54%) patients were on Tac monotherapy or combination therapy, whereas 11 of 71 (15%) were not on calcineurin inhibitors (CNIs). Average time to switching immunosuppression was 2.40 y from LTx (Figure 1).

Tacrolimus

Sixty of 74 (81%) patients enrolled to Tac remained on Tac either as a monotherapy or combination therapy. However, 11 of 74 (15%) patients were on CyA-ME or combination therapy at the last point of follow-up, and a total 3 of 74 (4%) were no longer on CNIs, with 2 of 3 maintained on other

TABLE 2.

Characteristics of 11 deaths in the cohort during extended follow-up

Patient	Baseline Immunosuppression	Immunosuppression switch	Cause of switch (d post-LTx to switch)	Cause of death	Time to death post-LTx, d	Primary graft (if not, cause of retransplantation)	Related to surgery or immunosuppression
1	CyA-ME	No	–	Intractable seizures	473	Yes	Seizures related to CyA-ME use
2	CyA-ME	Yes (Tac)	3rd episode of acute rejection (233)	CLF and HOCM	576	No (PVT)	CLF related to PVT after surgery. HOCM related to Tac use
3	CyA-ME	No	–	PTLD	1041	Yes	Related to immunosuppression
4	Tac	No	–	GI bleed	637	Yes	Surgical—Budd-Chiari
5	Tac	No	–	PTLD	658	Yes	Related to immunosuppression
6	Tac	Yes (CyA-ME)	Renal dysfunction (162)	Unknown	853	Yes	Unknown
7	CyA-ME	Yes (Tac)	Chronic rejection (520)	Multiple organ failure	4188	No (cryptogenic cirrhosis)	Unknown
8	CyA-ME	No	–	GI bleed	2221	Yes	Unknown
9	Tac	No	–	Respiratory failure and sepsis	2523	Yes	CF related
10	CyA-ME	Yes (Tac)	Steroid-resistant rejection (25)	Intraoperative death	2749	No (chronic rejection)	Complications during surgery
11	Tac	No	–	Hemolytic anemia causing multiorgan failure	3085	Yes	Pulmonary hypertension + noncompliant

CF, cystic fibrosis; CLF, chronic liver failure; CyA-ME, cyclosporine A microemulsion; GI, gastrointestinal; HOCM, hypertrophic cardiomyopathy; LTx, liver transplantation; PTLD, posttransplant lymphoproliferative disease; PVT, portal vein thrombosis; Tac, tacrolimus.

TABLE 3.

Causes of the 15 primary graft losses during extended follow-up

Patient	Baseline immunosuppression	Immunosuppression switch (switch immunosuppression)	Cause of switch (d after LTx to switch)	Cause of graft failure	Time to primary graft failure post-LTx, d	Related to surgery or immunosuppression
1	CyA-ME	No	–	Death (intractable seizures)	473	Seizures related to CyA-ME
2	CyA-ME	No	–	Death (PTLD)	1071	Immunosuppression related
3	CyA-ME	No	–	Death (GI bleed)	2221	Unknown
4	Tac	No	–	Death (disease recurrence)	398	None
5	Tac	No	–	Death (GI bleed)	637	Surgical (developed Budd-Chiari)
6	Tac	No	–	Death (PTLD)	658	Immunosuppression related
7	Tac	Yes (CyA-ME)	Renal dysfunction (162)	Death (unknown)	853	Unknown
8	Tac	No	–	Death (respiratory failure + sepsis)	2523	CF related
9	Tac	No	–	Hemolytic anemia + multiorgan failure	3085	Immunosuppression related
10	CyA-ME	Yes (Tac)	Lack of efficacy	Chronic rejection	372	Immunosuppression related
11	CyA-ME	Yes (Tac)	Low levels of CyA-ME (121)	Chronic rejection	1494	Immunosuppression related
12	CyA-ME	Yes (Tac)	Steroid-resistant rejection (25)	Chronic rejection	2546	Immunosuppression related
13	CyA-ME	Yes (Tac)	Chronic rejection (520)	Cirrhosis	527	Immunosuppression related
14	CyA-ME	Yes (Tac)	Acute rejection (711)	Cirrhosis	3829	Immunosuppression related
15	CyA-ME	No	–	Cirrhosis	4512	Surgical—patient had undergone dilation

