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Mulder, M.B.; Hoek, B. van; Berg, A.P. van den; Polak, W.G.; Alwayn, I.P.J.; Jong, K.P. de; ... ; Metselaar, H.J.

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Three-year results of renal function in liver transplant recipients on low-dose sirolimus and tacrolimus: a multicenter, randomized, controlled trial

Midas B. Mulder[1,2] | **Bart van Hoek**[3] | **Aad P. van den Berg**[4] | **Wojciech G. Polak**[5,2] | **Ian P.J. Alwayn**[6] | **Koert P. de Jong**[7] | **Brenda C.M. de Winter**[1,2] | **Elke Verhey-Hart**[8,2] | **Nicole S. Erler**[9,10] | **Caroline M. den Hoed**[8,2] | **Herold J. Metselaar**[8,2]

[1]Department of Hospital Pharmacy, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

[2]The Erasmus MC Transplant Institute, Rotterdam, The Netherlands

[3]Department of Gastroenterology and Hepatology, LUMC, Leiden University Medical Center, Leiden, The Netherlands

[4]Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[5]Department of Surgery, Division of HPB and Transplant Surgery, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

[6]Department of Surgery, LUMC, Leiden University Medical Center, Leiden, The Netherlands

[7]Department of Surgery, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[8]Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

[9]Department of Biostatistics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

[10]Department of Epidemiology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

Correspondence
Midas B. Mulder, Department of Hospital Pharmacy, Erasmus MC, University Medical Center Rotterdam, PO Box 2040, Rotterdam 3015 GD, The Netherlands. Email: m.b.mulder@erasmusmc.nl

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Abstract

The aim of this study was to investigate whether the combination of low-dose sirolimus (SRL) and low-dose extended-release tacrolimus (TAC) compared to normal-dose extended-release TAC results in a difference in the renal function and comparable rates of rejection, graft and patient survival at 36 months after transplantation. This study was an open-label, multicenter randomized, controlled trial. Patients were randomized to once-daily normal-dose extended-release TAC (control group) or once-daily combination therapy of SRL and low-dose extended-release TAC (interventional group). The primary endpoint was the cumulative incidence of chronic kidney disease (CKD) defined as grade ≥ 3 (estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2) at 36 months after transplantation. In total, 196 patients were included. CKD at 36 months was not different between the

Abbreviations: CKD, chronic kidney disease; CNIs, calcineurin inhibitors; eGFR, estimated glomerular filtration rate; FDA, Food and Drug Administration; IQR, interquartile range; ITT, intention-to-treat; LT, liver transplantation; MedDRA, Medical Dictionary for Regulatory Activities; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; NODAT, new onset diabetes after transplantation; NOTR, Dutch Organ Transplantation Registry; PP, per protocol; SAE, serious adverse event; SRL, sirolimus; TAC, tacrolimus; tBPAR, treated biopsy proven acute rejection.

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control and interventional group (50.8%, 95% CI: 39.7%–59.9%) vs. 43.7%, 95% CI: 32.8%–52.8%). Only at 6 months after transplantation, the eGFR was higher in the interventional group compared to the control group (mean eGFR 73.1 ± 15 vs. 67.6 ± 16 mL/min/1.73 m^2, $p = 0.02$) in the intention-to-treat population. No differences in the secondary endpoints and the number of serious adverse events were found between the groups. Once daily low-dose SRL combined with low-dose extended-release TAC does ultimately not provide less CKD grade ≥ 3 at 36 months compared to normal-dose extended-release TAC.

INTRODUCTION

Liver transplantation (LT) is the preferred treatment in patients with end-stage liver disease and HCC, with 1-year patient survival exceeding 80%. After LT, calcineurin inhibitors are the cornerstone of the immunosuppressive regimen, specifically tacrolimus (TAC).[1,2] The use of TAC has substantially decreased the risk of acute rejection and improved short-term outcomes.[3] However, prolonged use of TAC is associated with significant short-term and long-term toxicity, such as nephrotoxicity, diabetes mellitus, and hypertension.[4–6] Allen et al[7] and Tapirdamaz et al.[8] showed that 3 years after transplantation an overwhelming majority ($>50\%$) of LT recipients develop chronic kidney disease (CKD) defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2.

The impact of CNIs on renal function after LT resulted in a number of strategies to minimize CNI exposure. Several studies have shown that renal function can be effectively preserved by means of a delayed introduction of and reduced exposure to CNI agents in combination with a mammalian target of sirolimus (mTOR) inhibitor.[9–13] A meta-analysis by Lin et al.[14] showed that the eGFR increased by 10.2 mL/min (95% CI: 2.75–17.8) in patients using the mTOR inhibitor, everolimus, and low-dose CNI compared to normal-dose CNI at 12 months after the start of this combination.

To date, the combination of TAC and sirolimus (SRL), an mTOR inhibitor, has not been extensively studied on the long-term toxicity. Most studies evaluating the effect of SRL on renal function were small, short-term, or initially not designed for this evaluation.[15,16] Furthermore, an advantage of SRL is the fact that SRL is dosed once daily compared to the twice daily dosing regimen of everolimus. Therefore, the aim of this study was to investigate whether the combination of low-dose SRL and extended-release TAC compared to normal-dose extended-release TAC results in a difference in the renal function and comparable rates of rejection, graft survival and patient survival at 36 months after transplantation.

