Comparison of 2 immunosuppression minimization strategies in kidney transplantation: the ALLEGRO trial

Born, J.C. van den; Meziyerh, S.; Vart, P.; Bakker, S.J.L.; Berger, S.P.; Florquin, S.; ... ; Sanders, J.S.F.

Comparison of 2 Immunosuppression Minimization Strategies in Kidney Transplantation: The ALLEGRO Trial

Joost C. van den Born, MD, PhD,[1] Soufian Meziyerh, MD,[2,3,4] Priya Vart, PhD,[1,5] Stephan J.L. Bakker, MD, PhD,[1] Stefan P. Berger, MD, PhD,[1] Sandrine Florquin, MD, PhD,[6] Johan W. de Fijter, MD, PhD,[2] António W. Gomes-Neto, MD, PhD,[1] Mirza M. Idu, MD, PhD,[7] Robert A. Pol, MD, PhD,[8] Dave L. Roelen, PhD,[9] Marit S. van Sandwijk, MD, PhD,[10] Dorottya K. de Vries, MD, PhD,[3,4] Aiko P.J. de Vries, MD, PhD,[2,3,4] Frederike J. Bemelman, MD, PhD,[10] and Jan Stephan F. Sanders, MD, PhD[1]

Background. Evidence on the optimal maintenance of immunosuppressive regimen in kidney transplantation recipients is limited. **Methods.** The Amsterdam, LEiden, GROningen trial is a randomized, multicenter, investigator-driven, noninferiority, open-label trial in de novo kidney transplant recipients, in which 2 immunosuppression minimization strategies were compared with standard immunosuppression with basiliximab, corticosteroids, tacrolimus, and mycophenolic acid. In the minimization groups, either steroids were withdrawn from day 3, or tacrolimus exposure was reduced from 6 mo after transplantation. The primary endpoint was kidney transplant function at 24 mo. **Results.** A total of 295 participants were included in the intention-to-treat analysis. Noninferiority was shown for the primary endpoint; estimated glomerular filtration rate at 24 mo was 45.3 mL/min/1.73 m^2 in the early steroid withdrawal group, 49.0 mL/min/1.73 m^2 in the standard immunosuppression group, and 44.7 mL/min/1.73 m^2 in the tacrolimus minimization group. Participants in the early steroid withdrawal group were significantly more often treated for rejection ($P = 0.04$). However, in this group, the number of participants with diabetes mellitus during follow-up and total cholesterol at 24 mo were significantly lower. **Conclusions.** Tacrolimus minimization can be considered in kidney transplant recipients who do not have an increased immunological risk. Before withdrawing steroids the risk of rejection should be weighed against the potential metabolic advantages.

(*Transplantation* 2024;108: 556–566).

Received 13 March 2023. Revision received 7 July 2023.

Accepted 16 July 2023.

[1] Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands.

[2] Department of Internal Medicine, Division of Nephrology, Leiden University Medical Center, Leiden, the Netherlands.

[3] Transplant Center, Leiden University Medical Center, Leiden, the Netherlands

[4] Department of Surgery, Leiden University Medical Center, Leiden, the Netherlands.

[5] Department of Clinical Pharmacy and Pharmacology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands.

[6] Department of Pathology, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

[7] Department of Surgery, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

[8] Department of Surgery, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands.

[9] Department of Immunology, Leiden University Medical Center, Leiden, the Netherlands.

[10] Renal Transplant Unit, Department of Internal Medicine, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

Clinical Trial Notation: Steroid Free Immunosuppression or Calcineurin Inhibitor Minimization After Basiliximab Induction Therapy in Kidney Transplantation: Comparison With a Standard Quadruple Immunosuppressive Regimen (Allegro). ClinicalTrials.gov Identifier: NCT01560572.

S.P.B., J.W.d.F., A.P.J.d.V., F.J.B., and J.S.F.S. participated in the research design. J.C.v.d.B., S.M., P.V., S.J.L.B., R.A.P., M.S.v.S., A.P.J.d.V., F.J.B., and J.S.F.S. participated in writing of the article. S.J.L.B., S.P.B., S.F., M.M.I., R.A.P., D.L.R., M.S.v.S., D.K.d.V., A.P.J.d.V., F.J.B., and J.S.F.S. participated in the performance of the research. J.C.v.d.B., S.M., P.V., M.S.v.S., A.P.J.d.V., F.J.B., and J.S.F.S. participated in data analysis. All authors contributed to the discussion, and reviewed and edited the article.

The authors declare no conflicts of interest.

Astellas Pharma and Novartis provided financial support for the trial but did not have influence on protocol design, data acquisition, analysis, or reporting of the results.

Supplemental visual abstract; http://links.lww.com/TP/C881.

Correspondence: Jan Stephan F. Sanders, MD, PhD, Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands. (j.sanders@umcg.nl).

ISSN: 0041-1337/20/1082-556

DOI: 10.1097/TP.0000000000004776

INTRODUCTION

With current immunosuppressive medication, patient and kidney graft survival have significantly improved over time.[1] However, still approximately 20% of patients reach end-stage kidney disease within 5–8 y after kidney transplantation.[2,3] And, in the long term, morbidity and mortality are consistently high due to infections, malignancies, and cardiovascular events.[4,5] Steroids are known to increase the risk of diabetes, dyslipidemia, and hypertension, thereby adding to the increased cardiovascular risk of transplant recipients.[4,6] Most previous studies performed with withdrawal of steroids showed an increased risk of acute rejection,[7] although a recent study with steroid withdrawal in a low-immunological-risk population resulted in similar percentages of biopsy-proven acute rejection (BPAR) and equivalent kidney transplant function after both rabbit antithymocyte globulin or basiliximab induction.[8] In nearly all previous studies, withdrawal of steroids resulted in a reduction of posttransplant diabetes. However, most of these studies consisted of kidney transplant recipients with (very) low immunological risk and excluded deceased donors after circulatory death.

The majority of these immunosuppression minimization studies were performed with cyclosporine as calcineurin inhibitor,[9,10] whereas since the ELITE-Symphony study tacrolimus is the first choice calcineurin inhibitor.[11,12] However, in this study, tacrolimus trough levels were >6 ng/mL at 1 and 3 y after transplantation, although further reduction of trough levels might be beneficial from the perspective of renal and cardiovascular side effects.[13-15]

The Amsterdam, LEiden, GROningen (ALLEGRO) trial was designed to compare 2 different immunosuppression minimization strategies with a standard quadruple immunosuppressive regimen consisting of basiliximab induction, corticosteroids, tacrolimus, and mycophenolic acid[16] in low-to-intermediate-immunological-risk kidney transplant recipients. One immunosuppression minimization strategy was early steroid withdrawal at day 3 and the other consisted of tacrolimus minimization with reduced exposure from 6 mo after kidney transplantation. The aim of the study was to demonstrate noninferiority regarding kidney transplant function after 24 mo and reducing the side effects of immunosuppression.

MATERIALS AND METHODS

Study Design and Participants

We performed a 24-mo, prospective, randomized, open-label, multicenter study in 3 parallel groups of de novo kidney transplant recipients in which we compared standard immunosuppression with 2 different immunosuppression minimization strategies.

Three Dutch university medical centers participated—Amsterdam University Medical Center, Leiden University Medical Center, and University Medical Center Groningen. Approval from the Institutional Board of all participating institutions was obtained (UMCG Medical Ethical Committee number: 2010/171). The trial was conducted in compliance with the principles of Good Clinical Practice, the Declaration of Helsinki, and national laws and regulations. All participants provided written informed consent and could withdraw from the study at any time. The trial was registered at ClinicalTrials.gov with identifier: NCT01560572.

