ORIGINAL ARTICLE

ADHERE: randomized controlled trial comparing renal function in *de novo* kidney transplant recipients receiving prolonged-release tacrolimus plus mycophenolate mofetil or sirolimus

Oleg O. Rummo[1], Mario Carmellini[2], Lionel Rostaing[3], Rainer Oberbauer[4], Maarten H. L. Christiaans[5], Christiane Mousson[6], Robert M. Langer[7], Franco Citterio[8], Bernard Charpentier[9], Malcolm Brown[10], Gbenga Kazeem[11] & Frank Lehner[12] on behalf of the ADHERE study investigators[†]

1 Republican Scientific and Practical Center (RSPC) for Organ and Tissue Transplantation, Minsk, Belarus
2 Department of Medical, Surgical and Neuroscience, Policlinico Santa Maria alle Scotte, University of Siena, Siena, Italy
3 Department of Nephrology, Dialysis and Organ Transplantation, Toulouse University Hospital, INSERM U563, IFR–BMT, CHU Purpan, Toulouse, France
4 Department of Internal Medicine III, Nephrology and Dialysis, Medical University of Vienna, Vienna, Austria
5 Department of Internal Medicine/ Division of Nephrology, Maastricht University Medical Center, Maastricht, The Netherlands
6 Department of Nephrology–Transplantation, University Hospital, Dijon, France
7 Department of Transplantation and Surgery, Semmelweis University, Budapest, Hungary
8 Department of Surgery, Renal Transplantation, Catholic University, Rome, Italy
9 Department of Nephrology, Dialysis and Transplantation, University Hospital of Bicêtre, Le Kremlin Bicetre, France
10 Medical Affairs – Global, Astellas Pharma, Northbrook, IL, USA
11 Astellas Pharma Europe Ltd, Chertsey, UK
12 General, Visceral and Transplantation Surgery, Hannover Medical School, Hannover, Germany

SUMMARY

ADHERE was a randomized, open-label, Phase IV study comparing renal function at Week 52 postkidney transplant, in patients who received prolonged-release tacrolimus-based immunosuppressive regimens. On Days 0–27, patients received prolonged-release tacrolimus (initially 0.2 mg/kg/day), corticosteroids, and mycophenolate mofetil (MMF). Patients were randomized on Day 28 to receive either prolonged-release tacrolimus plus MMF (Arm 1) or prolonged-release tacrolimus (≥25% dose reduction on Day 42) plus sirolimus (Arm 2). The primary endpoint was glomerular filtration rate by iohexol clearance (mGFR) at Week 52. Secondary endpoints included eGFR, creatinine clearance (CrCl), efficacy failure (patient withdrawal or graft loss), and patient/graft survival. Tolerability was analyzed. The full-analysis set comprised 569 patients (Arm 1: 287; Arm 2: 282). Week 52 mean mGFR was similar in Arm 1 versus Arm 2 (40.73 vs. 41.75 ml/min/1.73 m^2; $P = 0.405$), as were the secondary endpoints, except composite efficacy failure, which was higher in Arm 2 versus 1 (18.2% vs. 11.5%; $P = 0.002$) owing to a higher postrandomization withdrawal rate due to adverse events (AEs) (14.4% vs. 5.2%). Results from this study show comparable renal function between arms at Week 52, with fewer AEs leading to study discontinuation with prolonged-release tacrolimus plus MMF (Arm 1) versus lower dose prolonged-release tacrolimus plus sirolimus (Arm 2).

Transplant International 2017; 30: 83–95

Key words
calcineurin antagonists, immunosuppression, kidney clinical, outcome

Received: 6 May 2016; Revision requested: 9 May 2016; Accepted: 14 October 2016; EV Pub Online 28 November 2016

[Correction added on 23 August 2017 after first online publication: The Legal Statement for the article has been updated.]

doi:10.1111/tri.12878

Introduction

For over 20 years, calcineurin inhibitors (CNIs), such as tacrolimus, have been the mainstay of immunosuppressive protocols for kidney transplantation, reducing the risk of graft failure and patient mortality compared with other therapies in both clinical trials and clinical settings [1,2]. Although short-term outcomes in Europe are satisfactory, ten-year kidney graft survival rates of approximately 56% [3] suggest that there is scope for further improvement in long-term outcomes for these patients. The causes of graft loss are diverse [4–6] and include some risk factors that could be managed through optimization of the immunosuppressive regimen [4,7–9]. Approaches taken to explore regimen optimization include the addition of other immunosuppressive treatments to CNIs, especially those with complementary mechanisms of action and different adverse event (AE) profiles [10–12]. Tacrolimus in combination with mycophenolate mofetil (MMF) is an effective immunosuppressive regimen for kidney transplant recipients [13]. The combination of tacrolimus and sirolimus, an inhibitor of mammalian target of rapamycin, has been shown to provide effective immunosuppression [14–17] and has been reported to be renal-sparing in some combinations [18], although sirolimus-related side effects have been associated with tolerability concerns and frequent discontinuations [16,19,20]. The strong synergistic immunosuppressive effect of tacrolimus plus sirolimus permits dose reduction of tacrolimus when used in combination with sirolimus. However, CNI avoidance, minimization, and withdrawal have not achieved improved long-term outcomes versus tacrolimus-based regimens [21], although two studies published in 2003/2004 reported similar efficacy outcomes with reduced and standard dosing of tacrolimus in combination with sirolimus [19,22].