PATIENTS AND METHODS

Study design and participants

This study was an open-label, multicenter randomized, controlled trial. Patients were enrolled between February 2011 and March 2018 and prospectively followed for 3 years or until death. Patients were randomized between 80 and 100 days after LT to (1) once daily normal-dose extended-release TAC (control group) or (2) once daily combination therapy of low-dose SRL and low-dose extended-release TAC (interventional group) (Figure 1). The immunosuppressive therapy could be switched to local practice in cause of patient safety, medical need or preference of treating physician. In the Netherlands, TAC monotherapy is the first line of immunosuppression after LT. In case of deterioration of the kidney function TAC monotherapy is switched to mycophenolic acid (MPA) in combination with low-dose TAC. Included were adult patients, between 18 and 70 years, after a primary LT or an early (within 14 days after the first LT) retransplantation with a patent hepatic artery, closed abdominal wound and transplanted in 1 of the 3 liver transplant centers in the Netherlands. All participants gave written informed consent before any study-related activity. Main exclusion criteria were multiorgan transplantation, biopsy-proven rejection 2 weeks prior to randomization, estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2, hyperlipidemia refractory to optimal medical therapy (cholesterol >9 mmol/l and/or triglycerides >8.5 mmol/L), signs of recurrent or de novo malignancies or non-HCC malignancies within the past 5 years, known hypersensitivity to SRL and the use of MPA.

The study was performed at 3 centers in the Netherlands: Erasmus University Medical Center Rotterdam, University Medical Center Groningen and Leiden University Medical Center. The study was approved by the institutional Ethical Committees at these institutions, registered in the EudraCT database (EudraCT: 2009-017843-32) and conducted in accordance with the principles of the declaration of Helsinki.

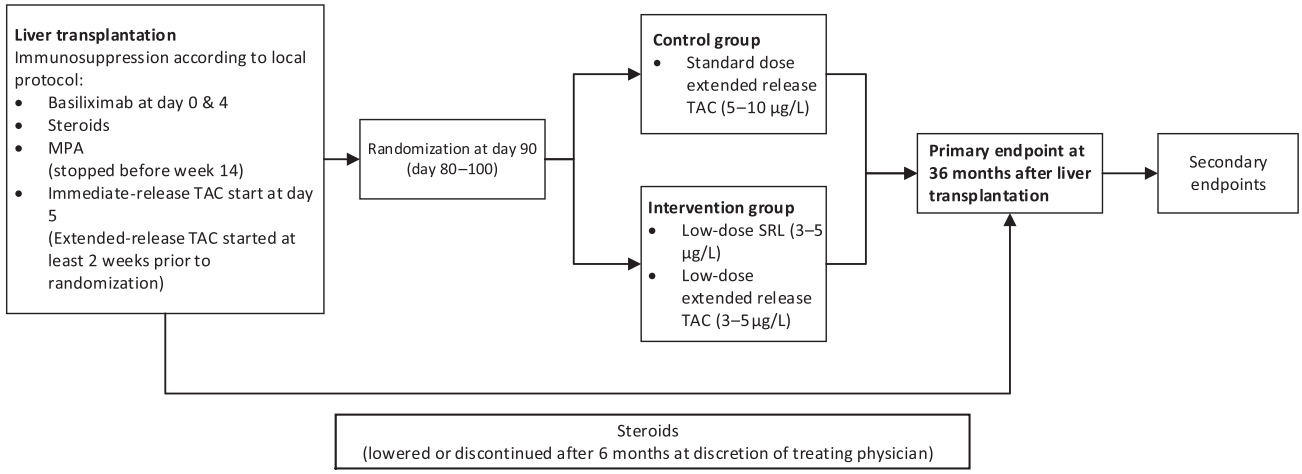

FIGURE 1 Overview of study design. Abbreviations: MPA indicates mycophenolic acid; SRL, sirolimus; TAC, tacrolimus.

Study endpoints

The primary endpoint was the cumulative incidence of CKD defined as grade ≥ 3 (eGFR <60 mL/min/1.73 m^2) at 36 months after LT. The renal function was measured by serum creatinine, and the estimated glomerular filtration rate was calculated using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation.[17] Secondary endpoints included: treated biopsy-proven acute rejection (tBPAR), retransplantation, mean eGFR, incidence of *de novo* diabetes mellitus (NODAT), incidence of and time to *de novo* or recurrent malignancy, tolerability and safety of the combination SRL and extended-release TAC.

NODAT is defined according to the definition of diabetes mellitus by the World Health Organization (ie, fasting plasma glucose value of 7.0 mmol/L measured at least on 2 different occasions or HbA1C > 65) and excludes the diagnosis of diabetes prior to LT.[18,19]

Randomization and masking

Participants were randomly assigned (1:1) to either the intervention group or the control group according to a computer-generated randomization list. Stratification was done by center, to ensure an equal distribution of both arms in the 3 participating centers. Blinding of participants and physicians was not applied.

Procedures

Participants were screened within 7 days before randomization. At the time of randomization MPA had to be discontinued. During the study, dose adjustments of extended-release TAC and SRL resulting in lower trough levels were allowed in case of severe adverse effects. Furthermore, according to common practice, in the control group higher TAC trough levels were aimed in the first 3 months after transplantation and gradually declined thereafter with a threshold of 5 ng/mL.

Control group: Participants were treated with extended-release TAC with trough levels: 5–10 μg/L and 7.5 mg prednisone. Steroids were lowered or discontinued after 180 days at the discretion of the treating physician.

Intervention group: Participants were treated with once daily combination therapy of SRL and low-dose extended-release TAC with trough levels: 3–5 μg/L for both SRL and TAC and 7.5 mg prednisone. Steroids were lowered or discontinued after 180 days at the discretion of the treating physician.

Data collection

Variables collected included recipient sociodemographic, clinical and transplantation parameters, donor details, the quality of life and fatigue severity score, serious adverse events (SAEs) and trough levels of SRL and extended-release TAC.

Statistical analysis

A sample size of 196 patients was planned for this study. On the basis of our preliminary data, the percentage of LT recipients with an eGFR <60 mL/min/1.73 m^2 in the control group at 3 years was estimated at 26.4%. The percentage of LT recipients with an eGFR <60 mL/min/1.73 m^2 in the interventional group is estimated to be 15% lower compared to the control group with an alpha (2-sided) of 0.05 and a power of 80%.

Variables were described using counts (%) for nominal and ordinal variables and mean (SD) or median (interquartile range) for the continuous variables, depending on the shape of the distribution.