Patients between the ages of 18 and 80 y who received a first or second kidney transplant from a living or deceased donor (both donation after circulatory death [DCD] and donation after brain death [DBD]) were eligible to participate in this study. Patients with more than 75% current or historic panel-reactive antibodies were excluded, as were patients with diabetes mellitus type 1, patients receiving a kidney from an HLA-identical living donor, and female patients who were unwilling to use contraception for the duration of the study.

Participants were randomly assigned in a 1:1:1 ratio to 3 different treatment groups: the early steroid withdrawal group, the standard-dose tacrolimus group, and the tacrolimus minimization group.

Randomization was stratified according to allocation center. No stratification was made based upon any patient or donor characteristics. Participants underwent randomization with the use of a centralized interactive voice-response system.

Study Medication

All participants received induction treatment with basiliximab (Simulect, Novartis), 20 mg intravenously on day 0 and day 4, and methylprednisone 500 mg, 250 mg, and 125 mg on days 0, 1, and 2, respectively. As standard immunosuppression, mycophenolate sodium (MyFortic, Novartis) was prescribed at 720 mg twice daily for the first 2 wk and then tapered to 540 mg twice daily for the remainder of the study. All participants received extended-release tacrolimus (Advagraf, Astellas) once daily.

Participants in the early steroid withdrawal group received no prednisolone maintenance from day 3 after transplantation onward. Whereas the standard immunosuppression group (group 2) and the tacrolimus minimization group (group 3) received 10mg prednisolone once daily for the first 6 wk and then prednisolone was tapered to 7.5 mg once daily for the remainder of the study.

Participants in the standard immunosuppression group received extended-release tacrolimus once daily with target trough levels of 8–12 ng/mL in the first 6 wk and trough levels of 6 to 10 ng/mL for the remainder of the study.

Participants in the tacrolimus minimization group received extended-release tacrolimus once daily as well, but target trough levels were lowered to 3–5 ng/mL from 6 mo after transplantation.

All participants received *Pneumocystis jirovecii* prophylaxis for the first 6 mo with trimethoprim/sulfamethoxazole. Additionally, participants with either donor or recipient cytomegalovirus seropositivity received valganciclovir prophylaxis for 3 mo.

Outcomes

The primary endpoint of the study was kidney function at 24 mo, measured as estimated glomerular filtration rate (eGFR) calculated following the Chronic Kidney Disease Epidemiology collaboration equation formula.[17] Additionally, creatinine clearance and proteinuria were measured in 24-h urine collections (1 single value).

Secondary endpoints of the study were patient survival, treated rejection, kidney failure (defined as primary

nonfunction or death-censored graft failure), discontinuation of study medication for more than 6 wk, and treatment failure (a composite endpoint consists of death, treated rejection, kidney failure, and discontinuation of study medication).[16] Additionally, protocol biopsies were taken at 12 and 24 mo. Furthermore, adverse events (AEs), serious AEs (SAEs), cardiovascular risk factors (blood pressure, lipid profile, and diabetes), and bone densitometry data were analyzed as secondary endpoints. Bone densitometry was performed at 2 wk and 12 mo after transplantation. Cytomegalovirus, EBV, and BK virus infection were defined as viremia with measurable viral load. A fasting glucose and an oral glucose tolerance test (OGTT) were performed 2 wk, 12 mo, and 24 mo after transplantation. Additionally, during follow-up glycated hemoglobin (HbA1c) was measured multiple times. Diabetes was defined following the American Diabetes Association criteria, for example, a fasting glucose was ≥126 mg/dL, and/or 2-h blood glucose ≥200 mg/dL with OGTT, and/or HbA1c ≥48 mmol/mol (6.5%). Additionally, when (post-transplant) diabetes was reported as AE, or OGTT was not performed because the participant already received treatment for diabetes, these participants were also considered as having diabetes.

Kidney Transplant Biopsies

Kidney transplant biopsies were performed at 12 and 24 mo after transplantation per study protocol. Additionally, indication biopsies were performed as deemed indicated by treating physicians.

Histochemical stainings were performed to assess morphology (HE, Silver, and PAS staining). Additionally, immunohistochemical staining was performed for SV40 large T antigen (Merck, Millipore, Amsterdam, NL) and immunofluorescent staining of frozen sections for C4d (BioRad, Hercules, CA).

All kidney biopsy specimens were analyzed by a single specialized nephropathologist in a blinded fashion and scored subsequently following the updated 2019 Banff classification.[18] Biopsies with <7 glomeruli or without an artery, were considered inadequate and excluded from analysis. In the case of more than 1 kidney biopsy on indication, the highest grade of rejection is presented. In the case of more than 1 biopsy with the same grade of rejection, chronological selection was applied. Data on rejection treatment were combined with BPAR to generate the variable-treated BPAR.

Donor-specific Antibodies After Transplantation

Serum samples of participants were analyzed for HLA antibodies after 24 mo of follow-up, using the Luminex-based single antigen bead technology. Positivity of HLA antibodies was defined according to the cutoff as proposed by Wisse et al,[19] and in accordance to instructions by the manufacturer. Donor-specific antibodies (DSAs) at 24 mo were determined by combining data on HLA antibodies and donor HLA typing. With available data for DSA at baseline, de novo DSA was evaluated.

Safety

All AEs were recorded and monitored. A data safety monitoring board investigated the rate of rejections and SAEs after 75 and 150 participants had been included in the study. The data safety monitoring board had the right to terminate the study if rejection rate was 30% or higher.

Statistical Analysis

The primary endpoint is analyzed by noninferiority analysis showing mean and confidence intervals (CIs). To investigate the noninferiority of intervention arm versus standard of care, the mean difference in eGFR at 24 mo together with 90% CIs was generated to ensure testing of a 1-sided hypothesis test with type I error of 0.05. The sample size was calculated with a power of 80% with a significance of 5%. Noninferiority was defined as a difference in eGFR of 10 mL/min/1.73 m^2 or less compared with the standard immunosuppression group. The SD for eGFR was estimated at 25 mL/min/1.73 m^2 based on earlier results. This resulted in a group size of 75 participants per group. Assuming a dropout of around 20%, 100 patients were included in each treatment group.

Statistical analyses were performed with the Statistical Package for Social Sciences (version 28, SPSS Statistics, IBM Corporation, Armonk, NY). Graphs were made in GraphPad Prism (GraphPad Software, La Jolla, CA). Distributions of variables were visualized with histograms and Q-Q plots. Normally distributed data are presented as mean and SD. Skewed data are presented as median and interquartile range.

In a sensitivity analysis, we repeated our analysis after imputing missing eGFR values at 24 mo. To impute missing values, we performed multivariate imputation with chained equation using variables age, sex, race, HLA-mismatch, donor type (living, DBD, or DCD), and dialysis status. Data from available cases were used, and each variable with a missing value on serum creatinine was imputed using a regression model conditional on all of the other variables specified in the imputation model. In total, 5 imputed datasets were created with 50 iterations. Pooled estimates were used for analysis. In case of graft failure, an eGFR of 0 mL/min/1.73 m^2 was imputed from the time of the event of graft failure. Linear regression was applied for the analysis of imputed eGFR.

Follow-up time was defined as the period from the date of transplantation until the date of event or the end of follow-up. For longitudinal data, Kaplan–Meier plots and log-rank tests were applied.