The ADHERE Phase IV study was designed to investigate renal function with once-daily, prolonged-release tacrolimus-based immunosuppression 1 year post *de novo* kidney transplantation. To the authors' knowledge, this is the first study to assess whether lower dose prolonged-release tacrolimus plus sirolimus (started on Day 28 after transplantation) improves renal function compared with higher dose prolonged-release tacrolimus plus MMF. This study is also the first large-scale clinical trial in kidney transplantation to use a primary endpoint of glomerular filtration rate measured by iohexol clearance (mGFR).

Patients and methods

Study design

ADHERE was a multicenter, randomized, open-label, two-arm, parallel-group comparative Phase IV study conducted at 58 sites in 18 European and Asia–Pacific countries between March 2011 and September 2013. The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, International Conference on Harmonization guidelines, and applicable laws and regulations. An independent ethics committee granted approval before initiation. Written informed consent was obtained from all participants.

Eligible patients were ≥18 years old with end-stage kidney disease, suitable for primary renal transplantation or retransplantation (unless the graft was lost from rejection within 6 months), and receiving a kidney transplant from a deceased or living (nonhuman leukocyte antigen identical) donor with compatible ABO blood type. Patients were excluded from the study if they had previously received an organ transplant other than a kidney, the cold ischemia time was >30 h, or the panel-reactive antibody grade was >20%. Patients were also excluded from the study if they received a graft from a donor after cardiac death (unless the donor was of Maastricht category 3, that is, withdrawal of support awaiting cardiac arrest), had significant liver disease, or required initial sequential or parallel therapy with immunosuppressive antibody preparation(s) or ongoing dosing with a systemic immunosuppressive drug prior to transplantation.

Randomization and masking

The randomization sequence was prepared by Pierrel Research Europe GmbH, Essen, Germany. Randomization was coordinated centrally using an interactive voice response system (managed by Cenduit GmbH, Allschwil, Switzerland) and block procedure. Eligible patients were randomized (1:1) on Day 28 following transplantation to receive either prolonged-release tacrolimus plus MMF (Arm 1) or prolonged-release tacrolimus (≥25% dose reduction on Day 42) plus sirolimus (Arm 2). Treatment allocation was stratified according to study center and donor type (deceased or living kidney donor). Patients who were experiencing an acute-rejection episode on Day 28 could have randomization delayed for up to 7 days after the end of treatment for the rejection episode.

Procedure

All patients received prolonged-release tacrolimus (Advagraf™; Astellas Pharma Europe BV, Leiden, the Netherlands) from Day 0 to Day 365 with an initial postoperative dose of 0.2 mg/kg/day. Doses were taken once daily, orally, and adjusted based on clinical efficacy and tolerability, taking into account recommended whole-blood trough concentrations. Tacrolimus target trough levels were 10–15 ng/ml until Day 14, then 8–12 ng/ml from Day 15 to Day 27 in both arms. Post-trandomization, Arm 1 tacrolimus target trough levels were 8–12 ng/ml between Day 28 and 41, and 6–10 ng/ml from Day 42 until the end of study (EOS). In Arm 2, tacrolimus target trough levels were 8–12 ng/ml between Day 28 and 41; the tacrolimus dose was then decreased by ≥25% to target tacrolimus trough levels of 4–5 ng/ml between Day 42 and the EOS.

All patients received oral MMF each day (1 g twice daily until Day 14, reduced to 0.5 g twice daily until Day 27). Patients in Arm 1 continued to receive MMF at a daily dose of 1 g. For patients randomized to Arm 2 only, MMF was discontinued on Day 28; these patients received sirolimus, orally, once daily from Day 28 to the EOS, with an initial daily dose of 1 mg and a target trough level range of 2–4 ng/ml (maximum dose 2 mg daily). All patients received a single dose of corticosteroids, administered as an intravenous bolus of ≤1000 mg on Day 0 in accordance with each center's policy. Oral corticosteroids were then tapered throughout the study (Day 1–13: 20 mg/day, Day 14–28: 15 mg/day, Day 29–42: 10 mg/day, Day 43–60: 5 mg/day, Day 60–365: ≤5 mg/day). Treatment of cytomegalovirus (CMV) was in accordance with each center's policy.

Primary and secondary efficacy variables

The primary endpoint was measured GFR by iohexol clearance (mGFR) at Week 52 post-transplant. Secondary efficacy variables included renal function at Week 52: estimated GFR [eGFR, Modification of Diet in Renal Disease-4 (MDRD4) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)] and creatinine clearance (CrCl) by the Cockcroft–Gault formula. Other secondary endpoints included the incidence of composite efficacy failure defined as patient withdrawal or graft loss (defined as retransplantation, transplant nephrectomy, death or dialysis ongoing at the EOS or at the time of discontinuation); clinical acute rejection (AR) and biopsy-confirmed AR (BCAR); patient and graft survival; delayed graft function (DGF) (defined as dialysis ≥1 day during the first 7 days post-transplant); and new-onset diabetes mellitus (NODM) as per the American Diabetic Association (ADA) 2010 criteria [23].