The primary endpoint was evaluated with Kaplan-Meier analysis and the log-rank test. Secondary endpoints were analyzed using the Student *t* test and the Pearson χ^2 test. For all statistical tests, a 2-sided *p* value of <0.05 was considered to indicate statistical significance.

A generalized mixed-effect model was fitted to examine kidney function over the course of the study. The model additionally included covariates shown to be relevant in previous studies: visit, study group, TAC trough levels, type of donation, recipient age and sex, lab MELD, initial cold and warm ischemic time and the usage of antihypertensive drugs as well as the interaction between visit and the study group. Participant specific random intercepts were included to account for correlation among repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. Missing data were considered as missing completely at random. To visualize the estimated associations, the expected kidney function across the course of the study was calculated while fixing the values of all other covariates to the median or reference category.

Data were approached in an intention-to-treat (ITT) and per protocol (PP) analysis. Patients with protocol violations in immunosuppressive therapy, a retransplantation or death were excluded in the PP analysis. All data were collected in the Dutch Organ Transplantation Registry (NOTR) and analysis were performed using R software (version 3.6.2).[20]

RESULTS

Table 1 presents the baseline characteristics of the ITT population at randomization. A total of 196 patients were included (Figure 2) and the majority of the patients were transplanted because of HCC (67/196, 34.2%), primary sclerosing cholangitis (37/196, 19.9%) or (non) alcoholic steatohepatitis (31/196, 15.8%). At baseline, the mean eGFR in the control and interventional group was 70.2 ± 16 and 71.8 ± 15 mL/min/1.73 m^2, more patients with NODAT were included in the control group compared to the interventional group (15.3% vs. 7.1%) and more patients in the interventional group experienced tBPAR (5.1% vs. 2%).

During the 3-year follow-up a switch in immunosuppressive therapy occurred in 48.9% (48/98) of the patients in the control group and in 44.9% (44/98) of the patients in the interventional group. In the control group the main reason for the switch in immunosuppressive therapy was deterioration of the kidney function (43/48, 89.6%). The other reason for a switch was recurrence of autoimmune hepatitis (5/48, 10.4%). In the interventional group multiple reasons for switching applied. The main reason for a switch were side effects of SRL and/or deterioration of the kidney function (29/44, 65.9%). The side effects consisted of pancytopenia (7/29, 24.1%), malaise (6/29, 20.7%), skin problems (n = 5/29, 17.2%), anemia (2/29, 6.9%), edema (2/29, 6.9%), hyperlipidemia (2/29, 6.9%), liver enzyme abnormalities (2/29, 6.9%), hypertension (1/29, 3.4%), proteinuria (1/29, 3.4%), and deep vein thrombosis (1/29, 3.4%). Other reasons for a switch were preference of treating physician with another immunosuppressive agent in case of deterioration of the kidney function (8/44, 18.2%), recurrence of viral infections (5/44, 11.4%), and recurrence of autoimmune hepatitis (2/44, 4.5%).

Immunosuppression

During the study, mean trough levels for TAC and SRL were within the target range for both groups (Table 2). At 6 months post-LT, the TAC trough levels in the control group were 7.1 ($\pm$2.5) μg/L and in the interventional group 5.0 ($\pm$1.9) μg/L. At the end of the study, the TAC trough levels in the control group were 5.0 ($\pm$2.3) μg/L and in the interventional group 3.9 ($\pm$1.5) μg/L. Most LT recipients in the control group in the ITT and PP population had TAC trough levels within the target range (5–10 μg/L). Whereas most LT recipients in the interventional arm in the ITT and PP population had TAC trough levels above or under the target range (3–5 μg/L). Over the period of 3-year follow-up, TAC and SRL trough levels above the target range of LT recipients in the interventional arm of the ITT and PP analysis varied between 10% and 40%.

After 1 and 3 years, corticosteroids were used in 25.5% (25/98) and 8.2% (8/98) of the patients in the control group and 29.6% (29/98) and 10.2% (10/98) of the patients in the interventional group. During the study, several switches in the immunosuppressive therapy in both groups were performed. In the interventional group: started were MPA (27 patients), everolimus (4 patients) and azathioprine (2 patients) and discontinued were SRL (42 patients) and TAC (9 patients). In the control group: started were MPA (40 patients), everolimus (6 patients), SRL (2 patients), azathioprine (5 patients) and cyclosporine (1 patient) and discontinued was TAC (6 patients). None of these patients was switched back during the study period.

Renal function: ITT population

The cumulative incidence of eGFR <60 mL/min/1.73 m^2 at 36 months post-LT was 50.8% (95% CI: 39.7%–59.9%) and 43.7% (95% CI: 32.8%–52.8%) of the patients in the control and interventional group ($p = 0.19$, Figure 3A). At 6 months, 1 year and 2 years, no evidence was found for a significant difference in the proportion of patients with eGFR

TABLE 1 Baseline characteristics of the ITT population at randomization (90 d after transplantation)