RESULTS

Participants and Study Medication

From June 2011 to August 2014, a total of 306 participants underwent randomization of which 295 participants were included in the intention-to-treat analysis, with 98 participants in the early steroid withdrawal group, 100 participants in the standard immunosuppression group, and 97 participants in the tacrolimus minimization group, respectively. In total, 219 participants (74.2%) completed the 24-mo visit (Figure 1). Baseline characteristics per treatment group are shown in Table 1. In terms of demographic characteristics, underlying kidney disease, comorbidity, and donor and surgical characteristics, the different treatment groups were comparable. The majority of tacrolimus

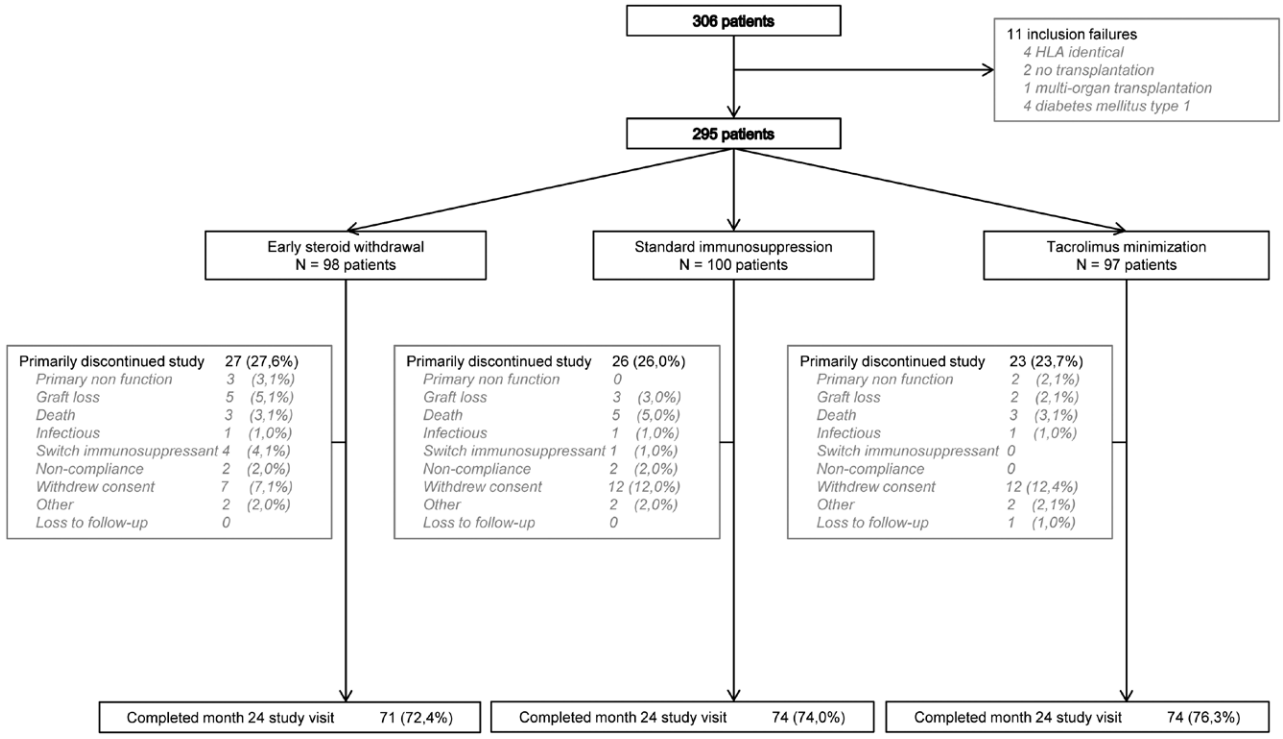

FIGURE 1. Flowchart of the enrolled patients. In total, 295 participants underwent a kidney transplantation, were randomized, and were included in the intention-to-treat (ITT) population. A total of 219 participants (74.2%) completed the month 24 visit.

trough levels were within the predefined boundaries (**Figure S1, SDC,** http://links.lww.com/TP/C869).

Efficacy—Primary Endpoint

The primary endpoint in the intention-to-treat population, kidney function as measured by eGFR at 24 mo, was 45.3 mL/min/1.73 m^2 (CI, 40.2–50.3) in the early steroid withdrawal group, 49.0 mL/min/1.73 m^2 (CI, 44.9–53.1) in the standard immunosuppression group, and 44.7 mL/min/1.73 m^2 (CI, 40.0–49.4) in the tacrolimus minimization group. These findings are consistent with noninferiority of early steroid withdrawal or tacrolimus minimization compared with standard immunosuppression with the predefined noninferiority margin of 10 mL/min/1.73 m^2 (Figure 2A). The difference between the early steroid withdrawal group and the standard immunosuppression group was −3.74 mL/min/1.73 m^2 (90% CI, −9.11 to 1.63), and −4.33 mL/min/1.73 m^2 (90% CI, −9.51 to 0.85) for the tacrolimus minimization versus the standard immunosuppression group. As sensitivity analysis, the primary endpoint analysis was repeated in the per-protocol population, and in the intention-to-treat population after imputing missing eGFR values at 24 mo (**Table S1, SDC,** http://links.lww.com/TP/C869). In the per-protocol population, no differences in eGFR after 24 mo were observed. In pooled analysis, the difference between the early steroid withdrawal group and the standard immunosuppression group was −3.76 mL/min/1.73 m^2 (90% CI, −9.25 to 1.73), and −4.80 mL/min/1.73 m^2 (90% CI, −9.98 to 0.37) for the tacrolimus minimization versus the standard immunosuppression group (**Table S1, SDC,** http://links.lww.com/TP/C869).

The course of eGFR over time in the 3 different treatment groups is shown in Figure 1B. Creatinine clearance was not different between the 3 treatment groups. (P = 0.73). Proteinuria was low and did not differ between treatment groups (P = 0.51; **Table S1, SDC,** http://links.lww.com/TP/C869).

Secondary Endpoints

In the early steroid withdrawal group, there were significantly more treated rejections compared with the other groups. Twenty-three participants (23.5%) in the early steroid withdrawal group received treatment for acute rejection versus 14 participants (14.0%) in the standard immunosuppression group, and 11 participants (11.3%) in the tacrolimus minimization group (Figure 3; log-rank test; P = 0.04).

Data on overall survival, graft survival, and death-censored graft survival at 1 and 2 y are provided in Table 2. Participant overall survival, interruption of study medication for more than 6 wk, kidney failure, and the predefined composite endpoint of treatment failure were not significantly different between the 3 treatment groups (**Figure S2A–D, SDC,** http://links.lww.com/TP/C869).

As a post hoc analysis, we analyzed delayed graft function for the different treatment groups, stratified by donor type (living, DBD, or DCD). No differences were observed (**Table S2, SDC,** http://links.lww.com/TP/C869). An additional post hoc analysis showed primary endpoint for different treatment groups, stratified by treated rejection or the occurrence of delayed graft function. No significant differences were shown. Primary endpoint was significantly different in participants with treated rejection (35.4 mL/min/1.73 m^2) compared with participants without treated rejection (48.1 mL/min/1.73 m^2; P = 0.001) (**Table S3, SDC,** http://links.lww.com/TP/C869).

TABLE 1.