Subgroup analyses

To assess the possibility that the treatment effect might differ in specific subgroups of patients, the subgroup-by-treatment interaction effect was tested in an analysis of covariance (ANCOVA) model similar to that used for the primary analysis. In addition, to further explore any significant subgroup-by-treatment interaction effect, observed analysis of the primary variable was also repeated separately for the subgroups of patients stratified by recipient age (<50 years, ≥50 years), donor age (<50 years, ≥50 years), donor status (deceased, living), and gender.

Other variables

Additional variables in this study included patient-reported EuroQoL 5-Dimensions Health Questionnaire (EQ-5D), assessment of adherence to the study drug using the Morisky Medication Adherence Scale-8, and the length and type of hospital stay.

Tolerability analyses

Tolerability was assessed by the evaluation of AEs and laboratory parameters, which were monitored throughout the study. Postrandomization AEs were defined as those with an onset date occurring on or after randomization; AEs that changed in severity on or after the date of randomization were included as postrandomization AEs. A serious AE was any untoward medical occurrence that was life threatening, resulted in death, persistent or significant disability or incapacity, congenital anomaly, birth defect, or required inpatient hospitalization. The relationship between the AE and the study medication was indicated as not related, possible, or probable.

Statistical methods

The safety-analysis set (SAF) was defined as all patients who took at least one dose of any study drug (prolonged-release tacrolimus, MMF or sirolimus). The intent-to-treat (ITT) population consisted of all patients who had been transplanted and randomized, and the full-analysis set (FAS) was defined as all patients who had been transplanted, randomized, and underwent postrandomization assessment of the primary endpoint

(i.e., evaluable iohexol sample measurements). Analysis of the primary and secondary efficacy variables on renal function, and the subgroup and subgroup-by-treatment interaction analyses, was undertaken on the FAS. Analyses of the nonrenal function secondary efficacy variables were undertaken on the ITT population. Tolerability analyses that occurred prior to randomization were performed using the SAF, and analysis of AEs with an onset date on or after Day 28 was performed using the ITT population.

To detect a clinically meaningful 6 ml/min increase in mGFR measured in Arm 2 versus Arm 1 with 90% power, 284 patients were required to have evaluable mGFR at Week 52 in each arm. With an assumed dropout rate of 16.5% over the 12-month period, it was estimated that 386 patients should be enrolled and transplanted per arm. The target enrollment of 772 patients had assumed <10% of the patients would fail to reach randomization; however, the prerandomization dropout rate was considerably greater than 10%. Therefore, the recruitment target was increased to 856 in order to reach the intended number of evaluable patients. Least square (LS) means of mGFR (FAS) were obtained from the ANCOVA, and the difference between the LS means and the 95% confidence intervals (CI) is presented. *P* values were derived from an ANCOVA model in which treatment arm, gender, race (black, nonblack), site, and donor status (deceased, living) were included as factors, and eGFR (MDRD4) at randomization and donor age were included as continuous covariates. A sensitivity analysis (ANCOVA) of the primary endpoint was conducted in the ITT population, with imputation of mGFR for patients with missing iohexol clearance and for those who experienced graft loss and/or death. For patients who experienced graft loss, retransplantation, dialysis at the end of the study, and/or death, the imputed value was set to zero. For patients with missing iohexol clearance who did not experience graft loss and/or death, a multiple imputation procedure was used to replace each missing value with a set of plausible values (based on the covariate values at baseline/randomization) that represent uncertainty about the correct value to impute. Similar multiple imputation data sets were analyzed for the secondary efficacy variables of eGFR and calculated CrCl using the ITT population. *P* values ≤0.05 were considered significant for all analyses. Kaplan–Meier survival analysis techniques were used for analysis of time-to-event endpoints, and the 95% CI for the difference between arms was calculated using the normal approximation method. In addition, the differences in incidence between arms were compared using a chi-square test. All data processing, summaries, and analyses were performed using SAS® software version 9.1.3 or higher on UNIX.

Results

Patient and donor demographics

Overall, 850 patients received ≥1 dose of study medication and were included in the SAF, and 120 patients in the SAF were excluded from the ITT as they were either not transplanted (12 patients) or transplanted but not randomized (108 patients). In total, 730 patients were included in the ITT (Fig. 1); 625 patients (73.5% of the SAF) completed the study. Postrandomization, 38 patients in Arm 1 and 67 patients in Arm 2 discontinued from the study; the main reason for discontinuation in both arms was AEs (Arm 1: 5.2%; Arm 2: 14.4%). Figure 2 shows the time and incidence of postrandomization study discontinuations in each arm. Patient demographics and baseline characteristics in the ITT were generally comparable between arms. Donors were mainly ≤65 years old (n = 648, 88.7%), male (n = 383, 52.5%), and deceased (n = 602, 82.5%). The overall mean (standard deviation, SD) cold and warm ischemia times were 10.9 (6.97) h and 38.4 (23.6) min, respectively, and were similar in both treatment arms. Recipient and donor baseline characteristics are shown in Table 1. In total, 569 (77.9%) patients from the ITT underwent a postrandomization assessment of the primary endpoint and were included in the FAS (Arm 1: 287; Arm 2: 282).