CF, cystic fibrosis; CyA-ME, cyclosporine A microemulsion; GI, gastrointestinal; LTx, liver transplantation; PTLD, posttransplant lymphoproliferative disease; Tac, tacrolimus.

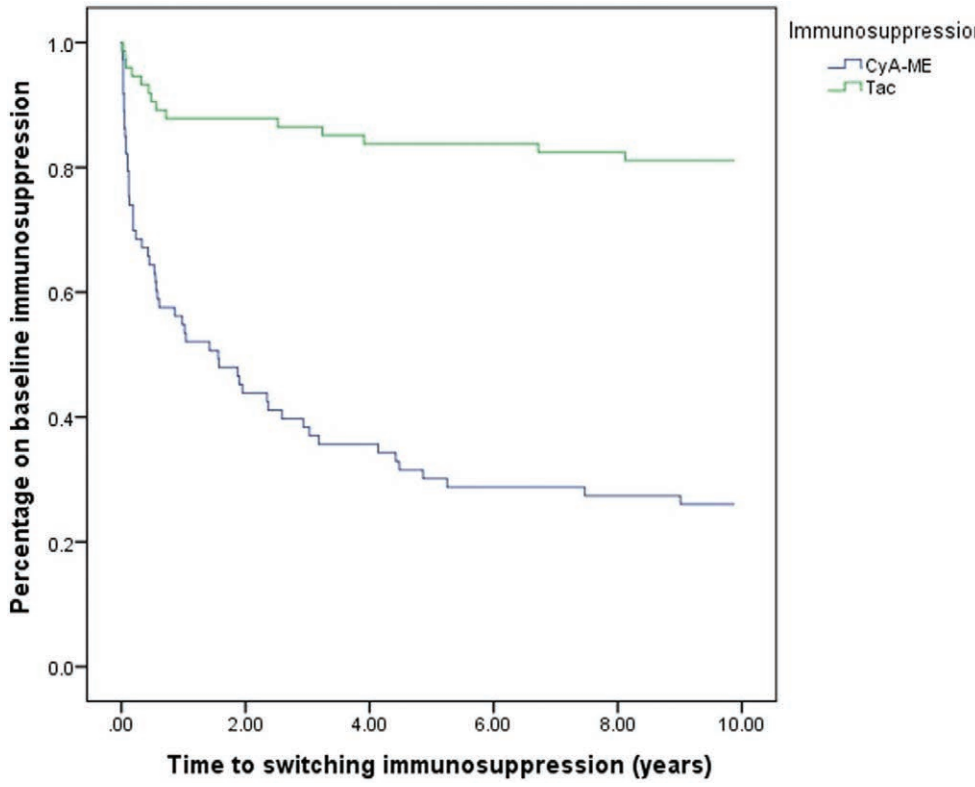

FIGURE 1. Kaplan-Meier curve illustrating the time to switching immunosuppression. CyA-ME, cyclosporine A microemulsion; Tac, tacrolimus.

immunosuppression regimes and 1 of 3 receiving no immunosuppression (Table 4). Average time to switching immunosuppression was 4.77 y from LTx.

Common reasons for changes in immunosuppressive regimes after 1 y included acute rejection, steroid-resistant rejection, chronic rejection, and cosmetic changes (Table 5).

Adverse Events

Although the rate of adverse events was similar in both groups, there was a higher incidence of cosmetic side effects in the CyA-ME cohort (Table 6). Thirteen patients (Tac n = 6; CyA-ME n = 7) reported to have experienced tremors, with 11 of 13 resolving with time. Convulsions were noted in 12 patients (Tac n = 6: CyA-ME n = 6), 3 of which had both convulsions and tremors (Tac = 2, CyA-ME = 1). No patient switched their immunosuppression regime because of these side effects.