Baseline characteristics

	Early steroid withdrawal	Standard immunosuppression	Tacrolimus minimization
n	98	100	97
Patient characteristics			
Mean age (y)	54.7 (14.5)	56.9 (12.1)	57.7 (13.6)
Male, n (%)	67 (68.4)	72 (72.0)	61 (62.9)
Mean BMI (SD) (kg/m^2)	26.5 (4.2)	26.3 (4.5)	27.2 (4.6)
Mean BSA (SD), m^2	1.94 (0.20)	1.96 (0.19)	1.97 (0.19)
Mean systolic blood pressure (SD), mmHg	141 (24)	142 (17)	140 (22)
Mean diastolic blood pressure (SD), mmHg	84 (18)	81 (14)	80 (12)
Race, n (%)			
Caucasian	81 (82.7)	82 (82.0)	79 (81.4)
Asian	3 (3.1)	5 (5.0)	5 (5.2)
Black	3 (3.1)	4 (4.0)	5 (5.2)
Other	11 (11.2)	9 (9.0)	8 (8.2)
Smoking, n (%)			
None	50 (51.0)	52 (52.0)	48 (49.5)
Current	14 (14.3)	18 (18.0)	16 (16.5)
Past	34 (34.7)	30 (30.0)	33 (34.0)
EBV-status IgG positive, n (%)	38 (82.6)	41 (80.4)	36 (78.2)
CMV-status IgG positive, n (%)	48 (49.0)	43 (43.0)	52 (54.2)
High-risk CMV-status (donor +/recipient –), n (%)	21 (21.6)	18 (18.2)	19 (19.8)
Cumulative HLA A/B/DR mismatch, n (%)			
<2	12 (12.2)	9 (9.0)	7 (7.2)
2–4	65 (66.3)	65 (65.0)	69 (71.1)
>4	21 (21.4)	26 (26.0)	21 (21.6)
Panel-reactive antibodies, n (%)			
0	41 (64.1)	42 (63.6)	37 (58.7)
≥5%	9 (14.1)	10 (15.2)	15 (23.8)
≥20%	4 (6.3)	3 (4.5)	6 (9.5)
Presence of DSAs, n (%)	1 (1.0)	1 (1.0)	2 (2.1)
Kidney disease characteristics			
Primary diagnosis, n (%)			
Diabetes mellitus type 2	8 (9.6)	10 (11.5)	7 (8.0)
Hypertension	22 (26.5)	18 (20.7)	25 (28.7)
Glomerulonephritis	8 (9.6)	17 (19.5)	12 (13.8)
ADPKD	20 (24.1)	20 (23.0)	16 (18.4)
FSGS	2 (2.4)	3 (3.4)	2 (2.3)
Other	23 (27.7)	19 (21.8)	25 (28.7)
Cardiovascular risk factors, n (%)			
Hypertension	81 (82.7)	77 (77.0)	76 (78.4)
Diabetes mellitus type 2	14 (14.3)	17 (17.0)	20 (20.6)
Hypercholesterolemia	24 (24.5)	24 (24.0)	25 (25.8)
Dialysis, n (%)			
Hemodialysis	36 (36.7)	38 (38.0)	46 (47.4)
Peritoneal dialysis	20 (20.4)	20 (20.0)	20 (20.6)
Both	15 (15.3)	16 (16.0)	12 (12.4)
None (preemptive transplantation)	27 (27.6)	26 (26.0)	19 (19.6)
Donor characteristics			
Mean age of the donor (SD) (y)	54.1 (14.4)	53.3 (14.5)	56.7 (11.5)
Male donor, n (%)	46 (46.9)	64 (64.0)	40 (41.2)
Living donor, n (%)	35 (35.7)	47 (47.0)	42 (43.3)
Deceased donor, n (%)	63 (64.3)	53 (53.0)	55 (56.7)
DBD, n (%)	25 (39.7)	13 (24.5)	25 (45.5)
DCD, n (%)	38 (60.3)	40 (75.5)	30 (54.5)
Surgical characteristics			
First or second kidney transplant, n (%)			
First	94 (95.9)	95 (95.0)	91 (93.8)

Continued next page

TABLE 1. (Continued)

	Early steroid withdrawal	Standard immunosuppression	Tacrolimus minimization
Second	4 (4.1)	5 (5.0)	6 (6.2)
Macroscopic atherosclerosis, n (%)			
Non/mild	73 (76.0)	77 (80.2)	73 (77.7)
Moderate	13 (11.5)	13 (13.5)	14 (14.9)
Severe	12 (12.5)	6 (6.3)	7 (7.4)
Mean cold ischemia time (SD) (h)			
Deceased donor	13.8 (4.7)	13.7 (3.8)	15.3 (5.0)
Living donor	2.6 (0.5)	2.7 (0.5)	2.8 (0.6)
Mean second warm ischemia time (SD) (min)	38.0 (10.8)	36.6 (11.7)	37.1 (10.5)
Perioperative complications, n (%)	6 (6.1)	9 (9.0)	5 (5.2)

Baseline characteristics showing patient characteristics, kidney disease, and donor and surgical characteristics. Perioperative complications included bleeding, reperfusion abnormalities, and the need to revise the anastomosis.
DBD, donation after brain death; DCD, donation after circulatory death; DSA, donor-specific antibody.

Safety

AEs occurred in nearly all participants. No significant differences were observed in AE or SAE between the different groups (Table 3). A more extensive and detailed table with all (S)AEs is shown in the supplements (**Table S4, SDC,** http://links.lww.com/TP/C869).

Kidney Biopsies on Indication—BPAR

A total of 174 kidney transplant biopsies in 111 participants were performed on indication. In total 116 biopsies in 89 participants were classified as adequate. There was no difference in BPAR between the different treatment groups. T-cell mediated rejection (TCMR) occurred in 25 participants overall: 13 participants (13.3%) in the early steroid withdrawal group, 6 participants (6.0%) in the standard treatment group, and 6 participants (6.2%) in the tacrolimus minimization group (P = 0.11; Table 4). Antibody-mediated rejection (AMR) or mixed AMR/TCMR was diagnosed in 7 participants and was not different among treatment groups (P = 0.93). Treated BPAR was not significantly different between the 3 treatment groups. Fourteen participants (14.3%) in the early steroid withdrawal group received treatment for BPAR versus 6 participants (6.0%) in the standard immunosuppression group, and 8 participants (8.2%) in the tacrolimus minimization group (P = 0.12).

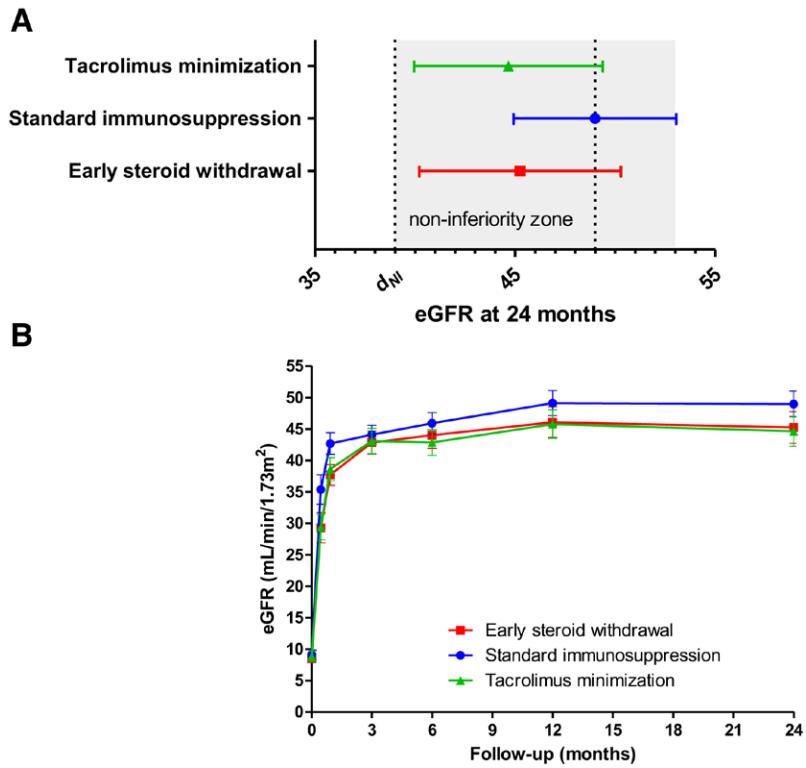

FIGURE 2. A, Primary endpoint, showing mean estimated glomerular filtration rate (eGFR, CKD-EPI 2009) with 95% confidence interval (CI) at 24 mo of follow-up. The left dashed line shows the predefined noninferiority margin (d_{NI}) of 10 mL/min/1.73 m^2 lower than the standard immunosuppression group. The light-gray area is the noninferiority zone. B, eGFR during follow-up, according to the 3 different treatment groups. Data are shown as mean ± SEM. In case of graft failure, an eGFR of 0 mL/min/1.73 m^2 was imputed.