Dosing and exposure

Mean prolonged-release tacrolimus dose was similar between arms up to randomization on Day 28 and remained comparable between arms until Day 42, when, in line with the ≥25% dose reduction in the study protocol, the mean dose was lower in Arm 2 versus Arm 1 to the EOS (Fig. 3a). Mean tacrolimus trough levels were comparable between arms until Day 28 and remained similar until Day 42; from Day 42 to EOS, mean trough levels were lower in Arm 2 versus Arm 1 (Fig. 3b). Mean (SD) EOS tacrolimus trough levels were similar between patients who discontinued due to AEs in Arm 2 [6.89 (3.44) ng/ml] compared with the overall Arm 2 population [5.73 (2.56) ng/ml] (including those who discontinued) (target range: 4–5 ng/ml).

Mean MMF dose was comparable between arms until Day 28; from Day 28 to EOS, the mean MMF dose

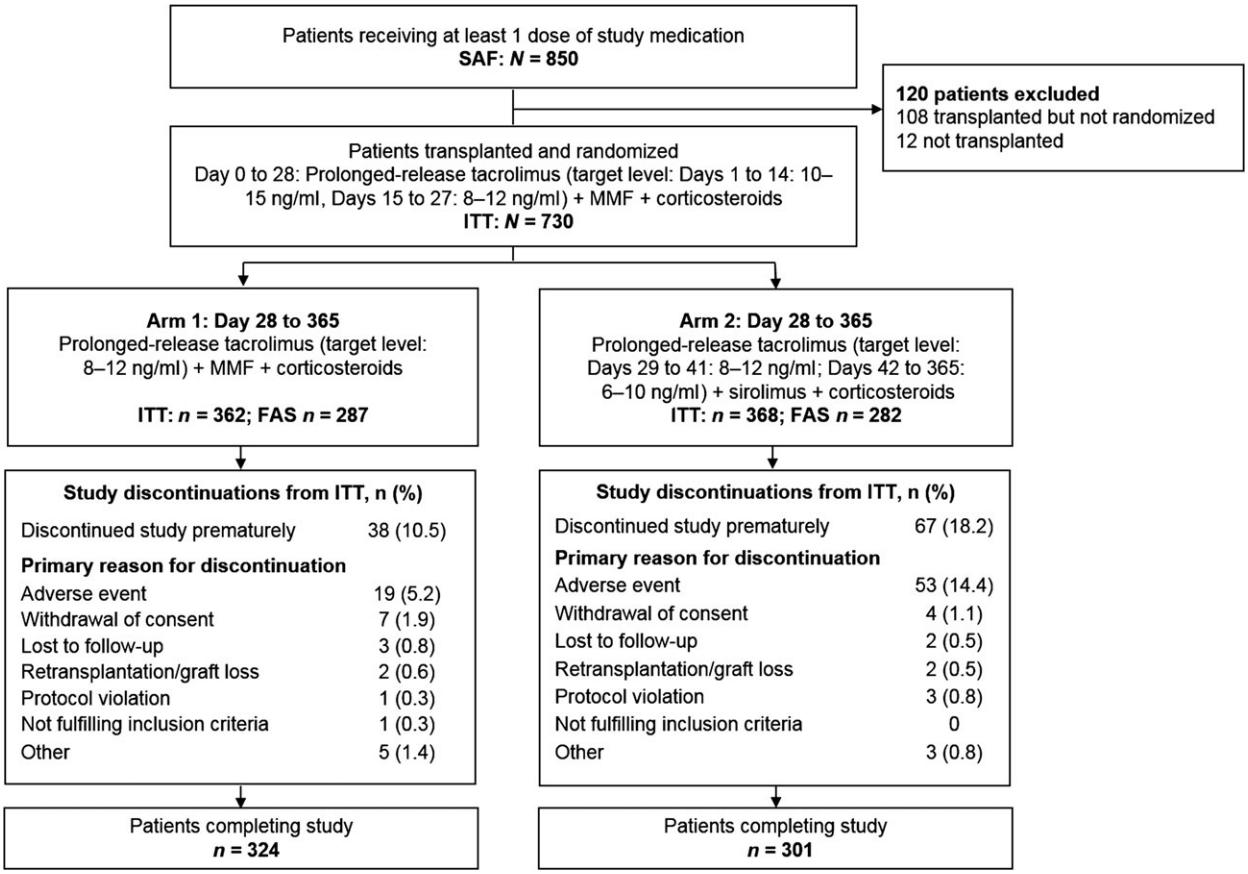

Figure 1 Flow of patients through the study, including reasons for discontinuation. The numbers presented in the figure are percentages. All patients received oral MMF until Day 27. For patients randomized to Arm 2 only, MMF was discontinued and sirolimus was initiated on Day 28 and continued throughout the study. FAS, full-analysis set; ITT, intent to treat; MMF, mycophenolate mofetil; SAF, safety-analysis set.

