Improving efficacy and reducing adverse effects of immunosuppression after liver transplantation

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CHAPTER 2

RANDOMIZED TRIAL OF CICLOSPORIN WITH 2-H MONITORING VS. TACROLIMUS WITH TROUGH MONITORING IN LIVER TRANSPLANTATION: DELTA STUDY

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J Clin Transl Hepatol. 2023;11(4):839-849

Abstract

Background & Aims
Previous trials comparing cyclosporine and tacrolimus after liver transplantation (LT) showed conflicting results. Most used trough monitoring for cyclosporine (C0), leading to less accurate dosing than with 2-hour monitoring (C2). Only one larger RCT compared C2 with tacrolimus based on trough level (T0) after LT, with similar treated biopsy-proven acute rejection (tBPAR) and graft loss , while a smaller RCT had less tBPAR with C2 compared to T0. Therefore it is still unclear which calcineurin inhibitor is preferred after LT. We aimed to demonstrate superior efficacy (tBPAR), tolerability, and safety of C2 versus T0 after first LT.

Methods
Patients after first LT were randomized to C2 or T0. tBPAR, patient- and graft survival, safety and tolerability were the main endpoints, with analysis by Fisher test, Kaplan-Meier survival analysis and log-rank test.

Results
In intention-to-treat analysis 84 patients on C2 and 85 on T0 were included. Cumulative incidence of tBPAR C2 versus T0 was 17.7% vs. 8.4% at 3 months (p=0.104), and 21.9% vs. 9.7% at 6 and 12 months (p=0.049). One-year cumulative mortality C2 vs. T0 was 15.5% vs. 5.9% (p=0.049) and graft loss 23.8% vs. 9.4% (p=0.015). Serum triglyceride and LDL-cholesterol was lower with T0 than with C2. Incidence of diarrhea in T0 vs C2 was 64% vs. 31% (p=0.001), with no other differences in safety and tolerability.

Conclusions
In the first year after LT immunosuppression with T0 leads to less tBPAR and better patient- and retransplant-free survival as compared to C2.

Introduction

Calcineurin inhibitors (CNIs) are the mainstay of immunosuppression after liver transplantation (LT). Initially, fast-release tacrolimus (Tac) was compared to the original ciclosporin (CsA) formulation, demonstrating that Tac had advantages over CsA with lower rejection rates but more adverse events (AEs) and discontinuation[1,2]. Later, microemulsion CsA, with improved pharmacological properties[3,4], led to less rejection and AEs than old CsA[5]. Several randomized controlled trials (RCTs) of (microemulsion) CsA vs. Tac in the first LT have been published[6-9], of which most reported one-year data[10-20]. In a 2016 meta-analysis, Tac with trough level monitoring (T0) compared to microemulsified CsA was associated with similar treated biopsy-proven acute rejection (tBPAR) rates, with no difference in one-year graft loss[21]. In contrast, an older meta-analysis from 2006 had shown less acute rejection and better graft- and patient survival for Tac compared to CsA after LT[22]. However, all except one of these larger studies used trough level monitoring of CsA (C0). In a smaller study Levy et al. found less rejection with C2 compared to T0, while in a larger RCT by this group in de novo LT comparing C2 vs. T0, no differences in mortality, acute rejection, or renal function were detected[17,18]. Using 2-hour CsA monitoring (C2) better reflects the area under the curve (AUC) and has been associated with less rejection and better renal function than C0[17,23,24]. This implies that it is still unclear which CNI is superior after LT. Therefore the objective of the present RCT was to demonstrate superior efficacy in terms of tBPAR, tolerability, and safety of either CsA with 2-hour monitoring or Tac with trough-level monitoring (T0) after first LT.

Methods

Study design and setting
The DELTA study was an open-label, parallel-group superiority parallel 2-arm investigator-initiated RCT in the three university medical centers of Leiden, Rotterdam, and Groningen performing LT in the Netherlands.

Patients, inclusion and exclusion criteria and randomization
All patients 18-75 years of age who underwent their first LT were included. Exclusion criteria were: combined or ABO-incompatible transplant, being not eligible to receive 10 mg/kg/day as initial dose of Neoral (CsA) (e.g., in case of severe renal insufficiency), seropositivity for human immunodeficiency virus (HIV) antibodies, urine production <200 mL within 12h after reperfusion, severe coexisting disease, unstable medical condition that could affect the study objectives, unlicensed drug or therapy administered within one month prior to study entry or instituted post-transplantation. Informed written consent was obtained prior

to transplantation. Baseline data were collected at that time and immediately before the transplantation.

Intervention

After randomization CsA (Neoral) or Tac (Prograft), comparable to standard practice, was administered within the first 48h postoperatively, based on adjusted body weight, for CsA at an initial oral dose of 10.0 mg/kg/day, and for Tac at an initial oral dose of 0.1 mg/kg/day, both in two divided doses (BID) daily on an empty stomach. The dose was adjusted to obtain the required blood drug levels daily for the first five days, then twice weekly, then weekly, and then at all visits. Target 2h (+/-15 min) blood CsA level for the first 3 months was 1000 (800-1200) µg/L, from 3 months on 800 (700-900) µg/L, while the target trough level for Tac during the first 3 months was 10 µg/L (8-15 µg/L), thereafter 5-10 µg/L, comparable to the institutional protocols. Short-term intravenous CsA or tacrolimus was allowed only if it could not be administered orally or per feeding tube. As monoclonal essays for measuring CsA C2 levels the Abbott FPIA AxSYM, Dade Behring Syva EMIT and Dade Behring Dimension were used in the three hospitals. For T0 level measurements, Abbott IMX MEIA, Dade Behring Syva EMIT, and Abbott FPIA TDz were used. The study duration was 6 months with an extension to 12 months and daily visits in the first 2 weeks, weeks 3 and 4, and at least 2, 3, 6, and 12 months.