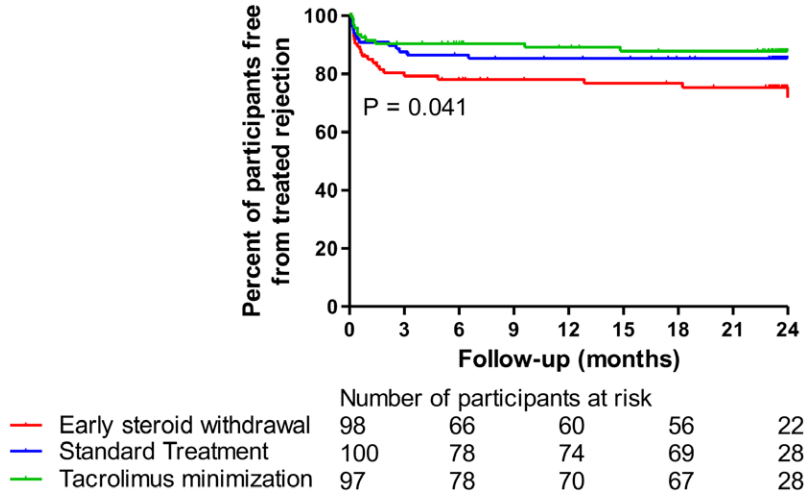

FIGURE 3. Kaplan–Meier plot for secondary endpoint, showing percentage of participants free from treated rejection, with significant difference among groups in treated rejection (log-rank test; $P = 0.04$).

Protocol Kidney Biopsies at 12 and 24 mo

In total, 142 protocol biopsies were performed at 12 mo. After exclusion of inadequate kidney biopsies, 118 biopsies of 295 participants (40%) were analyzed. Ninety-nine protocol biopsies were performed at 24 mo and 74 representative biopsies of 295 participants (25%) were included in the analysis (**Table S5, SDC,** http://links.lww.com/TP/C869). Inflammation within areas of interstitial fibrosis and tubular atrophyat 12 mo was significantly different among the 3 treatment groups. Scores were highest in the tacrolimus minimization group ($P = 0.04$). For other markers of graft fibrosis, no significant differences between treatment groups were found. Paired analysis of protocol biopsies did not change the results (data not shown). Post hoc analysis for subclinical rejection in 12 and 24 mo protocol kidney biopsies revealed no significant differences in the 3 treatment groups (**Table S6, SDC,** http://links.lww.com/TP/C869).

De Novo DSAs After Transplantation

The presence of de novo DSA at 24 mo did not differ between the 3 groups. In sera of 18 participants, de novo DSA was present at 24 mo; 8 participants (8.2%) in the early steroid withdrawal group, 5 participants (5.0%) in the standard treatment group, and 5 participants (5.1%) in the tacrolimus minimization group ($P = 0.58$). DSA at 24 mo did not differ between participants with or without TCMR.

Cardiovascular and Metabolic Outcomes and Bone Densitometry

More participants had diabetes mellitus after 24 mo of follow-up in the treatment groups containing steroids (Table 5; $P = 0.04$). The mean difference in glycated hemoglobin (HbA1c) in all participants (diabetic and nondiabetic) was significantly lower over time in the early steroid withdrawal group and tacrolimus minimization group compared with the standard immunosuppression group ($P = 0.003$). More detailed results of diabetes over time are given in **Table S7 (SDC,** http://links.lww.com/TP/C869). Also, cholesterol levels were significantly different between the 3 treatment groups at 24 mo, with a total cholesterol of 4.66 mmol/L in the early steroid group, versus 5.01 mmol/L in the standard immunosuppression group, and 5.34 mmol/L in the tacrolimus minimization group ($P = 0.03$). The use of lipid-lowering drugs after 24 mo did not differ significantly between the groups ($P = 0.26$). Differences in bone densitometry were not statistically significant between the treatment groups, although there was a tendency with a more reduced bone mass in the steroid groups ($P = 0.07$ for T-score of lumbar spine). The occurrence of osteoporosis after 12 mo did not differ between the 3 treatment groups ($P = 0.89$).

TABLE 2.

Overall survival and graft survival at 1 and 2 y

	Early steroid withdrawal	Standard immunosuppression	Tacrolimus minimization	*P*
After 1 y of follow-up				
Overall survival, n (%)	96 (98.0)	97 (97.0)	95 (97.9)	0.88
Graft survival, n (%)	89 (90.8)	94 (94.0)	91 (93.8)	0.62
Death-censored graft survival, n (%)	89 (92.7)	94 (96.9)	91 (95.8)	0.37
After 2 y of follow-up				
Overall survival, n (%)	95 (96.9)	95 (95.0)	94 (96.9)	0.71
Graft survival, n (%)	87 (88.8)	92 (92.0)	90 (92.8)	0.58
Death-censored graft survival, n (%)	87 (91.6)	92 (96.8)	90 (95.7)	0.23

TABLE 3.

Adverse events and serious adverse events

	Early steroid withdrawal	Standard immunosuppression	Tacrolimus minimization	*P*
Number of participants experiencing at least 1 event during the follow-up	98	100	97	
AEs, n (%)				
Any AE	98 (100.0)	99 (99.0)	96 (99.0)	0.61
Blood or lymphatic	51 (52.0)	44 (44.0)	44 (45.4)	0.48
Anemia	45 (45.9)	37 (37.0)	37 (38.1)	0.38
Leukopenia	6 (6.1)	5 (5.0)	4 (4.1)	0.82
Thrombocytopenia	10 (10.2)	9 (9.0)	6 (6.2)	0.59
Cardiac	17 (17.3)	24 (24.0)	24 (24.7)	0.39
Gastrointestinal	77 (78.6)	76 (76.0)	68 (70.1)	0.38
Infection of infestation	72 (73.5)	78 (78.0)	79 (81.4)	0.41
Pulmonary infection	6 (6.1)	13 (13.0)	10 (10.0)	0.26
Urinary tract infection	34 (34.7)	31 (31.0)	43 (44.3)	0.14
Viral infection	46 (46.9)	49 (49.0)	47 (48.5)	0.96
CMV infection	16 (16.3)	20 (20.0)	17 (17.5)	0.79
EBV infection	5 (5.1)	11 (11.0)	9 (9.3)	0.31
BK virus infection	18 (18.4)	19 (19.0)	22 (22.7)	0.72
Injury, poisoning of procedural complication	43 (43.9)	56 (56.0)	45 (46.4)	0.20
Complication of transplanted kidney	14 (14.3)	20 (20.0)	10 (10.0)	0.16
Wound complication	18 (18.4)	24 (24.0)	21 (21.6)	0.62
SAEs, n (%)				
Any SAEs	52 (53.1)	58 (58.0)	59 (60.8)	0.54
Infection	23 (23.5)	30 (30.0)	33 (34.0)	0.26
Pulmonary infection	1 (1.0)	6 (6.0)	7 (7.2)	0.10
Urinary tract infection	11 (11.2)	8 (8.0)	17 (17.5)	0.12
Viral infection	7 (7.1)	7 (7.0)	5 (5.2)	0.82
CMV infection	3 (3.1)	2 (2.0)	2 (2.1)	0.86
BK virus infection	1 (1.0)	2 (2.0)	0 (0.0)	0.38
Cancer	5 (5.1)	8 (8.0)	5 (5.2)	0.62
Kaposi's sarcoma	0	1	0	
Basal-cell carcinoma of the skin	2	2	0	
Squamous-cell carcinoma of the skin	0	1	2	
Lung	0	1	1	
Prostate	1	1	1	
Multiple myeloma	0	1	0	
Leukemia	1	0	0	
Renal cell carcinoma	0	1	0	
Breast	1	0	0	
Ovarium	0	0	1	

No significant differences in AEs were observed between the 3 treatment groups. Differences between groups were analyzed with χ^2 test.
AE, adverse event; SAE, serious adverse event.