	TAC (n = 98)	TAC + SRL (n = 98)
Recipient demographics at randomization		
Age, year (median, IQR)	57.00 (49.50–62.00)	54.50 (48.00–62.75)
Sex: male, n (%)	72 (73.5)	72 (73.5)
Body mass index, kg/m^2 (mean ± SD)	26.54 ± 4.03	25.88 ± 4.01
Ethnicity, n (%)		
Caucasian	85 (86.7)	81 (82.7)
Other[a]	8 (8.2)	12 (12.3)
Unknown	5 (5.1)	5 (5.1)
Primary disease, n (%)		
HCC	35 (35.7)	32 (32.7)
(Non)alcoholic steatohepatitis	16 (16.3)	15 (15.3)
Primary sclerosing cholangitis	21 (21.4)	16 (16.3)
Acute liver failure	5 (5.1)	10 (10.2)
Cryptogenic cirrhosis	4 (4.1)	4 (4.1)
Metabolic disease	5 (5.1)	4 (4.1)
Viral hepatitis	3 (3.1)	7 (7.1)
Other[b]	9 (9.2)	10 (10.2)
Hematology lab		
Hemoglobin, mmol/L (mean ± SD)	7.69 ± 0.89	7.56 ± 0.81
Leukocytes, 10^9/L (mean ± SD)	7.40 ± 2.71	7.17 ± 2.37
Neutrophil granulocytes, 10^9/L (mean ± SD)	5.64 (2.41)	5.32 ± 1.85
Platelets, 10^9/L (mean ± SD)	177.23 ± 67.55	189.10 ± 74.43
Prothrombin (s) (median, IQR)	13.00 (12.00–14.25)	13.00 (12.00–14.20)
Chemistry lab		
Albumin, g/L (mean ± SD)	44.01 ± 3.96	44.38 (3.83)
Bilirubin, μmol/L (median, IQR)	8.00 (6.00–12.00)	8.00 (6.00–11.00)
Creatinine, μmol/L (mean ± SD)	98.79 ± 21.37	95.33 ± 20.98
eGFR, ml/min/1.73 m^2 (mean ± SD)	70.23 ± 15.51	71.77 ± 14.86
Cholesterol total, mmol/L (mean ± SD)	4.76 ± 1.25	4.84 ± 1.11
Glucose, mmol/L (median, IQR)	7.30 (5.90–8.90)	6.95 (5.77–9.50)
HbA1c, mmol/mol (median, IQR)	38.00 (33.55–44.00)	39.00 (34.02–45.50)
HD lipoproteïn, mmol/L (mean ± SD)	1.45 ± 0.45	1.40 ± 0.53
LD lipoproteïn, mmol/L (mean ± SD)	2.81 ± 1.05	2.84 ± 0.92
Blood pressure		
Diastolic, mm HG (mean ± SD)	86.49 ± 10.72	82.28 ± 11.76
Systolic, mm HG (mean ± SD)	141.64 ± 20.82	136.67 ± 15.06
Heart rate, beats per minute (mean ± SD)	75.82 ± 11.22	77.70 ± 11.06
tBPAR, yes, n (%)	2 (2.0)	5 (5.1)
New onset diabetes after transplantation, yes, n (%)	15 (15.3)	7 (7.1)
Cholesterol medication use, yes, n (%)	5 (5.1)	2 (2.0)
Antihypertensive medication use, yes, n (%)	33 (33.7)	28 (28.9)
Mycophenolic acid use, yes, n (%)	14 (14.3)	4 (4.1)
Tacrolimus blood level, μg/L (mean ± SD)	7.9 ± 2.6	7.5 ± 2.8
Recipient demographics pretransplantation		
Lab MELD (median, IQR)	16.00 (10.00, 21.75)	17.00 (11.00, 22.00)
High urgency, yes, n (%)	7 (7.1)	11 (11.2)

TABLE 1. (*continued*)

	TAC (n = 98)	TAC + SRL (n = 98)
preexisting diabetes, yes, n (%)	16 (16.3)	26 (26.5)
Donor demographics		
Age, year (median, IQR)	53.00 (39.25–60.00)	52.50 (42.00–63.00)
Sex: male, n (%)	51 (52.0)	50 (51.0)
Type of donation, n (%)		
Donation after brain death	61 (62.2)	61 (62.2)
Donation after circulatory death	37 (37.8)	36 (36.7)
Living	—	1 (1.0)
Perioperative parameters		
Cold ischemia time (min) (mean ± SD)	417.56 ± 108.29	406.54 ± 131.05
Warm ischemia time (min) (median, IQR)	29.00 (25.00–37.00)	27.00 (24.00–38.00)

[a]Other includes Asian and Afro-American.
[b]Other includes primary biliary cirrhosis, secondary biliary cirrhosis, autoimmune cirrhosis, and polycystic liver disease.
Abbreviations: eGFR indicates estimated glomerular filtration rate; IQR, interquartile range; SRL, sirolimus; TAC, tacrolimus; tBPAR, treated biopsy proven acute rejection.

<0 mL/min/1.73 m^2 in the interventional group compared to the control group.

Figure 4A visualizes the individual kidney function measurements, the observed means per group, and the estimated group trajectories based on the linear mixed-effect model across the study period. The results of the model are shown in Supplementary Table 1 (http://links.lww.com/LVT/A1). After transplantation the eGFR was significantly improved in the interventional group compared to the control group at 6 months (mean eGFR