Endpoints

The primary objective of this study was to compare the efficacy of a C2 regimen to a T0 regimen in combination with steroids and induction therapy with basiliximab (anti-CD25 therapy) in the prevention of treated biopsy-proven acute rejection (tBPAR) after de novo LT. Cumulative incidence of tBPAR at 3 months after LT was the primary endpoint, cumulative incidences of tBPAR at 6 and 12 months were secondary endpoints. Acute rejection was suspected by a rise in liver enzymes with or without clinical signs. Biopsy-proven acute cellular rejection (BPAR) was defined as acute rejection confirmed by a liver biopsy according to the Banff classification of rejection after LT, and if anti-rejection treatment was administered this was called treated BPAR (tBPAR)[25]. If histological confirmation was not possible an acute rejection could be treated according to standard protocol; these rejections together with the tBPAR cases formed the category of treated acute clinical rejection (tACR). The pathologists were blinded for treatment groups. The decision for treatment versus no treatment for rejection was left to the discretion of the transplant team.

Other secondary endpoints included chronic rejection diagnosed according to the adjusted Banff criteria[26], histological grading of tBPAR[25], retransplantation, patient survival, combinations thereof, biometrics (blood pressure and weight), biochemistry, safety and tolerability, conversion of immunosuppression, causes graft loss (by retransplantation or mortality) and long-term outcome.

Safety analysis was performed in all randomized patients. Hypertension and hyperlipidemia were defined by the updated World Health Organization (WHO) criteria[27,28].

Safety endpoints measured throughout the study included renal function, occurrence of malignancies, infections, and any adverse or serious adverse events (AEs), classified according to the Medical Dictionary for Regulatory Activities (MeDRA) classification[29]. Infections were considered clinically significant, as defined by the Centers for Disease Control (CDC)[30]. Post-transplant diabetes mellitus (PTDM), new-onset hypertension and new-onset hyperlipidemia were defined by use of medication for these conditions during but not before the study.

Sample size

The sample size calculation yielded *n* of 124 (62 per group), based on a 20% reduction in tBPAR risk of 30% vs. 10% (two-sided chi-square test), based on Levy et al. (11% tBPAR with C2 vs. 36% with T0)[17], and α=5% as the critical p-value for superiority of either drug, with a power of 1-ß=80%. To compensate for early discontinuations in the first 3 months, and between 3 and 12 months, the minimum number of included patients was 150 and 171 respectively with 1:1 randomization. This sample size was also sufficient to show equivalence between the groups, with a non-inferiority margin of 5%.

Randomization, data management and IRB approval

Randomization was performed within 24h post LT, and 1: 1 to C2 or T0, in blocks with random numbers by drawing blinded treatment allocation envelopes. Non-stratified randomization was reviewed by a Biostatistics Quality Assurance group and locked after approval. Patients who discontinued the study were excluded from the study. All data were immediately uploaded using TRIALINK software and secured on a locked server with an audit trail for all data changes. Only in case of severe renal dysfunction, prescription of mycophenolate mofetil (MMF) 500-1000 mg BID or azathioprine 50-150 mg QD was allowed. As study medication was often delayed to the second day after LT for impaired renal function, an amendment also allowing a delay in first study medication to a maximum of 48h (instead of 24h in the original protocol) postoperatively was approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) and Research Ethics Board (REB). A safety board was not required. This study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The study protocol and amendments were approved by the IRB or the REB of each participating center. The trial was registered in the Dutch Trial Registry (number NTR489 and Clinicaltrials. gov, number NCT00149994).

Statistical analysis

The trial was designed as an RCT with intention-to-treat (ITT) analysis. In addition, as defined in the protocol, per-protocol (PP) analysis was performed for at least 6 months on the allocated treatment. All subjects who were randomized and received at least one dose of study medication were included in the safety analysis. The study medication was not blinded and the initial statistical analysis was blinded. Comparisons between the two treatment groups were assessed using the Wilcoxon rank-sum test for continuous variables and the two-sided Fisher's exact test for categorical variables. To assess the comparability of blood biochemistry results, a mixed-model analysis with fixed effects was used. Time-to-event outcomes were analyzed using Kaplan-Meier survival analysis with standard error of the mean (SE) and log-rank test with hazard ratio (HR) with 95% confidence intervals (CI) for comparison, as specified in the protocol. Statistical significance was set at $p<0.05$. SPSS version 24 (SPSS Inc., Chicago IL, USA) and R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analyses. For more details on methods see the Supplementary File 1.

Results

Patients

Of 187 patients eligible for informed consent, 11 were not transplanted and were not randomized; five were transplanted but died immediately postoperatively. Thus, the safety population included 171 randomized patients. All patients underwent LT with the whole liver obtained from a deceased donor and were randomized 2002 through 2006. One patient was excluded from the ITT analysis due to an administrative problem, randomized but not transplanted at that time and one patient was excluded for protocol violation, leaving 169 patients (84 on C2 and 85 on T0) for ITT analysis (Figure 1). In total, 151 patients (69 at C2 and 82 at T0) fulfilled the predefined requirements for per-protocol analysis. Except for the etiology of acute liver failure, the patient characteristics were similar between the groups (Table 1). The drug levels for C2 and T0 are shown in the Supplementary File 1. As shown, it took five days to reach target C2, staying on target thereafter, while T0 was on target immediately, adjusted to a T0 just above 10 for the first three months, and 5-10 µg/L thereafter The use of additional immunosuppressants in cases of severe renal dysfunction did not differ between the C2 and T0 groups and during the study there was significantly more study drug conversion in the C2 group (11/84) than in the T0 group (1/85, p=0.0024, as shown in the Supplementary File 1).