Thirty-five patients developed hypertrichosis (Tac n = 8, CyA-ME n = 27), and of the 8 Tac patients who developed the condition, 4 patients had been switched to CyA-ME for other adverse events and developed hypertrichosis after switching. Twenty-six patients developed gum hyperplasia (Tac n = 6; CyA-ME n = 20), of which 3 of 6 Tac patients had been switched to CyA-ME for other adverse events. Sixteen patients had both hypertrichosis and hyperplasia

TABLE 4.

Last known immunosuppression

	Tac (n = 74), n (%)	CyA-ME (n = 71), n (%)
CyA-ME	4 (5)	12 (17)
Tac	30 (41)	19 (27)
Cya-ME combination	7 (9)	10 (14)
Tac combination	30 (41)	19 (27)
MMF	0 (0)	1 (1)
+ Steroids	0 (0)	5 (7)
+ Everolimus	0 (0)	1 (1)
Steroids	1 (1)	1 (1)
Sirolimus (sirolimus + steroids)	1/0 (1)	1/2 (1/2)
None	1 (1)	0

CyA-ME, cyclosporine A microemulsion; MMF, mycophenolate mofetil; Tac, tacrolimus.

TABLE 5.

Causes of change in immunosuppression after 1 y

	Tac (n = 14), n (%)	CyA-ME (n = 49), n (%)
Rejection		
Acute rejection	4 (28.6)	12 (24.5)
Steroid-resistant rejection	0	8 (16.3)
Chronic rejection	2 (14.3)	5 (10.2)
Cosmetic	0	8 (16.3)
Graft hepatitis/dysfunction	0	2/2 (8.2)
Renal dysfunction	0	5 (10.2)
Allergy	3 (21.4)	0
PTLD	2 (14.3)	0
Other	3 (21.4)	8 (16.3)

CyA-ME, cyclosporine A microemulsion; PTLD, posttransplant lymphoproliferative disease; Tac, tacrolimus.

TABLE 6.

Adverse events for patients randomized to Tac and CyA-ME

Adverse event	Tac (n = 40), n (%)	CyA-ME (n = 74), n (%)
Tremors	10 (12.7)	13 (16.9)
Convulsions	6 (7.6)	6 (7.8)
Hypertrichosis	8 (10.1)	27 (35.0)
Gum hyperplasia	6 (7.8)	20 (30.0)
Bone disease	8 (10.1)	8 (10.4)
Diabetes	1 (1.3)	0 (0.0)
HOCM	1 (1.3)	0 (0.0)

CyA-ME, cyclosporine A microemulsion; HOCM, hypertrophic cardiomyopathy; Tac, tacrolimus.

(Tac n = 2; CyA-ME n = 14), in which both Tac patients had been switched to CyA-ME.

There was only 1 report of disturbed glucose metabolism requiring insulin treatment 11 y after transplantation. It is noted that this patient was noncompliant (drinking/smoking). He developed diabetes in January 2009 and was transferred to adult services in 2007.

There was no difference in the rate of bone disease and fractures, occurring in 16 patients overall (Tac n = 8, CyA-ME n = 8). Although 23 patients developed eczema (Tac n = 16, CyA-ME n = 7), the majority of reported cases resolved over time.

Height and Weight

Height and weight were seen to improve equally in both groups in the years after transplantation. Table 7 details the changes in height and weight scores over the 10-y period.