remained consistent in Arm 1 (~1 g/day). From Day 28, patients in Arm 2 received sirolimus; mean (SD) sirolimus whole-blood trough levels in the FAS were 3.27 (2.20) ng/ml [median: 2.70 (range: 0.0–14.1) ng/ml] on Day 28 and 3.73 (1.44) ng/ml (3.50 (1.0–8.8) ng/ml) at Week 52. Although there was no requirement to titrate the sirolimus dose, patients remained within the target sirolimus trough range (2–4 ng/ml) except for on Day 56 [4.21 (2.32) ng/ml]. In total, 54.1% of patients in Arm 2 had more than one sirolimus dose >1 mg/day between Day 28 and Week 52. Mean (SD) EOS sirolimus trough levels were 3.29 (1.82) ng/ml for patients who discontinued due to AEs in Arm 2 compared with 3.68 (1.61) ng/ml for the overall Arm 2 population. The mean and median daily doses of sirolimus at the EOS, in patients who withdrew due to AEs, were 1.29 and 1.0 mg/day, respectively. All randomized patients received steroids; the mean (SD) steroid cumulative doses were comparable between arms [Arm 1: 2111 (1450) mg versus Arm 2: 2219 (1503) mg, respectively].

Renal function (primary and secondary endpoints; FAS)

The primary efficacy endpoint GFR (iohexol clearance) at Week 52 was comparable between Arm 1 and Arm 2 (LS mean: 40.7 vs. 41.8 ml/min/1.73 m^2, respectively; $P = 0.405$) (Table 2). When the methods used for the primary analysis were repeated using the ITT, with imputed data for patients with missing GFR by iohexol clearance measurements, similar results were obtained. The secondary efficacy endpoints comparing renal function showed no significant differences between arms for LS means of eGFR (MDRD4: 50.5 vs. 51.0 ml/min/1.73 m^2; $P = 0.720$; CKD-EPI: 51.5 vs. 51.8 ml/min/1.73 m^2; $P = 0.823$) or CrCl (Cockcroft–Gault: 56.6 vs. 57.1 ml/min/1.73 m^2; $P = 0.736$) (Table 2).

Other secondary endpoints (ITT/SAF)

Other secondary endpoints (from randomization on Day 28 to Week 52; ITT) and all time-to-event secondary

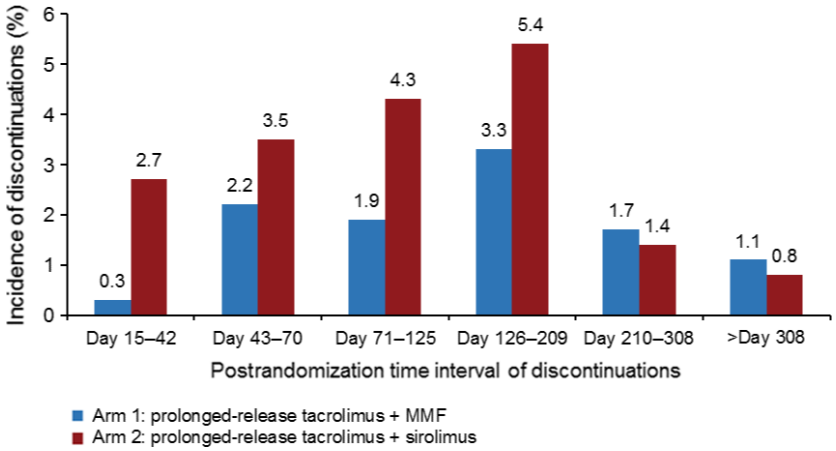

Figure 2 Incidence of study discontinuations in each arm stratified by time interval postrandomization (ITT). The graph presents postrandomization discontinuation data for all patients who were transplanted and randomized (ITT population). All patients received oral MMF until Day 27. For patients randomized to Arm 2 only, MMF was discontinued and sirolimus was initiated on Day 28 and continued throughout the study; primary reason for discontinuation (after Day 28): adverse event, Arm 1 n = 19 (5.2%) versus Arm 2 n = 53 (14.4%); withdrawal of consent, Arm 1 n = 7 (1.9%) versus Arm 2 n = 4 (1.1%); lost to follow-up, Arm 1 n = 3 (0.8%) versus Arm 2 n = 2 (0.5%); retransplantation/graft loss, Arm 1 n = 2 (0.6%) versus Arm 2 n = 2 (0.5%); protocol violation, Arm 1 n = 1 (0.3%) versus Arm 2 n = 3 (0.8%); not fulfilling inclusion/exclusion criteria, Arm 1 n = 1 (0.3%) versus Arm 2 n = 0; other, Arm 1 n = 5 (1.4%) versus Arm 2 n = 3 (0.8%). ITT, intent to treat; MMF, mycophenolate mofetil.

efficacy endpoints (AR, BCAR, patient and graft survival, and NODM) were generally comparable between arms at Week 52 with the exception of composite efficacy failure (Table 3). The Kaplan–Meier estimate of composite efficacy failure was significantly higher in Arm 2 versus Arm 1 (18.2% vs. 11.5%; $P = 0.002$). The difference between arms, postrandomization, was driven by patient withdrawal due to AEs, which was significantly higher in Arm 2 versus Arm 1 (14.4% vs. 5.2%; $P < 0.001$). Kaplan–Meier estimates of DGF including events prior to randomization were 23.1% in nonrandomized patients, 11.9% in Arm 1 and 11.1% in Arm 2 (SAF).

Other outcomes (ITT)

Patient-reported outcomes assessed at Week 52 using EQ-5D showed no significant differences between arms ($P = 0.711$). The changes from baseline on the Visual Analog Scale, scored from "best imaginable health state" to "worst imaginable health state," were similar between arms, with both arms showing an increase in quality of life compared with baseline data.