Figure 1: CONSORT Patient flow chart for ITT and per-protocol analysis. ITT, intent-to-treat.

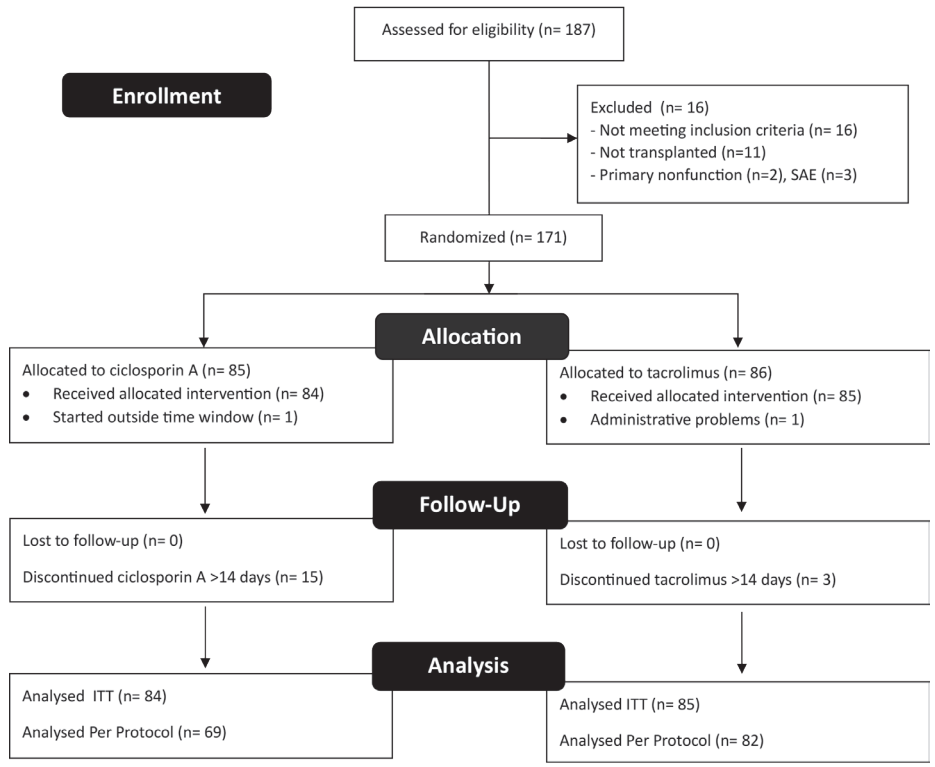


Table 1. Patient demographics and baseline characteristics of the Intention-To-Treat Population.

Treatment Allocation	Cyclosporin	Tacrolimus	p-value
n=	84	85	
Site G/L/R	18/27/39	16/29/40	0.908
Sex male	53 (63,1)	55 (64,7)	0.873
Age	48,1 yrs, ± 12,1	49,9, ± 9,9	0.287
Ethnicity			0.779
Caucasian	67 (79,8)	67 (78,8)	1.000
Afro-European	4(4,8)	6 (7,1)	0.746
Oriental/Asian	9 (10,7)	10 (11,8)	1.000
Other	4 (4,8)	2 (2,4)	0.443
Cause of underlying liver disease			
viral hepatitis (B,C,D)	14 (16,7)	17 (20)	0.692
alcoholic liver disease	14 (16,7)	18 (21,2)	0.557
hepatocellular/cholangiocarcinoma**	2 (2,4)	5 (5,9)	0.443
autoimmune liver disease***	29 (34,5)	32 (37,6)	0.749
metabolic liver disease	2 (2,4)	4 (4,7)	0.681
non-alcoholic fatty liver disease	1(1,2)	0	0.497
acute liver failure	5(6,0)	0	0.030
cryptogenic, drug induced, other*	17 (20,2)	9(10,6)	0.092
Child-Pugh score at randomization	7,64 ± 1,912	8,22 ± 2,154	0.123
MELD score at randomization	14,62 ± 7,776	15,6 ± 6,912	0.896
Cold Ischemia Time (CIT)	8h13min ±2h55min	8h33min ± 2h36min	0.438
Warm Ischemia Time	35min ±10,3min	33min ±10,9min	0.279

Values with ± are the mean ±SD; otherwise, n (%). The underlying causes of liver disease were grouped into 20 separate categories. Race was self-reported. Ad *) Other causes of liver disease in the CsA group (n=11) were cirrhosis due to cystic fibrosis, familial amyloid polyneuropathy(n=5), polycystic liver disease, Caroli syndrome (n=2), Budd-Chiari syndrome, and epithelioid hemangioendothelioma. Other causes of liver disease in the tacrolimus group (n=7) were familial amyloid polyneuropathy, Budd-Chiari syndrome (n=2), Rendu-Osler-Weber syndrome, polycystic liver disease (n=2), and vanishing bile duct syndrome without prior transplantation. Ad **) Cholangiocarcinoma in primary sclerosing cholangitis (PSC) in one as incidental finding after OLT. Ad ***) autoimmune hepatitis, primary biliary cholangitis, or PSC.