DISCUSSION

Over the past decade, LTx has become the established treatment for patients with end-stage liver disease. Organ scarcity, which was the main restriction to transplantation, has been in part relieved from modernized surgical techniques. Therefore, interest now is in long-term follow-up and exploring the methods to optimize graft survival and prevent complications. This study is an extension of a randomized, multicenter trial exploring the efficacy and safety of 2 commonly used CNIs, Tac and CyA-ME, over a 10- to 14-y period in 10 European centers. Several reports have explored the advantages of Tac over Cya-ME for immunosuppression in adult LTx, particularly with regard to acute and corticosteroid resistant rejection in short-term follow-up.[14-16] However, there is little evidence about the long-term safety and efficacy of Tac or CyA-ME immunosuppression in children.

This prospective randomized study indicates similar incidences in longer-term rejection-free rate and graft and patient survival between the 2 treatment arms. However, although the rate of death was similar in both groups, the causes differed and were mostly infectious/malignancy in the Tac group and chronic rejection/liver failure in the CyA group. Furthermore, all causes of graft loss in the Tac cohort were non–graft related. Overall, this may suggest that the immunosuppression was less effective in maintaining graft function in the CyA group compared with the Tac cohort. It is possible that in this early study of Tac use, there may have been initial overimmunosuppression in the Tac cohort. However, our analysis is limited by the high degree of crossover between the study groups.

Furthermore, we observed no increased risk of EBV and PTLD in any patient group, and long-term renal function and growth were normal. However, a high number of patients on CyA switched immunosuppression because of rejection-associated complications, as well as renal dysfunction. In addition, there was a higher incidence of cosmetic adverse events in the CyA-ME cohort compared with Tac.

A significant proportion of the patient remained on steroid therapy at the study end. Following the end of the original 1-y clinical trial, all centers reverted to their standard practice for prophylactic immunosuppression regime. A majority of centers in Europe, as per their local practice, prescribed low-dose maintenance steroids except for Birmingham where steroids would be discontinued after 3 mo. However, Birmingham modified treatment according to need and biopsy reports. Patients were admitted for follow-up biopsies, and if they demonstrated hepatitis, inflammation, and fibrosis, low-dose steroids were prescribed to reduce this graft injury, and patients were then maintained on this low dose. Additionally, patients who experienced late graft rejection (primarily CyA group) had steroids introduced to reverse rejection and patients were then maintained on steroids to prevent further episodes.

To our knowledge, this is the first study to have conclusively assessed, across a broad range of outcomes, the long-term effects of Tac and CyA-ME. In a retrospective analysis of 233 patients,[17] Jain et al reported a significantly reduced rate of graft failure, patient death, and rejection in a 9-y follow-up among Tac-treated patients compared with CyA-ME (all $P < 0.005$). Although the rate of PTLD was not significantly different between the cohorts ($P = 0.13$), survival was significantly better for Tac-treated patients than the CyA-ME cohort. As such, our inability to detect significant differences in these outcomes may stem from our reduced sample size or because more patients transferred from CyA-ME to Tac. However, the study by Jain et al failed to randomize its patients at baseline and did not conduct a multivariate analysis for their outcomes. Therefore, we cannot be sure that selection bias has not affected their findings.

A separate analysis by Hasenbein et al[18] specifically explored renal function and side effects in a cohort of 42 children receiving Tac and 87 receiving CyA-ME. In the 5-y follow-up, no significant difference was seen in renal function between the 2 groups; however, a higher incidence of cosmetic side effects

TABLE 7.

Changes in height and weight *z*-scores

	12 mo		24 mo		36 mo		48 mo		60 mo		120 mo	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight	Height	Weight
Tac	−0.50	−0.14	−0.25	−0.02	−0.14	0.14	−0.24	0.48	−0.02	0.86	0.24	0.49
CyA-ME	−0.58	−0.62	−0.84	−0.76	−0.60	−0.62	−0.59	−0.48	−0.69	0.03	−0.75	0.07

CyA-ME, cyclosporine A microemulsion; Tac, tacrolimus.