For the patients who were randomized, the mean number of days in hospital from baseline to EOS was 21.1 in Arm 1 versus 17.4 in Arm 2. A total of 22 patients in Arm 1 were admitted to the intensive care unit compared with 17 patients in Arm 2. The mean duration of stay for patients in Arm 1 was 9.9 days compared with 12.4 days for the patients in Arm 2. A similar number of patients in Arm 1 and Arm 2 had dialysis during the study (82 vs. 80 patients, respectively); the mean number of days that patients spent in hospital on dialysis was 8.6 days in Arm 1 versus 6.9 days in Arm 2. Treatment adherence was high in both arms, and the majority of patients took their medication regularly.

Subgroup analyses (FAS)

When the FAS was analyzed using similar methods to the primary analysis, with the treatment-by-donor age interaction effect included, a significant interaction effect was observed for the treatment-by-donor age effect ($P = 0.027$); however, the overall treatment effect did not reach statistical significance ($P = 0.602$). When the analysis was repeated separately for patient subgroups, there were no significant differences in mean GFR (iohexol clearance) between arms except for patients with donor age <50 years. In this subgroup (donor age <50 years), mGFR was significantly higher in Arm 2 versus Arm 1 (61.9 vs. 57.7; difference: 4.2 ml/min/1.73 m^2; $P = 0.038$).

Tolerability analyses (SAF/ITT)

The incidence of mortality was low overall and comparable between arms. Of the 16 deaths reported, nine occurred during the study period (six

Table 1. Baseline characteristics of recipients and donors in each treatment arm (FAS).

		Arm 1: prolonged-release tacrolimus + MMF (*n* = 287)	Arm 2: prolonged-release tacrolimus + sirolimus (*n* = 282)
Donor			
Gender, male, *n* (%)		153 (53.3)	150 (53.2)
Race, *n* (%)			
White		144 (90.6)	157 (89.7)
Asian		14 (8.8)	17 (9.7)
Other*		1 (0.6)	1 (0.6)
Not recorded		128	107
Mean age, years (SD)		48.2 (15.5)	49.9 (14.5)
Donor type			
Living related, *n* (%)		32 (11.1)	40 (14.2)
Living nonrelated, *n* (%)		10 (3.5)	13 (4.6)
Deceased, *n* (%)		245 (85.4)	229 (81.2)
ABO identical†, *n* (%)		272 (94.8)	260 (92.2)
ABO compatible‡, *n* (%)		15 (5.2)	22 (7.8)
Mean total HLA mismatch, *n*		2.87	2.99
CMV recipient/donor, *n* (%)	Negative/negative	35 (12.2)	21 (7.4)
	Negative/positive	56 (19.5)	45 (16.0)
	Positive/positive	139 (48.4)	166 (58.9)
	Positive/negative	47 (16.4)	33 (11.7)
	Positive/unknown	2 (0.7)	5 (1.8)
	Unknown/positive	5 (1.7)	8 (2.8)
	Unknown/negative	3 (1.0)	4 (1.4)
EBV, *n* (%)	Positive	154 (53.7)	161 (57.1)
Mean PRA grade§ (SD)		0.75 (2.8)	0.77 (2.7)
Recipient			
Gender, male, *n* (%)		179 (62.4)	186 (66.0)
Race, *n* (%)			
White		256 (92.4)	251 (91.9)
Asian		18 (6.5)	16 (5.9)
Other*		3 (1.1)	6 (2.2)
Not recorded		10	9
Mean age, years (SD)		49.6 (13.2)	49.2 (13.0)
Mean BMI¶, kg/m^2 (SD)		25.3 (4.2)	25.4 (4.3)
Primary reason for kidney transplantation, *n* (%)	PKD	62 (21.6)	43 (15.2)
	Glomerulonephritis	49 (17.1)	59 (20.9)
	HN (including HNy)	32 (11.2)	34 (12.1)
	Unknown	31 (10.8)	29 (10.3)
	Other**	113 (39.4)	117 (41.5)
HBV, *n* (%)	Positive	6 (2.1)	2 (0.7)
EBV, *n* (%)	Positive	222 (77.4)	223 (79.1)

All patients received oral MMF until Day 27. For patients randomized to Arm 2 only, MMF was discontinued and sirolimus was initiated on Day 28 and continued throughout the study.

BMI, body mass index; CMV, cytomegalovirus; EBV, Epstein–Barr virus; FAS, full-analysis set; HBV, hepatitis B virus; HLA, human leukocyte antigen; HN, hypertensive nephrosclerosis; HNy, hypertensive nephropathy; PKD, polycystic kidney disease; PRA, panel-reactive antibody; SD, standard deviation.

*Other race: Black, Native Hawaiian, Pacific Islander, or other.

†Recipient and donor had the same blood group.

‡A or B recipient received kidney from donor with same or O group; AB recipient received organ from an O, A, or B donor.

§PRA grade: Arm 1, *n* = 283, Arm 2, *n* = 281.

¶Recipient BMI: Arm 1, *n* = 279, Arm 2, *n* = 278.