Endpoints of ITT analysis

The results of the ITT analysis of the primary and main secondary endpoints for both the raw incidence rates and for the Kaplan-Meier survival estimate are shown in Table 2.

tBPAR

The cumulative incidence of tBPAR-censored for death and re-transplantation in KM analysis within 3 months after LT was numerically but not statistically higher for C2 than for T0: 17.7% (95% CI 9.3-26.1) for C2 vs. 8.4% (95% CI 2.5-14.3) for T0 (HR 2.088 [95% CI 0.866-5.026], p=0.10). At both 6 and 12 months this cumulative incidence of tBPAR was significantly higher with C2 than with T0 (21.9% for C2 and 9.7% for T0 at both 6 and 12 months , p=0.049) (Table 2, Figure 2).

Chronic rejection

Chronic rejection occurred within 12 months in 3/84 (4%) of C2 treated patients versus 0/85 in the T0 group (Fisher exact test p=0.12, log-rank p=0.07).

Mortality

The Kaplan-Meier estimate for the cumulative incidence of mortality within 3 months was not different between C2 and T0 at 3 and 6 months, but was significantly higher at 12 months with C2 compared to T0 (15.5% vs. 5.9%, logrank p=0.049; Table 2, Figure 3).

Re-transplantation

Cumulative incidence of re-transplantation (re-LT or death-censored graft failure) was numerically more frequent in the C2 group as compared to the T0 group, but this was not a statistically significant difference (Table 2).

Re-transplantation-free survival

In the Kaplan-Meier analysis, the combined endpoint of re-transplantation or mortality within 12 months after LT was more frequent in the C2 group than in the T0 group (23.8% vs. 9.4%, p=0.015), so re-transplantation-free survival within 12 months was better with T0 than with C2. This and causes of graft loss are shown in the Supplementary File 1.

Table 2. Cumulative raw incidences (n,%), and Kaplan-Meier estimates of the cumulative incidences with hazard ratio (HR) C2 versus T0 of patients reaching endpoints at 3, 6 and 12 months after LT in the C2 group (n=84) versus those in the T0 group (n=85) (ITT analysis).

		Cumulative raw incidence			Kaplan-Meier estimate of cumulative incidence and HR			
	Group	C 2 (n=84)	T0 (n=85)	Fisher p	C2 (CI)	T2 (CI)	HR (CI)	logrank p
Endpoint		N (%)	N (%)					
tBPAR	3 mo	14 (16.7%)	7 (8.2%)	0.11	17.7% (9.3-26.1)	8.4% (2.5-14.3)	2.088 [0.866-5.026]	0.10
	6 mo	**17 (20.2%)**	**8 (8.4%)**	0.054	**21.9% (12.7-31.1)**	**9.7% (3.4-16.0)**	**2.269 [1.003-5.155]**	**0.049**
	12 mo	**17 (20.2%)**	**8 (8.4%)**	0.054	**21.9% (12.7-31.1)**	**9.7% (3.4-16.0)**	**2.275 [1.003-5.155]**	**0.049**
Mortality	3 mo	5 (6.0%)	4 (4.7%)	0.75	4.2% (0.9-11.1)	4.7% (0.2-9.2)	1.276 [0.371-4.389]	0.72
	6 mo	5 (6.0%)	5 (5.9%)	0.99	7.1% (1.6-12.6)	5.9% (0.8-11.0)	1.227 [0.398-3.787]	0.74
	12 mo	**13 (15.5%)**	**5 (5.9%)**	**0.049**	**15.5% (7.9-22.1)**	**5.9% (0.8-11.0)**	**2.704 [1.005-7.284]**	**0.049**
re-LT	3 mo	5 (6.0%)	3 (3.5%)	0.50	6.0% (0.9-11.1)	3.5% (0.0-7.4)	1.707 [0.451-6.467]	0.46
	6 mo	5 (6.0%)	3 (3.5%)	0.50	6.0% (0.9-11.1)	3.5% (0.0-7.4)	1.707 [0.451-6.467]	0.46
	12 mo	8 (9.5%)	4 (4.7%)	0.25	9.5% (3.2-15.7)	4.7% (0.2-9.2)	2.065 [0.663-6.446]	0.23
re-LT or	3 mo	9 (11.0%)	6 (7.0%)	0.43	10.7% (10.0-11.4)	7.3% (1.6-12.6)	1.549 [0.574-4.177]	0.40
mortality	6 mo	10 (12.0%)	7 (8.0%)	0.46	11.9% (5.0-18.6)	8.2% (2.3-6.1)	1.480 [0.583-3.758]	0.42
	12 mo	**20 (24.0%)**	**8 (9.0%)**	**0.01**	**23.8% (14.8-32.8)**	**9.4% (3.1-15.7)**	**2.662 [1.197-5.916]**	**0.015**
tBPAR	3 mo	23 (27.4%)	13 (15.3%)	0.06	27.4% (17.8-37.0)	15.3 (7.7-22.9)	1.858 [0.954-3.628]	0.07
or re-LT	**6 mo**	**27 (32.1%)**	**15 (17.6%)**	**0.03**	**32.1% (22.1-42.1)**	**17.6 (9.6-25.6)**	**1.930 [1.039-3.596]**	**0.04**
ormortality	**12 mo**	**33 (39.3%)**	**16 (18.8%)**	**0.004**	**39.3% (28.9-49.7)**	**18.8 (10.6-27.0)**	**2.268 [1.261-4.096]**	**0.006**

Figure 2: Cumulative incidence of tBPAR, censored for death and retransplantation. ITT with hazard ratio and 95% confidence interval (Kaplan–Meier and logrank analysis). Solid line: Tacrolimus T0. Interrupted line: Cyclosporin C2.