was seen among CyA-ME patients. Similar results regarding renal function are seen in other studies.[17,19,20] Finally, Martinelli et al conducted a 20-y follow-up study of 127 pediatric LTx recipients in France, of which 93.7% (n = 119) of the cohort received CyA-ME at study initiation. The study reported 83% survival at 10 y. A further 11 patients showed histological features of chronic rejection in long-term follow-up, and growth was normal in most patients. At study end, 48 patients were still receiving Tac, compared with 45 on CyA-ME. Reasons for switching immunosuppression were not discussed.[21]

There are few published papers on the incidence of PTLD among pediatric LTx recipients, and what single-center reports there are present similar rates of PTLD occurrence as we have observed.[22-24] These are further corroborated among analyses of pediatric solid organ transplant recipients receiving Tac immunosuppression.[25,26]

There was a higher general rate of adverse effects in the CyA-ME cohort, particularly hypertrichosis and gum hyperplasia. Furthermore, "cosmetic" was the largest reason for immunosuppression switch among all participants. Overall, the cosmetic effects of CyA-ME have been well documented in the literature and recommendations have already been made to avoid using CyA-ME in teenage girls.[27,28]

A significant limitation to this study is the high rate of crossover and switching of immunosuppression between the 2 drugs. This prevented us from conducting accurate statistical analyses. Furthermore, we have not concurrently analyzed how many patients received further immunosuppressive medications (eg, azathioprine/mycophenolate mofetil). However, we believe the reasons as to why patients switched immunosuppressive regimens form part of the descriptive results of our study and inform us as to the practical effects of long-term CNI therapy.

In conclusion, our findings show that within our cohort, Tac-based immunosuppression may have provided improved patient and graft outcomes over patients receiving CyA-ME (albeit this is hard to determine given the high rate of crossover between the 2 study arms). Most significantly, the incidence of cosmetic adverse events was significantly reduced with Tac, and this was a major cause of switching the patient's immunosuppression regimen from CyA-ME. Finally, most deaths in the Tac cohort were infection/malignancy related and not associated with chronic graft failure. From our retrospective cohort study, we would conclude that Tac and Tac combination therapy appear to be a safe and effective form of long-term immunosuppression for pediatric LTx recipients.