**Other reasons for primary kidney transplantation: obstructive uropathy (including chronic pyelonephritis), diabetic nephropathy, IgA nephropathy, tubular and interstitial disease, not recorded, focal segmental glomerulonephritis, membranoproliferative glomerulonephritis, hereditary nephropathy, systemic lupus erythematosus, other systemic vasculitis.

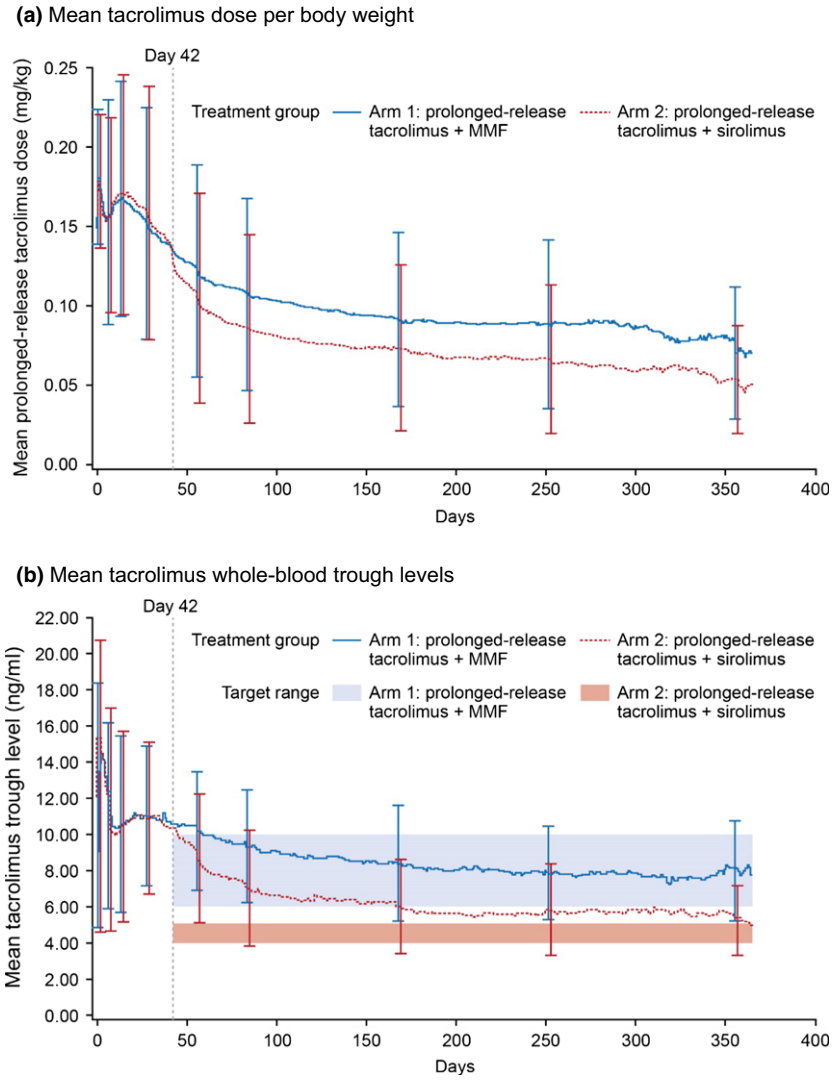

Figure 3 Tacrolimus (a) dose per body weight and (b) trough levels stratified by arm over 52 weeks of treatment (ITT). All patients received oral MMF until Day 27. For patients randomized to Arm 2 only, MMF was discontinued and sirolimus was initiated on Day 28 and continued throughout the study and tacrolimus dose was decreased by ≥25% between Day 42 and the EOS; error bars (SD) correspond to visit days (Days 1, 7, 14, 28, 56, 84, 168, 252, and 356). ITT, intent to treat; MMF, mycophenolate mofetil; SD, standard deviation.

prerandomization, three postrandomization), two of which were considered to be related to treatment (pneumonia, one patient in each arm). Seven deaths occurred after the end-of-treatment visit or withdrawal from the study, but within 1 year post-transplant. In the whole study period, 205 (24.1%) patients in the SAF reported ≥1 AE of special interest (NODM, proteinuria, pulmonary complications, mouth ulcers, and impairment of wound healing).

Prerandomization tolerability analyses (SAF)

A total of 717 (84.4%) patients reported AEs post-transplant but prerandomization. AEs prerandomization were reported in 304 (84.0%) vs. 314 (85.3%) patients in Arm 1 versus Arm 2, respectively; 99 (82.5%) of the 120 patients not randomized experienced ≥1 AE, and six (5%) of these patients died. Causes of death included bradycardia, pulmonary sepsis, gastrointestinal disorder, sudden death, operative hemorrhage, and aortic dissection.