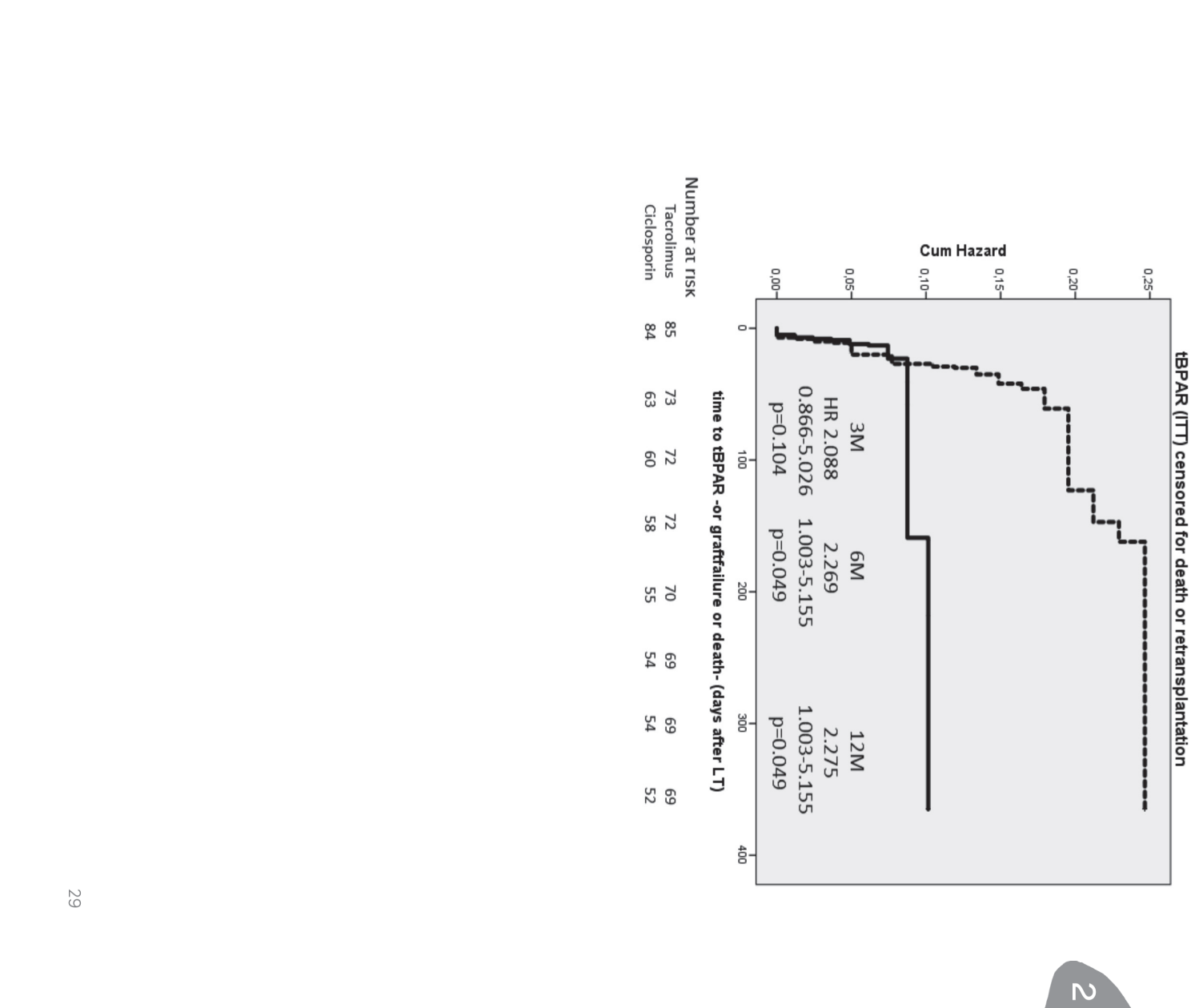
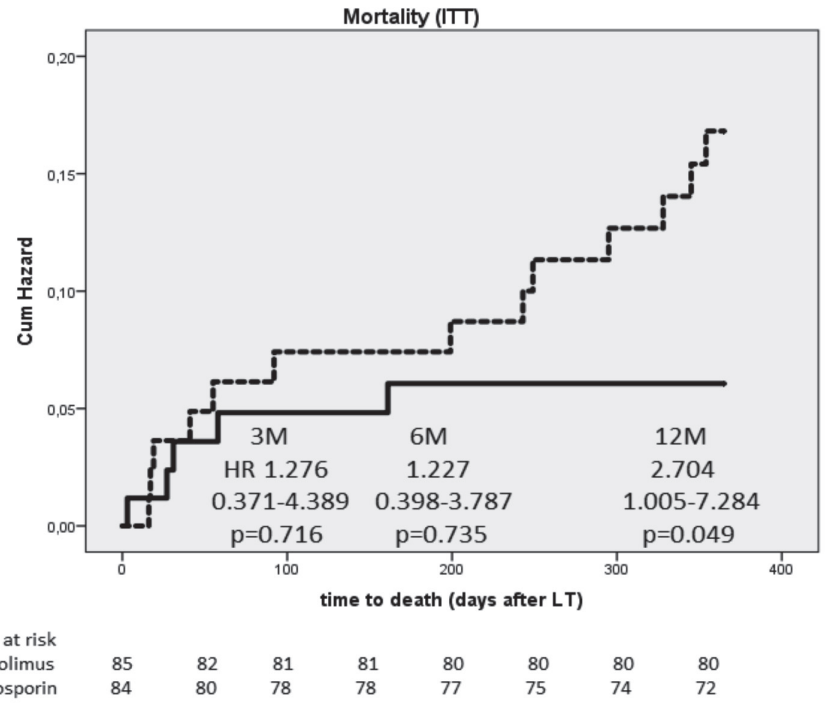


Figure 3: Cumulative incidence of mortality. ITT with hazard ratio and 95% confidence interval (Kaplan–Meier and log-rank analysis). Solid line: Tacrolimus T0. Interrupted line: Cyclosporin C2. ITT, intent-to-treat.