DISCUSSION

This study shows that minimization of immunosuppression, either with early steroid withdrawal at day 3, or with lower tacrolimus trough levels from 6 mo after transplantation, is noninferior to standard immunosuppression for kidney transplant function at 2 y after kidney transplantation. This is in accordance with the existing literature on steroid-free immunosuppression after kidney transplantation.[8,20-23]

The ALLEGRO study is the first randomized clinical trial comparing 2 different immunosuppression minimization strategies with a currently prevailing quadruple standard regimen containing basiliximab induction, prednisolone, extended-release tacrolimus, and mycophenolic acid in kidney transplant recipients of both living, DBD, and DCD kidney transplant donors with a low to medium immunological risk.

Participants in the early steroid withdrawal group experienced significantly more treated rejections during follow-up. This is in line with previous studies and a meta-analysis.[7,9,24,25] However, no significant difference in BPAR (any TCMR of AMR) or treated BPAR was found between the treatment groups. This difference can partly be explained by the 7 biopsies which were excluded from the analysis (with <7 glomeruli or an absent artery). In another 10 transplant kidney biopsies in participants with treated rejection, the analysis did not provide enough evidence to mark it as TCMR or AMR despite histological suspicious lesions. Finally, the unblinded set-up of this study could

TABLE 4.

Kidney biopsies on indication—Biopsy-proven acute rejection

	Early steroid withdrawal	Standard immunosuppression	Tacrolimus minimization	*P*
Participants, n	98	100	97	
Participants with at least one kidney biopsy, n (%)	34 (34.7)	24 (24.0)	31 (32.0)	
Borderline TCMR, n (%)	4 (4.1)	2 (2.0)	4 (4.1)	0.86
Any TCMR, n (%)	13 (13.3)	6 (6.0)	6 (6.2)	0.11
TCMR grade IA, n (%)	1 (1.0)	0 (0.0)	0 (0.0)	0.44
TCMR grade IB, n (%)	1 (1.0)	0 (0.0)	0 (0.0)	0.44
TCMR grade IIA, n (%)	5 (5.1)	2 (2.0)	4 (4.1)	0.76
TCMR grade IIB, n (%)	4 (4.1)	2 (2.0)	1 (1.0)	0.44
TCMR grade III, n (%)	2 (2.0)	2 (2.0)	1 (1.0)	0.71
AMR or mixed AMR/TCMR, n (%)	3 (3.1)	2 (2.0)	2 (2.1)	0.93
Treated BPAR	14 (14.3)	6 (6.0)	8 (8.2)	0.12

Biopsies with <7 glomeruli were excluded from analysis. χ^2 test was applied for all parameters.
AMR, antibody-mediated rejection; BPAR, biopsy-proven acute rejection; TCMR, T-cell mediated rejection.

also partly explain the difference between treated rejection and treated BPAR.

Importantly, early steroid withdrawal was not associated with the formation of de novo DSA at 24 mo of follow-up. Because most rejections in our study occurred within the first 3 mo after transplantation, it could be hypothesized that steroid withdrawal on the third day might be too early in the course of kidney transplantation. However, previous studies with steroid withdrawal at 3 mo,[24,26-32] or 6 mo[33] after kidney transplantation also resulted in an increased risk of rejection from the moment of steroid withdrawal.

In this study, tacrolimus minimization 6 mo after transplantation did not result in differences in kidney transplant function, with a comparable incidence of treated rejection and BPAR. This is in line with the TRANSFORM trial,[34,35] comparing standard-exposure calcineurin inhibitors and MPA with reduced exposure calcineurin inhibitors and mTOR inhibitor everolimus. In the TRANSFORM study the tacrolimus trough levels were comparable to our study. However, in the TRANSFORM trial–in contrast with our study–no DCD donors were included. In the current study, additional protocol biopsies were performed at 12 and 24 mo. However, no histological beneficial effects of tacrolimus minimization were detected. One of the hallmarks of calcineurin inhibitor nephrotoxicity, arteriolar hyalinosis, is not significantly different in the protocol biopsies at 12 of 24 mo. One explanation could be that the follow-up was too short to detect these histological differences between 2 regimens with tacrolimus though levels of ~7.5 ng/mL versus ~4.4 ng/L. The higher scores of interstitial fibrosis and tubular atrophy in the tacrolimus minimization group in the 12 mo biopsies may be a result of more inflammation due to lower immunosuppression, however, and especially because this effect is not sustained in protocol biopsies at 24 mo, it may as well be an incidental finding based on a type I error. Additional post hoc analysis of subclinical rejection in protocol biopsies revealed no differences between the 3 treatment groups. It should be noted that protocol biopsies were performed in a minority of the participants, 40% of participants at 12 mo and 25% at 24 mo. Previous research has shown that lower trough levels of tacrolimus are associated with an increased risk of developing de novo DSA, acute rejection, and death-censored graft loss.[36-38] However, in our study, lower target trough levels of tacrolimus (3.0–5.0 ng/mL) from 6 mo after kidney transplantation did not result in higher rejection rates during follow-up or de novo DSA at 24 mo, compared with higher trough levels of tacrolimus (6.0–10.0 ng/mL).

From a metabolic perspective, analysis of incidence of diabetes mellitus and lipid metabolism showed that steroid-free immunosuppression was associated with a more beneficial cardiovascular risk profile with lower total cholesterol and lower percentage of participants with diabetes mellitus 24 mo after transplantation. Remarkably, also in the tacrolimus minimization group, the increase of HbA1c over time was significantly lower compared with the standard immunosuppression group. These findings are in line with previous studies,[9,39] and especially in the light of the known cardiovascular burden of transplantation patients in the long term,[40,41] an important outcome. Although 2 wk is early in the course after transplantation, together with the OGTT results at 12 and 24 mo, these analyses provide robust data on posttransplantation diabetes. Additionally, bone densitometry also suggests beneficial outcomes of steroid-free immunosuppression, albeit not significant. This is in accordance with previous literature showing that hip fractures remain an important complication after kidney transplantation and early steroid withdrawal is associated with reduced fracture risk.[42,43]

The study had several limitations. It was not blinded, and therefore treating physicians could have been biased in rejection treatment or interpretation of kidney biopsy outcomes. Additionally, follow-up was limited to 2 y, and the rate of study discontinuation was relatively high, whereas 7%–12% of participants withdrew consent for different reasons. Unfortunately, protocol biopsies were performed with a selection of participants. The study was powered to show noninferiority on the primary endpoint, therefore it could be that the power of the study is not adequate to compare other outcomes such as BPAR, side effects, or cardiovascular outcomes. Lastly, the incidence of BPAR in our study was rather low, as only patients with low to medium immunological risk were included, and patients at high risk for rejection were specifically excluded. Therefore, the results of this study should not be generalized to patients with an increased immunological risk.

In summary, although no direct advantages on kidney transplant function or histology could be demonstrated in

TABLE 5.