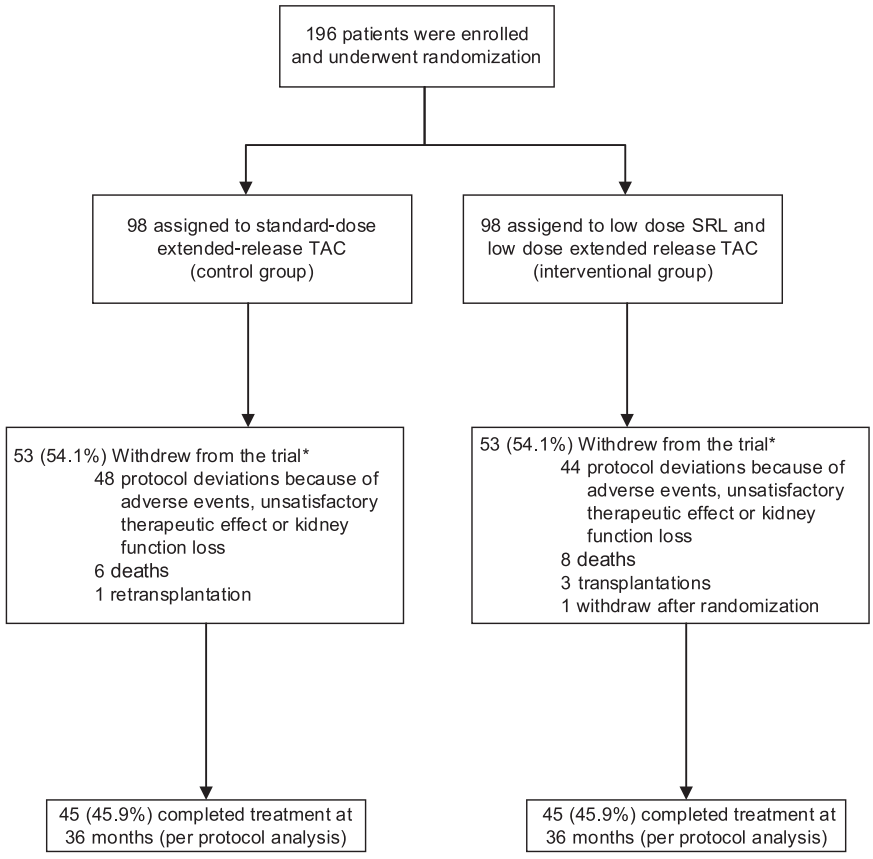

FIGURE 2 Enrollment, randomization, and follow-up. Abbreviations: LT indicates liver transplantation; SRL, sirolimus; TAC, tacrolimus. *Some LT recipients experiencing protocol deviations, died or had a retransplantation.

TABLE 2 Secondary endpoints

	Month 6, n (%)		Year 1, n (%)		Year 2, n (%)		Year 3, n (%)		Overall at end of study period, n (%)		
	TAC control (n = 98)	Low-dose TAC + SRL (n = 98)	TAC control (n = 96)	Low-dose TAC + SRL (n = 97)	TAC control (n = 95)	Low-dose TAC+SRL (n = 95)	TAC control (n = 92)	Low-dose TAC + SRL (n = 92)	TAC control (n = 92)	Low-dose TAC+SRL (n = 92)	*p* value[a]
Malignancy, yes	—	—	1 (1.1)	2 (2.1)	2 (2.2)	5 (5.4)	3 (3.3)	2 (2.3)	6 (6.5)	9 (9.8)	0.59
NODAT, yes	1 (1.0)	2 (2.0)	—	—	1 (1.1)	1 (1.1)	—	1 (1.1)	17 (18.5)	11 (11.9)	0.31
Recovery NODAT, yes	1 (1.0)	—	2 (2.1)	—	5 (5.4)	3 (3.3)	3 (3.3)	2 (2.3)	11 (11.9)	5 (5.4)	0.19
tBPAR, yes	—	1 (1.0)	2 (2.1)	—	1 (1.1)	1 (1.1)	—	1 (1.1)	5 (5.4)	8 (8.7)	0.57
Drop out during study period											
Death, yes	2 (2.1)	—	1 (1.1)	—	2 (2.2)	2 (2.1)	1 (1.1)	6 (6.5)	6 (6.5)	8 (8.7)	0.78
Retransplantation, yes	—	—	—	2 (2.1)	1 (1.1)	1 (1.1)	—	—	1 (1.1)	3 (3.3)	0.62
Withdraw after randomization, yes	—	1 (1.0)	—	—	—	—	—	—	—	1 (1.1)	—
Immunosuppressive drug trough levels											
Tacrolimus, μg/L, mean (SD)	7.1 (2.5)	5.01 (1.9)	6.5 (2.8)	5.3 (2.5)	5.6 (2.4)	4.4 (1.9)	5.0 (2.3)	3.9 (1.5)	—	—	—
Number of recipients in target range tacrolimus	70 (71.4)	39 (39.8)	58 (60.4)	37 (38.1)	59 (62.1)	40 (42.1)	46 (50)	38 (41.3)	—	—	—
Number of recipients above target range tacrolimus	11 (11.2)	43 (43.9)	10 (10.4)	42 (43.3)	3 (3.2)	28 (29.5)	5 (5.4)	20 (21.7)	—	—	—
Sirolimus, mg/L, mean (SD)	—	4.1 (1.5)	—	4.9 (1.9)	—	4.6 (1.8)		4.2 (2.1)	—	—	—
Number of recipients in target range sirolimus	—	36 (36.7)	—	22 (22.7)	—	25 (26.3)	—	24 (26.1)	—	—	—
Number of recipients above target range tacrolimus	—	23 (23.5)	—	26 (26.8)	—	18 (18.9)	—	10 (10.9)	—	—	—

[a]*P* value for testing differences in column overall at end of study period based on Pearson χ^2 test.

Abbreviations: eGFR indicates estimated glomerular filtration rate; NODAT, new onset diabetes after transplantation; SRL, sirolimus; TAC, tacrolimus; tBPAR, treated biopsy proven acute rejection.