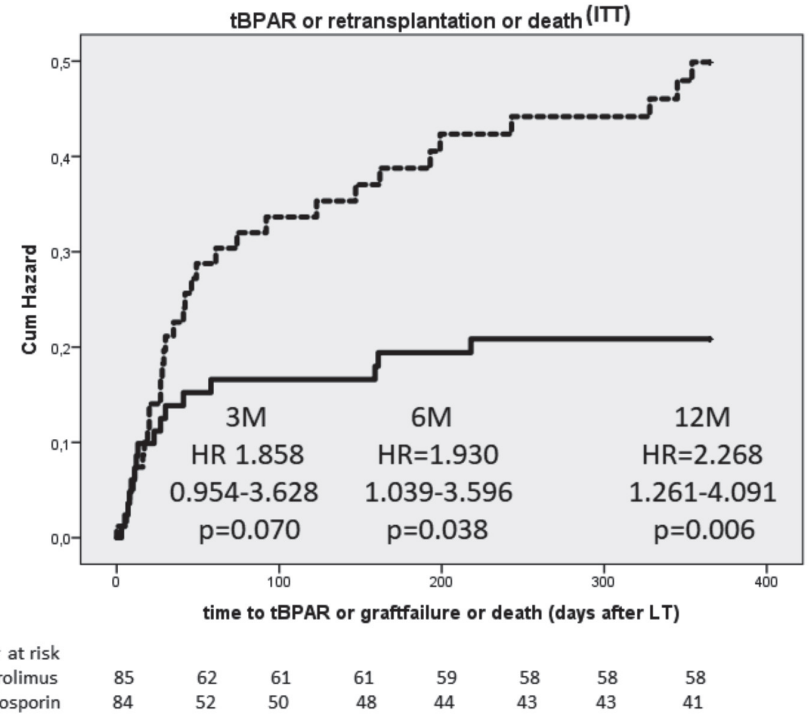
Combined endpoint of treated BPAR or re-transplantation or mortality

In the Kaplan-Meier analysis, the combined endpoint of tBPAR, re-transplantation or mortality occurred more frequently in the C2 group as compared to the T0 group, both within 6 months after LT (32.1% vs. 17.6% respectively, p=0.04) and within 12 months after LT (39.3% vs. 18.8% respectively, p=0.006; Figure 4). More secondary endpoints are shown in the Supplementary File 1.

Biometrics and biochemistry

Systolic blood pressure was higher with C2 at 6 months than at baseline (p=0.014), and diastolic blood pressure was higher than at baseline after 6 months for both T0 (p=0.002) and C2 (p=0.001), with no difference between C2 and T0. No significant intra- or between-group differences were observed throughout the study in terms of the incidence of hypertension or body weight. These data are shown in the Supplementary File 1.

Figure 4: Cumulative incidence of combined endpoint of tBPAR or retransplantation or mortality. ITT with hazard ratio and 95% confidence interval (Kaplan–Meier and log-rank analysis). Solid line: Tacrolimus T0. Interrupted line: Cyclosporin C2. ITT, intent-to-treat.



As shown in Table 3, serum triglyceride and LDL cholesterol levels were lower with T0 than with C2 (mean 1.7 ±1.0 mmol/L vs. 1.9 ± 1.2 mmol/L, p=0.03, and mean 2.3 ±1.3 mmol/L vs. 2.8 ±1.2 mmol/L, p=0.01 respectively) after 12 months. No differences were found between the groups in terms of changes in HDL cholesterol levels. Fasting glucose and serum creatinine levels did not differ between T0 and C2 at 3, 6, and 12 months. The mean calculated creatinine clearance was similar between T0 and C2 at the start (107 mL/min vs. 113 mL/min (p=0.41). Creatinine clearance at 3 months was lower for T0 than C2 (79 vs. 91 mL/min) (p=0.029), but was similar at 6 (80 mL/min vs. 88 mL/min), and 12 months of treatment (83 mL/min vs. 87 mL/min, p=0.470) in T0 vs. C2, respectively, with a similar decrease in creatinine clearance after 12 months compared to baseline (-25.9 mL/min vs -27.8 mL/min respectively, p=0.47).

Table 3. Laboratory measurements of glucose, lipids and renal function with T0 and C2 (ITT analysis)

Laboratory data per treatment group								
	Tacrolimus				Ciclosporin			
Mean	**baseline**	**3 months**	**6 months**	**12 months**	**baseline**	**3 months**	**6 months**	**12 months**
Fasting blood glucose (mmol/l)[a]	6,9(±3,6)	6,3(±2,3)	6,2(±3,5)	5,8(±2,6)	6,8(±2,7)	6,3(±2,4)	5,7(±2,2)	5,4(±1,8)
LDL-cholesterol (mmol/l)[c]	2,1(±1,2)	2,6(±1,0)	2,3(±0,8)	2,3(±0,9)	2,4(±1,3)	3,2(±1,3)	3,1(±1,3)	2,8(±1,3)
HDL-cholesterol (mmol/l[b])	1,1(±0,6)	1,5(±0,6)	1,5(±0,5)	1,3(±0,4)	1,2(±0,6)	1,3(±0,4)	1,2(±0,5)	1,2(±0,4)
Triglycerides (mmol/l)[d]	1,1(±0,6)	1,7(±1,1)	1,6(±1,0)	1,7(±1,0)	1,3(±0,7)	2,2(±1,7)	2,1(±1,5)	1,9(±1,2)
Serum creatinin[e]	84,6(±28,0)	108(±30,9)	109(±31,1)	107(±30,3)	84(±41,6)	99(±29,2)	103(±33,3)	105(±35,1)
Creatinin clearance (Cockroft Gault, ml/min)	107(±42,1)	79(±28,1)	80(±29,2)	83(±31,3)	113(±48,0)	91(±35,7)	88(±35,6)	87(±35,7)