Cardiovascular and metabolic outcomes and bone densitometry

	Early steroid withdrawal			Standard immunosuppression			Tacrolimus minimization			*P*
	Baseline	24 mo visit	Difference	Baseline	24 mo visit	Difference	Baseline	24 mo visit	Difference	
Patient characteristics										
Systolic blood pressure (SD), mmHg	143.3 (21.2)	142.7 (18.7)	−0.7 (25.8)	139.6 (18.0)	139.4 (15.2)	−0.2 (21.5)	140.4 (25.7)	139.6 (15.7)	−0.8 (29.3)	0.99
Diastolic blood pressure (SD), mmHg	88.1 (17.8)	82.1 (11.8)	−5.3 (18.5)	81.4 (15.4)	80.8 (10.1)	−0.6 (16.8)	82.0 (13.1)	80.2 (13.7)	−1.8 (17.2)	0.40
BMI (SD) (kg/m^2)	26.7 (4.1)	27.0 (4.0)	0.3 (2.3)	26.1 (5.1)	26.5 (5.7)	0.5 (2.1)	27.4 (4.8)	28.6 (5.3)	1.2 (3.5)	0.23
Participants with diabetes mellitus (%)	14 (14.3)	40 (40.8)	26 (26.5)	17 (17.0)	53 (53.0)	36 (36.0)	20 (20.6)	57 (58.8)	37 (38.1)	0.04*
HbA1c, mmol/mol	37.3 (6.5)	40.7 (6.8)	3.3 (4.8)	34.7 (4.8)	47.2 (18.0)	12.4 (15.5)	39.2 (10.6)	41.9 (6.2)	2.6 (9.0)	0.003**
% of participants taking lipid-lowering drugs at 24 mo		39.8			50.0			40.2		0.26
Total cholesterol (SD), mmol/L	4.67 (0.96)	4.66 (0.89)	−0.01 (1.10)	4.22 (1.22)	5.01 (0.92)	0.80 (1.22)	4.42 (0.97)	5.34 (1.33)	0.92 (1.56)	0.03*
HDL cholesterol (SD), mmol/L	1.28 (0.39)	1.29 (0.43)	0.01 (0.41)	1.25 (0.34)	1.36 (0.34)	0.11 (0.39)	1.26 (0.36)	1.51 (0.43)	0.25 (0.37)	0.11
LDL cholesterol (SD), mmol/L	2.51 (0.79)	2.73 (0.83)	0.22 (0.89)	2.08 (0.83)	2.87 (0.96)	0.80 (1.07)	2.40 (0.91)	3.16 (1.16)	0.76 (1.40)	0.14
Triglycerides (SD), mmol/L	2.09 (0.92)	1.57 (0.69)	−0.52 (0.98)	2.10 (1.58)	1.90 (0.88)	−0.21 (1.52)	1.98 (1.09)	1.71 (0.73)	−0.27 (0.85)	0.61
Bone densitometry	2 wk	12 mo visit	Difference	2 wk	12 mo visit	Difference	2 wk	12 mo visit	Difference	
% of participants with osteoporosis at 12 mo		15.4			18.8			17.0		0.89
T score total hip	−1.09 (1.07)	−1.09 (1.17)	0.00 (0.45)	−1.20 (0.92)	−1.28 (0.99)	−0.08 (0.83)	−1.08 (1.13)	−1.11 (1.10)	−0.04 (0.46)	0.80
Z score total hip	−0.38 (0.98)	−0.33 (1.08)	0.05 (0.46)	−0.38 (0.83)	−0.47 (0.78)	−0.09 (0.49)	−0.21 (1.02)	−0.24 (0.99)	−0.03 (0.43)	0.27
T score lumbar spine	−0.63 (1.60)	−0.64 (1.45)	0.00 (0.44)	−0.82 (1.38)	−1.07 (1.32)	−0.25 (0.63)	−0.45 (1.46)	−0.54 (1.36)	−0.08 (0.62)	0.07
Z score total hip	−0.04 (1.67)	−0.03 (1.58)	0.01 (0.43)	−0.06 (1.40)	−0.27 (1.37)	−0.21 (0.63)	0.36 (1.45)	0.33 (1.44)	−0.03 (0.65)	0.10

Data are shown as increase (delta) between value at baseline and value at 24-mo visits. Bone densitometry data are shown as increase (delta) between value at 2 wk and 12 mo after transplantation. Data were analyzed with ANOVA test for delta parameters if normally distributed. χ^2 test was applied for participants with diabetes mellitus during follow-up, lipid-lowering drugs at 24 mo, and participants with osteoporosis at 12 mo. Osteoporosis was defined according to the WHO criteria: a T-score (hip and/or lumbar spine) ≤2.5 SDs.

*$P < 0.05$.

**$P < 0.01$.

the current study, lower target trough levels of tacrolimus seem equally safe and as effective as higher trough levels of tacrolimus with regard to AEs, rejection rates, and graft function. Therefore, lower target trough levels of tacrolimus could be considered in kidney transplant recipients with low to medium immunological risk in the first 2 y after kidney transplantation.

Early steroid withdrawal was associated with better outcomes on cardiovascular risk factors. However, as this was associated with more treated rejections shortly after steroid withdrawal, the potential metabolic advantages should be carefully weighed against the risk of rejection. And although kidney transplant function was not different between different treatment groups after 24 mo, participants with treated rejection had significantly lower eGFR at 24 mo compared with participants without treated rejection in a post hoc analysis (**Table S3, SDC,** http://links.lww.com/TP/C869).

The ALLEGRO study showed that the 3 immunosuppressive regimens studied resulted in equal outcome of kidney transplant function at 2 y, and that therefore other factors including cardiovascular and immunological risk, and AEs should be taken into account when personalizing immunosuppressive therapy in kidney transplant recipients.

ACKNOWLEDGMENTS

The authors thank G.N. Nieuwenhuizen, T.S.M. Standaar, I. Bunck, G.P.A. Clerx, and T.C. Timmer (Renal Transplant Unit, AMC), S.A.M. van Berkel and S. Hendriksen (Clinical Research Unit, Department of Medicine, LUMC), M. van Dijk (Department of Nephrology, UMCG), and S. Boontje, B. Scheerder, and H. Strooisma (Trial Coordination Center, UMCG) for their contribution and collection of the data. The authors

also thank R.J. Hene†, R.T. Gansevoort, and E.F. de Maar for their participation in the data safety monitoring board (DSMB).