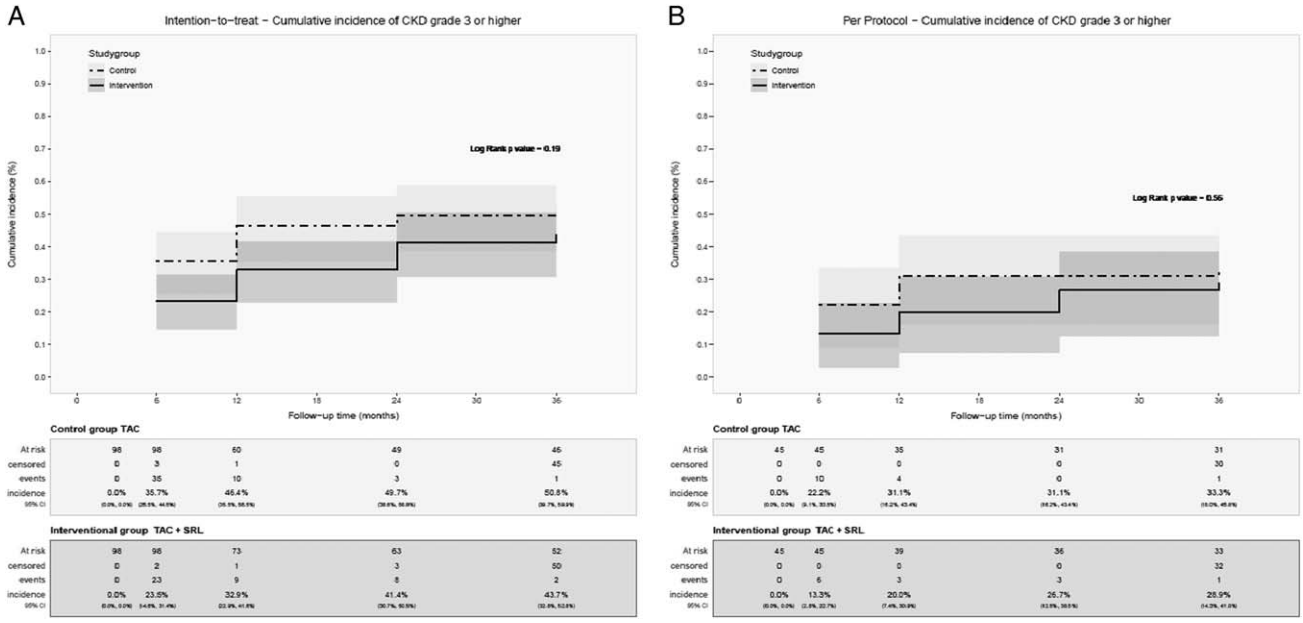

FIGURE 3 Overall cumulative incidence of chronic kidney disease grade ≥3 in the intention-to-treat and per protocol population. (A) The cumulative incidence of chronic kidney disease grade ≥3 in the intention-to-treat population. (B) The cumulative incidence of chronic kidney disease grade ≥3 in the per protocol population. Abbreviations: SRL indicates sirolimus; TAC, tacrolimus.

73.1 ± 15 vs. 67.6 ± 16 mL/min/1.73 m^2, $p = 0.02$). No evidence for a significant difference in the eGFR was shown between the interventional group and the control group at 1 year (mean eGFR 70.1 ± 17 vs. 65.9 ± 16 mL/min/1.73 m^2, $p = 0.08$), 2 years (mean eGFR 69.6 ± 17 vs. 67.4 ± 16 mL/min/1.73 m^2, $p = 0.39$) and 3 years (mean eGFR 67.7 ± 17 vs. 68.5 ± 17 mL/min/1.73 m^2, $p = 0.77$). Consistent with these results, the linear mixed-effect model did not identify significant differences in the kidney function across the study period.

Renal function: PP population

The cumulative incidence of eGFR <60 mL/min/1.73 m^2 at 36 months post-LT was 33.3% (95% CI: 18%–45.8%) and 28.9% (95% CI: 14.3%–41.0%) of the patients in the control and interventional group ($p = 0.56$, Figure 3B). At 6 months, 1 year and 2 years, no evidence was found for a significant difference in the proportion of patients with eGFR <60 mL/min/1.73 m^2 in the interventional group compared to the control group.

Figure 4B visualizes the individual kidney function measurements, the observed means per group, and the estimated group trajectories based on the linear mixed-effect model across the study period. The results of the model are shown in Supplementary Table 1, http://links.lww.com/LVT/A1. No relevant differences in the eGFR between the interventional group and the control group were found at 6 months (mean eGFR 77.4 ± 13 vs. 72.4 ± 12 mL/min/1.73 m^2, $p = 0.07$), 1 year (mean eGFR 75.5 ± 15 vs. 71.7 ± 12 mL/min/1.73 m^2, $p = 0.20$), 2 years (mean eGFR 74.5 ± 16 vs. 73.9 ± 11 mL/min/1.73 m^2, $p = 0.84$), and 3 years (mean eGFR 73.5 ± 15 vs. 73.3 ± 13 mL/min/1.73 m^2, $p = 0.96$). The linear mixed-effect model had results consistent with this.

Secondary endpoints

Table 2 shows the incidence of death, retransplantation, malignancies, NODAT, and tBPAR for the ITT population. No significant differences were demonstrated between the 2 groups.

Safety

Table 3 shows the SAEs according to the MedDRA and the severity of the SAEs during the study period. In total, 191 SAEs were reported: 50.8% (97/191) in the control group and 49.2% (94/191) in the interventional group. SAEs most frequently reported were fever (23%, 44/191), infections (17.8%, 34/191) and cholangitis and bile duct obstruction (16.2%, 31/191). More patients in the control group experienced a SAE due to fever (25.8%) compared to the interventional group (10.6%). No differences in proteinuria and cardiovascular events were found. Hepatic artery thrombosis did not occur in both study groups during the study period.

DISCUSSION

In this 36-month randomized, controlled trial we demonstrated that once daily low-dose SRL combined with low-dose extended-release TAC compared to