(SD): standard deviations between brackets; a: Fasting glucose was lower at 12 months compared to baseline in both groups, p=0,004; b: HDL-cholesterol was higher in the Tac group at 3 and 6 months (p=0,005 & 0,020), but not after 1 year compared to baseline; c: LDL-cholesterol was significantly higher in the CsA group at 3 and 6 months (p=0,026 & 0,005), though not after 1 year; d: In the Tac group triglyceride level was higher at 12 months compared to baseline, p=0,005. For CsA, triglycerides were only higher at 3 and 6 months compared to baseline, p = 0,005; e: Between the two treatment groups, there are no significant differences in serum creatinin at any measured time point; f: Between treatment groups, only at 3 months a difference in creatinin clearance could be found (p=0.029). After 3 months creatinin clearance did not significantly change between 3-6 and 6-12 months.

Treatment-emergent AEs

All patients experienced one or more AE, with no differences between the two treatment arms in the total number of treatment emergent AEs or SAEs, as shown in the Supplementary File 1. Patients in the T0 group experienced more diarrhea (65%) than those in the C2 group (31%) (p<0.001). More patients with T0 than C2 experienced an infection (51% T0 vs. 49% C2, HR 0.375, 95% CI 0.144-0.979, p=0.045). The total number of infections did not differ between groups (321 vs. 342 clinically significant infections with T0 versus C2, respectively), nor was there any difference found in the site or type of pathogen. Post-hoc analysis demonstrated that more patients in the C2 group than in the T0 group experienced one of the more early infection episodes, defined as less than one month after LT (127 vs. 104, p=0.049). In contrast, the T0 group experienced more late infections, defined as between 1 and 12 months after transplantation, than the C2 group (100 vs. 63, p=0.002). Sepsis tended to be a more common cause of death with C2 (n=7) than T0 (n=2), but the difference was not significant. In patients with 3 or more months of re-transplant-free survival, treatment for (new-onset) PTDM occurred in 14/79 (17.7%) patients on T0 versus 11/75 (14.7%) patients on C2 (p=0.77); treatment for new-onset hypertension after transplantation occurred in 18/79 (22.8%) patients on T0 vs. 26/75 (34.7%) patients on C2 (p=0.15); treatment for new-onset lipidemia after transplantation occurred in 3/79 (3.8%) patients at T0 vs. 4/75 (5.3%) patients on C2 (p=0.71). Except for renal function, which was the indication for prescription, there were no significant differences in the primary or secondary endpoints between patients using or not using mycophenolate mofetil or azathioprine (not shown). More ITT and PP results and more details are shown in the Supplementary File 1.

Discussion

In this RCT, de novo Tac (T0) with trough-level monitoring and cyclosporine (CsA) with 2h monitoring (C2) after adult LT were compared. At 6 and 12 months, but not yet at 3 months, after LT, T0 was superior to C2 for preventing tBPAR. At 12 months, Tac was also superior in terms of mortality and re-transplantation-free survival. This was partially because chronic ejection only occurred with cyclosporine. The composite endpoint of BPAR, re-transplantation or mortality had a very significantly lower incidence with T0 than with C2 at 6 and 12 months after LT. A higher conversion rate was observed from C2 to T0 than vice versa, often in relation to rejection. The secondary endpoints renal function, weight, blood pressure, glucose, incidence of BPAR (treated or untreated) and tACR (with or without liver biopsy) did not differ between the two arms. After 12 months, serum triglyceride and LDL-cholesterol levels were lower with T0 than with C2, HDL-cholesterol was similar between groups. The incidence of treatment for PTDM, hyperlipidemia or hypertension did not differ between C2 and T0. Diarrhea was

twice as frequent with T0 as compared to C2 treatment, without a clear explanation; there was no additional prescription of MM in this group. More patients treated with T0 experienced an infection after the first month, which may be related to the stronger immunosuppressive effect of Tac, as indicated by the lower tBPAR rate with T0 at 6 and 12 months. However there were more infections in the first month with cyclosporine, and a non-significant trend towards more deaths for sepsis with C2. This may be related to more difficult dose adjustments with C2 than with T0, leading to over-immunosuppression in some. The incidence of other AEs and SAEs was comparable. The most recent meta-analysis of RCTs comparing de novo Tac vs. CsA after first LT found no difference in tBPAR rates and in one-year graft loss, but better one-year patient survival, less hypertension, and more PTDM[21]. Ten of these studies used trough level monitoring of CsA (C0). Using C2 monitoring has been associated with less rejection and better renal function than C0[17,24,25]. However, in the only larger previous RCT in de novo LT comparing C2 vs. T0, no differences in mortality, acute rejection, or renal function but more PTDM with T0 were detected[18]. In this RCT with C2 vs. T0 the findings were clearly different. The study also did not find a difference in PTDM, but was not designed to detect such a difference. C2 better reflects AUC than C0, therefore C2 leads to more accurate dosing[24,31]. This may explain why no differences in renal function and hypertension were found between C2 and T0 in the current study. A patient- and graft survival advantage of Tac compared to CsA was also seen in the largest RCT by O'Grady et al[11] and in previous meta-analysis, with no difference in death-censored graft survival, as in the current study[21]. The higher mortality with C2 than with T0 in the current study tended to be related to more sepsis and chronic rejection. The etiology of the liver disease was not a risk factor in this study. In a previous study, a survival advantage for CsA was explained by more deaths due to hepatitis C (HCV) recurrence with Tac[32]. However, the REFINE study, designed to demonstrate the superiority of CsA over Tac for LT in HCV cirrhosis, did not show any differences in survival or other parameters between Tac and CsA[33]. With the current highly effective HCV therapies, the influence of HCV on post-transplant graft or patient survival is even more unlikely.