REFERENCES

1. Hariharan S, Israni AK, Danovitch G. Long-term survival after kidney transplantation. *N Engl J Med*. 2021;385:729–743.
2. Gondos A, Döhler B, Brenner H, et al. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. *Transplantation*. 2013;95:267–274.
3. Coemans M, Süsal C, Döhler B, et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int*. 2018;94:964–973.
4. Arend SM, Mallat MJ, Westendorp RJ, et al. Patient survival after renal transplantation; more than 25 years follow-up. *Nephrol Dial Transplant*. 1997;12:1672–1679.
5. Shirali AC, Bia MJ. Management of cardiovascular disease in renal transplant recipients. *Clin J Am Soc Nephrol*. 2008;3:491–504.
6. Ojo AO, Hanson JA, Wolfe RA, et al. Long-term survival in renal transplant recipients with graft function. *Kidney Int*. 2000;57:307–313.
7. Haller MC, Royuela A, Nagler EV, et al. Steroid avoidance or withdrawal for kidney transplant recipients. *Cochrane Database Syst Rev*. 2016;2016:CD005632.
8. Thomusch O, Wiesener M, Opgenoorth M, et al. Rabbit-ATG or basiliximab induction for rapid steroid withdrawal after renal transplantation (harmony): an open-label, multicentre, randomised controlled trial. *Lancet*. 2016;388:3006–3016.
9. Vincenti F, Schena FP, Paraskevas S, et al; FREEDOM Study Group. A randomized, multicenter study of steroid avoidance, early steroid withdrawal or standard steroid therapy in kidney transplant recipients. *Am J Transplant*. 2008;8:307–316.
10. Matas AJ, Kandaswamy R, Gillingham KJ, et al. Prednisone-free maintenance immunosuppression-a 5-year experience. *Am J Transplant*. 2005;5:2473–2478.
11. Ekberg H, Tedesco-Silva H, Demirbas A, et al; ELITE-Symphony Study. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med*. 2007;357:2562–2575.
12. Ekberg H, Bernasconi C, Tedesco-Silva H, et al. Calcineurin inhibitor minimization in the symphony study: Observational results 3 years after transplantation. *Am J Transplant*. 2009;9:1876–1885.
13. Stegall MD, Cornell LD, Park WD, et al. Renal allograft histology at 10 years after transplantation in the tacrolimus era: Evidence of pervasive chronic injury. *Am J Transplant*. 2018;18:180–188.
14. Cockfield SM, Wilson S, Campbell PM, et al. Comparison of the effects of standard vs low-dose prolonged-release tacrolimus with or without ACEi/ARB on the histology and function of renal allografts. *Am J Transplant*. 2019;19:1730–1744.
15. Wojciechowski D, Wiseman A. Long-term immunosuppression management: opportunities and uncertainties. *Clin J Am Soc Nephrol*. 2021;16:1264–1271.
16. van Sandwijk MS, de Vries APJ, Bakker SJL, et al. Early steroid withdrawal compared with standard immunosuppression in kidney transplantation - interim analysis of the Amsterdam-Leiden-Groningen randomized controlled trial. *Transplant Direct*. 2018;4:e354.
17. Levey AS, Stevens LA, Schmid CH, et al; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
18. Loupy A, Haas M, Roufosse C, et al. The Banff 2019 kidney meeting report (I): updates on and clarification of criteria for T cell- and antibody-mediated rejection. *Am J Transplant*. 2020;20:2318–2331.
19. Wisse BW, Kamburova EG, Joosten I, et al. Toward a sensible single-antigen bead cutoff based on kidney graft survival. *Transplantation*. 2019;103:789–797.
20. Laftavi MR, Stephan R, Stefanick B, et al. Randomized prospective trial of early steroid withdrawal compared with low-dose steroids in renal transplant recipients using serial protocol biopsies to assess efficacy and safety. *Surgery*. 2005;137:364–371.
21. Rostaing L, Cantarovich D, Mourad G, et al; CARMEN Study Group. Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. *Transplantation*. 2005;79:807–814.
22. Woodle ES, First MR, Pirsch J, et al; Astellas Corticosteroid Withdrawal Study Group. A prospective, randomized, double-blind, placebo-controlled multicenter trial comparing early (7 day) corticosteroid cessation versus long-term, low-dose corticosteroid therapy. *Ann Surg*. 2008;248:564–577.
23. Krämer BK, Klinger M, Vítko S, et al. Tacrolimus-based, steroid-free regimens in renal transplantation: 3-year follow-up of the ATLAS trial. *Transplantation*. 2012;94:492–498.
24. Ponticelli C, Carmellini M, Tisone G, et al. A randomized trial of everolimus and low-dose cyclosporine in renal transplantation: with or without steroids? *Transplant Proc*. 2014;46:3375–3382.
25. Mourad G, Glyda M, Albano L, et al; Advagraf-based immunosuppression regimen examining new onset diabetes mellitus in kidney transplant recipients (ADVANCE) study investigators. Incidence of posttransplantation diabetes mellitus in de novo kidney transplant recipients receiving prolonged-release tacrolimus-based immunosuppression with 2 different corticosteroid minimization strategies: ADVANCE, A randomized controlled trial. *Transplantation*. 2017;101:1924–1934.
26. Ahsan N, Hricik D, Matas A, et al. Prednisone withdrawal in kidney transplant recipients on cyclosporine and mycophenolate mofetil--a prospective randomized study. steroid withdrawal study group. *Transplantation*. 1999;68:1865–1874.
27. Gulanikar AC, Belitsky P, MacDonald AS, et al. Randomized controlled trial of steroids versus no steroids in stable cyclosporine-treated renal graft recipients. *Transplant Proc*. 1991;23(1 Pt 2):990–991.
28. Isoniemi H, Ahonen J, Eklund B, et al. Renal allograft immunosuppression. II. A randomized trial of withdrawal of one drug in triple drug immunosuppression. *Transpl Int*. 1990;3:121–127.
29. Lebranchu Y. Comparison of two corticosteroid regimens in combination with CellCept and cyclosporine A for prevention of acute allograft rejection: 12 month results of a double-blind, randomized, multi-center study. M 55002 study group. *Transplant Proc*. 1999;31:249–250.
30. Kim HC, Chang KJ, Kwon JK, et al. Long-term results of cyclosporine monotherapy in renal transplantation. *Transplant Proc*. 1998;30:3539–3540.
31. Sola E, Alférez MJ, Cabello M, et al. Low-dose and rapid steroid withdrawal in renal transplant patients treated with tacrolimus and mycophenolate mofetil. *Transplant Proc*. 2002;34:1689–1690.
32. Pascual J, van Hooff JP, Salmela K, et al. Three-year observational follow-up of a multicenter, randomized trial on tacrolimus-based therapy with withdrawal of steroids or mycophenolate mofetil after renal transplant. *Transplantation*. 2006;82:55–61.
33. Smak Gregoor PJ, de Sévaux RG, Ligtenberg G, et al. Withdrawal of cyclosporine or prednisone six months after kidney transplantation in patients on triple drug therapy: a randomized, prospective, multicenter study. *J Am Soc Nephrol*. 2002;13:1365–1373.
34. Pascual J, Berger SP, Witzke O, et al; TRANSFORM Investigators. Everolimus with reduced calcineurin inhibitor exposure in renal transplantation. *J Am Soc Nephrol*. 2018;29:1979–1991.
35. Berger SP, Sommerer C, Witzke O, et al; TRANSFORM Investigators. Two-year outcomes in de novo renal transplant recipients receiving everolimus-facilitated calcineurin inhibitor reduction regimen from the TRANSFORM study. *Am J Transplant*. 2019;19:3018–3034.
36. Davis S, Gralla J, Klem P, et al. Lower tacrolimus exposure and time in therapeutic range increase the risk of de novo donor-specific antibodies in the first year of kidney transplantation. *Am J Transplant*. 2018;18:907–915.
37. Davis S, Wiebe C, Campbell K, et al. Adequate tacrolimus exposure modulates the impact of HLA class II molecular mismatch: a validation study in an American cohort. *Am J Transplant*. 2021;21:322–328.
38. Gold A, Tönshoff B, Döhler B, et al. Association of graft survival with tacrolimus exposure and late intra-patient tacrolimus variability in pediatric and young adult renal transplant recipients-an international CTS registry analysis. *Transpl Int*. 2020;33:1681–1692.
39. Knight SR, Morris PJ. Steroid avoidance or withdrawal after renal transplantation increases the risk of acute rejection but decreases cardiovascular risk. A meta-analysis. *Transplantation*. 2010;89:1–14.
40. Kasiske BL. Epidemiology of cardiovascular disease after renal transplantation. *Transplantation*. 2001;72(6 Suppl):S5–S8.
41. Jardine AG, Fellström B, Logan JO, et al. Cardiovascular risk and renal transplantation: Post hoc analyses of the assessment of Lescol in renal transplantation (ALERT) study. *Am J Kidney Dis*. 2005;46:529–536.
42. Nair SS, Lenihan CR, Montez-Rath ME, et al. Temporal trends in the incidence, treatment and outcomes of hip fracture after first kidney transplantation in the united states. *Am J Transplant*. 2014;14:943–951.
43. Nikkel LE, Mohan S, Zhang A, et al. Reduced fracture risk with early corticosteroid withdrawal after kidney transplant. *Am J Transplant*. 2012;12:649–659.