REFERENCES

1. Bucuvalas J. Long-term outcomes in pediatric liver transplantation. *Liver Transpl.* 2009;15(suppl 2):S6–S11.
2. Kelly DA, Bucuvalas JC, Alonso EM, et al; American Association for the Study of Liver Diseases; American Society of Transplantation. Long-term medical management of the pediatric patient after liver transplantation: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Liver Transpl.* 2013;19:798–825.
3. Soltys KA, Mazariegos GV, Squires RH, et al; SPLIT Research Group. Late graft loss or death in pediatric liver transplantation: an analysis of the SPLIT database. *Am J Transplant.* 2007;7:2165–2171.
4. Miloh T, Barton A, Wheeler J, et al. Immunosuppression in pediatric liver transplant recipients: unique aspects. *Liver Transpl.* 2017;23:244–256.
5. van Mourik ID, Thomson M, Kelly DA. Comparison of pharmacokinetics of Neoral and Sandimmune in stable pediatric liver transplant recipients. *Liver Transpl Surg.* 1999;5:107–111.
6. Panicker VV, Mathew A, Dhamramaratnam A. Cosmetically disfiguring side effects of cyclosporine. *Int J Trichology.* 2012;4:50.
7. Ng VL, Fecteau A, Shepherd R, et al; Studies of Pediatric Liver Transplantation Research Group. Outcomes of 5-year survivors of pediatric liver transplantation: report on 461 children from a North American multicenter registry. *Pediatrics.* 2008;122:e1128–e1135.
8. LaRosa C, Baluarte HJ, Meyers KEC. Outcomes in pediatric solid-organ transplantation. *Pediatr Transplant.* 2011;15:128–141.
9. McLin VA, Anand R, Daniels SR, et al; SPLIT Research Group. Blood pressure elevation in long-term survivors of pediatric liver transplantation. *Am J Transplant.* 2012;12:183–190.
10. Kelly D, Jara P, Rodeck B, et al. Tacrolimus and steroids versus ciclosporin microemulsion, steroids, and azathioprine in children undergoing liver transplantation: randomised European multicentre trial. *Lancet.* 2004;364:1054–1061.
11. McDiarmid SV, Busuttil RW, Ascher NL, et al. FK506 (tacrolimus) compared with cyclosporine for primary immunosuppression after pediatric liver transplantation. Results from the U.S. multicenter trial. *Transplantation.* 1995;59:530–536.
12. Cox KL, Freese DK. Tacrolimus (FK506): the pros and cons of its use as an immunosuppressant in pediatric liver transplantation. *Clin Invest Med.* 1996;19:389–392.
13. Tzakis AG, Reyes J, Todo S, et al. Two-year experience with FK 506 in pediatric patients. *Transplant Proc.* 1993;25:619–621.
14. Euorpean FK506 Multicentre Liver Study Group. Randomised trial comparing tacrolimus (FK506) and cyclosporin in prevention of liver allograft rejection. *Lancet*. 1994;344:423–428.
15. Fung J, Abu-Elmagd K, Jain A, et al. A randomized trial of primary liver transplantation under immunosuppression with FK 506 vs cyclosporine. *Transplant Proc.* 1991;23:2977–2983.
16. U.S. Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. *N Engl J Med*. 1994;331:1110–1115.
17. Jain A, Mazariegos G, Kashyap R, et al. Comparative long-term evaluation of tacrolimus and cyclosporine in pediatric liver transplantation. *Transplantation.* 2000;70:617–625.
18. Hasenbein W, Albani J, Englert C, et al. Long-term evaluation of cyclosporine and tacrolimus based immunosuppression in pediatric liver transplantation. *Pediatr Transplant.* 2006;10:938–942.
19. Wayman KI, Cox KL, Esquivel CO. Neurodevelopmental outcome of young children with extrahepatic biliary atresia 1 year after liver transplantation. *J Pediatr.* 1997;131:894–898.
20. Loo RM, Ariyarajah V, Oh C, et al. Comparison between effects of cyclosporine and tacrolimus on glomerular filtration rate in pediatric post-orthotopic liver transplant patients. *Pediatr Transplant.* 2006;10:55–59.
21. Martinelli J, Habes D, Majed L, et al. Long-term outcome of liver transplantation in childhood: a study of 20-year survivors. *Am J Transplant.* 2018;18:1680–1689.
22. D'Alessandro AM, Knechtle SJ, Chin LT, et al. Liver transplantation in pediatric patients: twenty years of experience at the University of Wisconsin. *Pediatr Transplant.* 2007;11:661–670.
23. Jain A, Nalesnik M, Reyes J, et al. Posttransplant lymphoproliferative disorders in liver transplantation: a 20-year experience. *Ann Surg.* 2002;236:429–436.
24. Pinho-Apezzato ML, Tannuri U, Tannuri ACA, et al. Multiple clinical presentations of lymphoproliferative disorders in pediatric liver transplant recipients: a single-center experience. *Transplant Proc.* 2010;42:1763–1768.
25. Shapiro R, Nalesnik M, McCauley J, et al. Posttransplant lymphoproliferative disorders in adult and pediatric renal transplant patients receiving tacrolimus-based immunosuppression. *Transplantation.* 1999;68:1851–1854.
26. LaCasce AS. Post-transplant lymphoproliferative disorders. *Oncologist.* 2006;11:674–680.
27. McKaig SJ, Kelly D, Shaw L. Investigation of the effect of FK506 (tacrolimus) and cyclosporin on gingival overgrowth following paediatric liver transplantation. *Int J Paediatr Dent.* 2002;12:398–403.
28. Kelly DA. Optimal immunosuppression in teenagers. *Pediatr Transplant.* 2002;6:480–487.