This study has limitations. A limitation was that CsA and Tac were not administered based on the AUC, which leads to the most accurate dosing[34,35]. For practical reasons in the outpatient clinic and based on the existing literature and recommendations, T0 and C2 as single levels best reflecting the AUC, were used in the current study[34,35]. Another limitation is the drop-out of patients from the C2 arm, limiting power. Drop outs in the C2 arm was due to larger than expected crossover from C2 to T0 for rejection, and from re-transplantation and mortality. Obviously, this is also an indication in favour of Tac. Another limitation was that target C2 levels were not reached within 5 days in many patients, as was reported by Levy[17]. However, all patients in this study received basiliximab, protecting most patients from rejection in at least the first week. A limitation is that some patients were treated for rejection without liver biopsy; however, that did

not influence results, as they were not included in the primary endpoint of tBPAR. Some of those patients may not have had rejection. Also, some with tBPAR and only mild rejection were not treated, but this was similar for both treatment arms. Moreover, the primary endpoint of tBPAR allowed for the best comparison between treatment groups and with other studies that all used the same endpoint[20,21]. Despite the use of basiliximab, which may have reduced rejection in the first weeks after LT, differences in rejection rates and survival became apparent. Use of mycophenolate mofetil or zathioprine was allowed if needed, usually in case of severe renal dysfunction, but this was similar in frequency in both arms. Moreover, renal function did not differ between both arms. That strongly reduced the possibility that the use of these drugs in some patients and the associated dose reduction of Tac or CsA influenced the outcomes. Currently more once-daily prolonged formulation of Tac is used. However, based on a previous study, it is likely that the current results also apply to once-daily prolonged release tacrolimus[36]. The study was not powered to identify differences in secondary outcomes or adverse effects; therefore, the results of secondary endpoints must be interpreted as exploratory. Some long-term data have been added in the Supplementary File 1, but interpretation of these data is difficult because of changes in immunosuppression over time. The fact that more early infections occurred in the CsA group and more late infections occurred in the Tac group was remarkable and not mentioned in other studies, but the limitation is that this was a post-hoc analysis.

Recently, it has been shown that -in contrast to cessation, lowering Tac dosage by adding mycophenolate, everolimus or sirolimus soon after transplantation may be beneficial for short-term but not long-term renal function[37]. It has been shown that early conversion to a calcineurin-free regimen may spare renal function, but that it may lead to more rejection, and the long-term effect of such changes are yet unknown[37,38]. The current study found no differences in PTDM and lipids, except for slightly higher LDL-cholesterol and triglycerides with CsA. In a meta-analysis of existing data, Tac tends to exhibit higher diabetogenicity than CsA and sirolimus in the short-term (2-3 years), while in the long-term, sirolimus was associated with more PTDM than Tac or CsA[39]. It is also likely that CNIs increase the long-term cardiovascular risk[40]. Therefore, larger studies assessing long-term risks and comparing different maintenance regimens are warranted. While most LT centers now prefer Tac after the 2006 meta-analysis, there are still LT centers and parts of the world where CsA is widely used after LT. In a recent consensus statement of the ILTS, no preference for Tac or CsA after LT was mentioned[41]. While the most recently published meta-analysis from 2016 found no significant difference in rejection rates, and while the only large previous study with C2 vs. T0 after LT found no differences in acute rejection, mortality, and renal function, the implication of the current RCT is that Tac is to be preferred over cyclosporine even with 2h monitoring in the first year after LT de novo, because of less rejection (tBPAR), lower mortality, and better re-transplant-free survival.

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Abbreviations

AE	Adverse events
ACR	Acute clinical rejection
AUC	Area under the concentration-time curve
AZA	Azathioprine
BPAR	Biopsy proven acute rejection
C0	CsA with blood concentration pre-dose (trough level)
C2	CsA with blood concentration 2 hours after drug intake
CI	Confidence interval
CNI	Calcineurin inhibitor
CR	Chronic rejection
CsA	Ciclosporine A (microemulsion form)
GFR	Glomerular filtration rate
HCV	Hepatitis C virus
HDL	High density lipoproteins
IEC	Independent ethics board
IRB	Institutional review board
ITT	Intent-to-treat
KM	Kaplan-Meier
LDL	Low density lipoproteins
MeDRA	Medical Dictionary for Regulatory Activities
MMF	Mycophenolate mofetil
OLT	Orthotopic liver transplantation
PP	Per-protocol
PTLD	Post-transplant lymphoproliferative disorder
SAE	Serious adverse events
SCr	Serum creatinine
Tac	Tacrolimus
T0	Tac with blood concentration pre-dose (trough level)
tACR	Treated ACR
tBPAR	Treated BPAR

Supplementary Files