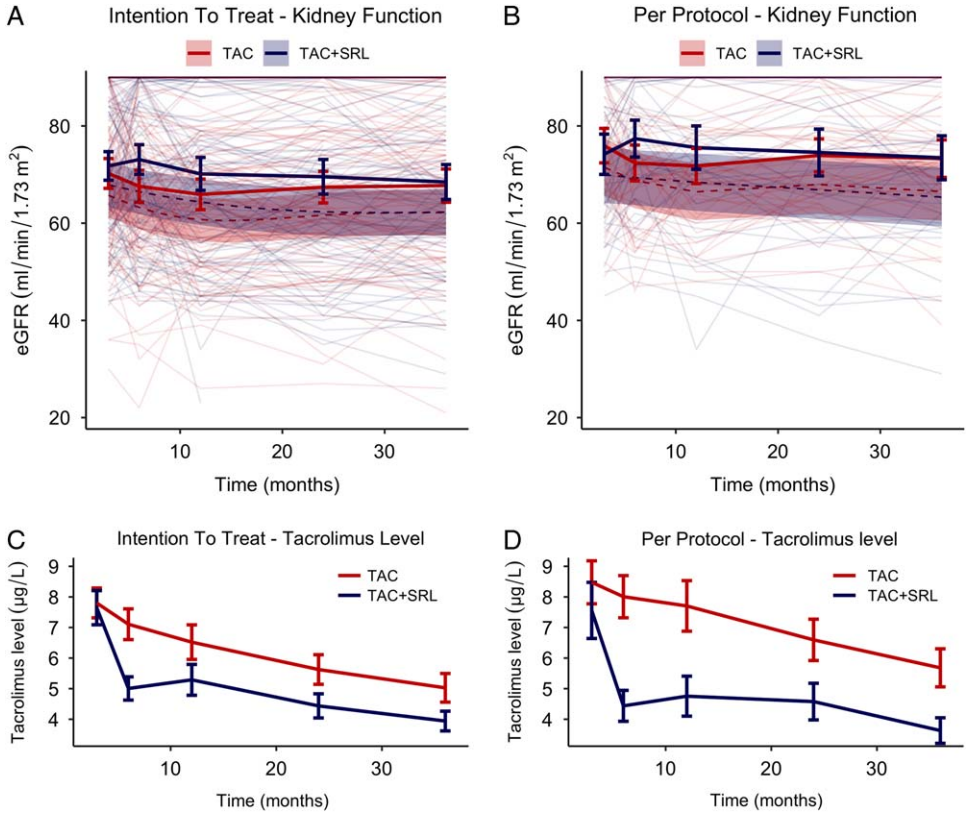

FIGURE 4 Kidney function and tacrolimus levels in the intention–to-treat and per protocol population. (A) Individual estimated glomerular filtration rate (eGFR) trajectories [chronic kidney disease (CKD)-Epidemiology Collaboration (EPI) formula] and group-wise mean with 95% CI during the course of the study of the intention-to-treat (ITT) population represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95% CI from the generalized mixed-effect model (values for the covariates: tacrolimus trough levels, type of donation, recipient age and sex, lab MELD, initial cold and warm ischemic time and the usage of antihypertensive drugs were set to the population median or reference category). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. Missing data were considered as missing completely at random. (B) Individual eGFR trajectories (CKD-EPI formula) and group-wise mean with 95% CI during the course of the study of the per protocol (PP) population represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95% CI from the generalized mixed-effect model (values for the covariates: tacrolimus trough levels, type of donation, recipient age and sex, lab MELD, initial cold and warm ischemic time and the usage of antihypertensive drugs were set to the population median or reference category). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. Missing data were considered as missing completely at random. (C) Mean tacrolimus level (µg/L) with 95% CI during the course of the study of the ITT population. (D) Mean tacrolimus level (µg/L) with 95% CI during the course of the study of the PP population.

normal-dose extended-release TAC does not result in an improvement of the kidney function in the long term. The mean eGFR in both study groups did not differ between the moment of randomization and the end of the study. Low-dose SRL combined with extended-release TAC could be a valuable strategy to minimize TAC exposure in LT recipients, with rates of rejection, graft survival and patient survival that are comparable in both arms. The combination significantly improved the renal function at 6 months after transplantation. However, this combination did ultimately not provide a better renal function at 36 months compared to normal-dose extended-release TAC.

Our results are in line with the findings of Buchholz et al,[16] who evaluated the effect of an SRL-based immunosuppressive regimen in combination with CNI minimization on renal function as a subset of the SiLVER study.[21] They showed that an SRL-based immunosuppressive regimen protects renal function in the short term, that is, for 3 months after LT.

Our findings contradict the studies of Fischer et al[13] and Sterneck et al,[22] in which the mTOR inhibitor everolimus showed a clinically relevant renal benefit at 36 months compared to normal-dose TAC. In these studies patients were randomized after 4 weeks whereas in our study patients were randomized at day 90. Furthermore, Fischer and colleagues and Sterneck and colleagues aimed for TAC trough levels between 6 and 12 µg/L, whereas we aimed for TAC trough levels between 5 and 10 µg/L. An explanation for the difference in clinical relevant renal benefit could be the height of the TAC trough levels in the control groups. The TAC trough levels at 36 months were ~1.6 µg/L higher in the control groups in the studies of Fischer and colleagues

TABLE 3 SAEs according to the MedDRA

	TAC (n = 97)		TAC + SRL (n = 94)	
	No. of patients with event	No. of events (%)	No. of patients with event	No. of events (%)
SAEs				
Death	6	6 (6.2)	8	8 (8.5)
Cholangitis and bile duct obstruction	13	18 (18.6)	8	15 (15.9)
Fever[a]	12	25 (25.8)	6	10 (10.6)
Infections[b]	19	21 (21.4)	20	22 (23.4)
Liver transplant rejection	1	1 (1.0)	4	4 (4.3)
Renal failure	2	3 (3.1)	1	2 (2.1)
Other	18	23 (23.7)	27	33 (35.1)
Severity				
Mild	2	2 (2.1)	3	3 (3.2)
Severe	42	88 (90.7)	38	80 (85.1)
Life threatening	7	7 (7.2)	10	11 (11.7)

[a]Fever with an unspecified cause and no overlap with the SAEs for cholangitis or infections.
[b]Infections includes every viral or bacterial infection that occurred during the study period excluding cholangitis.
Abbreviations: SAE indicates serious adverse event; SRL, sirolimus; TAC, tacrolimus.

and Sterneck and colleagues, compared to the control group in our study. Another explanation for the difference in clinical relevant renal benefit could be the lower difference in TAC trough levels between the study groups in our study compared to both other studies. In the interventional group of the study by Sterneck and colleagues, TAC was tapered and discontinued and the study by Fischer and colleagues aimed for TAC trough levels of 3 to 5 μg/L corresponding to our target levels.[13,22] The difference in mean TAC trough levels between the study groups in the studies by Fischer and colleagues and Sterneck and colleagues was > 3 μg/L at the end of the study period, whereas we had a difference in mean TAC trough levels between the study groups at the end of the study period of ~1 μg/L. CNI-induced nephrotoxicity is thought to be irreversible in the long term due to interstitial fibrosis and glomerular sclerosis in the kidney.[23] Several studies show a prevalence of > 50% of the LT recipients with a CKD defined as an eGFR of <60 mL/min/1.73 m^2.[7,8] Our ITT and PP analysis showed lower rates with a prevalence of 30% to 50% of the LT recipients having an eGFR of <60 mL/min/1.73 m^2. This difference could be explained by the height of the TAC trough levels. We demonstrated that in the control group further progression of the CNI-induced nephrotoxicity could be prevented for by reducing the TAC trough levels to eventually 5 μg/L after 36 months in this study. In the past, higher TAC trough levels were aimed for resulting in too much immunosuppression and progressive CNI-induced nephrotoxicity. Reducing the TAC trough levels will prevent deterioration of the kidney function for the majority of the LT recipients. Moreover, for some LT recipients a CNI-free dosing regimen might be considered in case of severe deterioration of the kidney function.

Another important finding in our study is that we did not find a higher risk of hepatic artery thrombosis or increased mortality in the SRL-based group. This is in contrast with the FDA statement.[24] Our finding also contradicts the results of a study by Teperman and colleagues that showed an increased risk of rejection in patients treated with an SRL-based regimen without CNIs compared to patients treated with a CNI-based regimen. However, we combined SRL with low-dose extended-released TAC and randomized the LT recipients after 90 days, whereas Teperman et al[12] randomized LT recipients at 4 to 12 weeks (median: 54 d). This might explain the fact that we did not find a higher risk of hepatic artery thrombosis or increased mortality in the SRL-based group. The introduction of a SRL-based regimen after 3 months might for some patients be a valuable addition to the existing immunosuppressive strategies and more patient-friendly compared to everolimus because of the once daily dosing regimen.

Interestingly, in the first year more LT recipients in the interventional arm had TAC trough levels above the target range than within the target range. This could have resulted in more TAC-induced nephrotoxicity and as a consequence the kidney function in the interventional arm might have been higher in the first year when more LT recipients had TAC trough levels within the target range. We have confidence that low TAC trough levels of 3 to 5 μg/L in combination with another immunosuppressive agent in the first year are feasible. In our LT population, in the first year after transplantation we experience the most problems with infections and bile

duct problems and very little early or late graft rejection. In addition, the study by Fischer et al[13] showed that the study group treated with everolimus and reduced TAC (3–5 μg/L) did not experience more tBPAR compared to the control TAC group (6–10 μg/L).

SRL could also be a valuable addition in the immunosuppressive strategy to increase the antibody response after SARS-CoV-2 vaccines in the current pandemic. Several studies show that MPA use is a strong predictor of a low antibody response to SARS-CoV-2 vaccines regardless the height of the MPA trough levels.[25,26] MPA inhibits both T and B lymphocytes proliferation, whereas mTOR inhibitors deplete only the T lymphocytes and indirectly the B lymphocytes resulting in higher antibody formation after vaccination.

There are several strengths to note in this study. First, this is the first randomized controlled trial testing the effect of the combination of low-dose SRL and extended-release TAC on renal function and safety. Second, the study had a long follow-up and clinicians were allowed to lower the dose of TAC in the control group, which reflects the clinical practice setting.

There is one major limitation to our study, namely the fact that almost half of the patients in both groups switched immunosuppressive therapy because of deterioration of the kidney function, side effects or preference of the treating physician. This is a significant deviation and the high number of patients switching the immunosuppressive regimen could introduce selection bias and therefore difficulties with interpreting the ITT and PP results. Overall, the results in our ITT analysis might be underestimating the actual effect of the interventional regimen. Since a large proportion in the control group switched to combination therapy, where after lower TAC levels were aimed for, less TAC-induced nephrotoxicity might been experienced resulting in higher kidney functions in the control group. The selection bias is a consequence of the use of immunosuppressive agents in a study with long-term follow-up and has been addressed in several other studies.[27,28] Patients consistent with their randomized immunosuppressive regimen at the end of the follow-up are not necessarily representative of the total study population since these patients experience less severe renal insufficiency. Although the ITT and PP analysis needs to be cautiously interpreted, our results are consistent in the ITT and PP analysis supporting the null hypothesis that a once daily SRL-based regimen does not result in a difference in the renal function in LT recipients in the long term.

In conclusion, in this study once-daily low-dose SRL combined with low-dose extended-release TAC does ultimately not provide less grade ≥ 3 chronic renal dysfunction at 36 months compared to normal-dose extended-release TAC. However, the combination improves the renal function at the short term after transplantation and could be a valuable strategy to minimize TAC exposure in LT recipients.

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CONFLICT OF INTEREST

Bart van Hoek advises for Norgine and Abacus and has received grants from Sandoz, Astellas, and Chiesi.

AUTHOR CONTRIBUTIONS

Herold J. Metselaar designed the study. Midas B. Mulder, Elke Verhey-Hart, Koert P. de Jong, Aad P. van den Berg, Bart van Hoek, Ian P.J. Alwayn, Brenda C.M. de Winter, Wojciech G. Polak, Caroline M. den Hoed, and Herold J. Metselaar were involved in the execution of the study. Midas B. Mulder, Caroline M. den Hoed, and Herold J. Metselaar had access to and verified the underlying data. Midas B. Mulder and Nicole S. Erler analyzed the data. Midas B. Mulder wrote the manuscript with input from all other authors. All authors participated in data interpretation, manuscript writing, review, and approval of the final version of the manuscript for submission.

DATA AVAILABILITY STATEMENT

Requests for access to the study data can be emailed to the corresponding author.

ORCID

Bart van Hoek http://orcid.org/0000–0001–6527–764X
Wojciech G. Polak http://orcid.org/0000–0002–3096–4893
Ian P.J. Alwayn http://orcid.org/0000–0003–0603–9849
Brenda C.M. de Winter http://orcid.org/000–0002–4452–8443
Nicole S. Erler http://orcid.org/0000–0002–9370–6832

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