Postrandomization tolerability analyses (ITT)

Postrandomization AEs and study drug-related AEs were comparable between arms [AEs Arm 1: 307 of 362 (84.8%) versus Arm 2: 309 of 368 (84.0%); study drug-related AEs Arm 1: 212 of 362 (58.6%) versus Arm 2: 215 of 368 (58.4%)]. The most commonly reported postrandomization adverse events are presented in Table 4. Postrandomization AEs of special interest were lower in Arm 1 versus Arm 2 [41 of 362 (11.3%) vs. 70 of 368 (19.0%)]. Postrandomization, Arm 1 versus Arm 2 had a higher incidence of CMV infection [43 of 362 (11.9%) vs. 14 of 368 (3.8%)], bacterial urinary tract infection [33 of 362 (9.1%) vs. 18 of 368 (4.9%)], and leukopenia [47 of 362 (13.0%) vs. 9 of 368 (2.4%)]. In contrast, patients in Arm 1 versus Arm 2 had a lower incidence

Table 2. Renal function at Week 52 as assessed by primary and secondary endpoints.

	Arm 1: prolonged-release tacrolimus + MMF (n = 287)	Arm 2: prolonged-release tacrolimus + sirolimus (n = 282)	P value*
Primary endpoint			
GFR by iohexol clearance (ml/min/1.73 m^2)			
Mean	40.73	41.75	0.405
Difference†	1.02		
95% CI for mean difference	–1.39, 3.44		
Secondary endpoints			
eGFR by MDRD4 (ml/min/1.73 m^2)			
Mean	50.54	51.03	0.720
Difference†	0.49		
95% CI for mean difference	–2.21, 3.20		
eGFR by CKD-EPI (ml/min/1.73 m^2)			
Mean	51.46	51.77	0.823
Difference†	0.31		
95% CI for mean difference	–2.44, 3.07		
Calculated CrCl by Cockcroft–Gault (ml/min)			
Mean	56.61	57.14	0.736
Difference†	0.53		
95% CI for mean difference	–2.54, 3.59		

All patients received oral MMF until Day 27. For patients randomized to Arm 2 only, MMF was discontinued and sirolimus was initiated on Day 28 and continued throughout the study.

CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; FAS, full-analysis set; GFR, glomerular filtration rate; LS, least square; MDRD4, modification of diet in renal disease-4; MMF, mycophenolate mofetil.

*Derived from an ANCOVA model in which treatment arm, gender, race (black, nonblack), site, and donor status (deceased, living) were included as factors, and eGFR (MDRD4) at randomization and donor age were included as continuous covariates.

†LS mean difference for Arm 2 minus Arm 1; ANCOVA: analysis of covariance.

Table 3. Kaplan–Meier estimates of secondary efficacy variables at Week 52 (ITT).

	Patients with postrandomization event*		Kaplan–Meier estimate difference†	
	Arm 1: prolonged-release tacrolimus + MMF (n = 362)	Arm 2: prolonged-release tacrolimus + sirolimus (n = 368)	% Difference (95% CI)	P value‡
Composite efficacy failure, n (%)	40 (11.5)	67 (18.2)	6.7 (1.5, 11.9)	0.002
Acute rejection, n (%)	26 (7.3)	30 (8.3)	1.0 (−2.9, 4.9)	0.624
BCAR, n (%)	14 (4.3)	13 (3.6)	–0.7 (−3.6, 2.3)	0.892
Graft loss, n (%)	10 (2.9)	8 (2.2)	–0.7 (−3.0, 1.6)	0.676
Patient death, n (%)	4 (1.1)	1 (0.3)	–0.9 (−2.1, 0.4)	0.177
NODM, n (%)	24 (8.5)	36 (12.8)	4.3 (−0.9, 9.5)	0.183

All patients received oral MMF until Day 27. For patients randomized to Arm 2 only, MMF was discontinued and sirolimus was initiated on Day 28 and continued throughout the study.

BCAR, biopsy-confirmed acute rejection; CI, confidence interval; ITT, intent to treat; MMF, mycophenolate mofetil; NODM, new-onset diabetes mellitus.

*Events that happened at or after Week 52 were grouped into Week 52+.

†Kaplan–Meier survival estimates for the incidence of patients with the event (Arm 2–Arm 1).

‡Wilcoxon–Gehan test.

of physician-reported diabetes mellitus [14 of 362 (3.9%) vs. 25 of 368 (6.8%)], hyperlipidemia [12 of 362 (3.3%) vs. 24 of 368 (6.5%)], proteinuria [6 of 362 (1.7%) vs. 22 of 368 (6.0%)], and peripheral edema [42 of 362 (11.6%) vs. 66 of 368 (17.9%)]. A total of three patients in the SAF died postrandomization, two in Arm 1 and one in Arm 2 (Arm 1: acute myocardial infarction and organizing pneumonia; Arm 2: pneumonia).

Discussion

The results from this large, comprehensive, multicenter study show that at Week 52, patients maintained similar renal function with standard-dose, prolonged-release tacrolimus plus MMF compared with lower dose, prolonged-release tacrolimus plus sirolimus. The efficacy and safety profiles of the immunosuppressive regimens were generally comparable overall, with similar incidences of graft and patient survival, AR, BCAR, NODM, and DGF. However, a significantly higher incidence of composite efficacy failure was reported in Arm 2 (sirolimus) versus Arm 1 (MMF); this difference was driven by a higher number of early withdrawals due to AEs in Arm 2. Overall, treatment adherence was high in both arms, and the majority of patients took their medication regularly.

Mean target tacrolimus trough levels were achieved early after prolonged-release tacrolimus initiation in both arms, and tacrolimus trough levels in Arm 1 remained within the target window throughout the study. In Arm 2, after the protocol-stipulated dose reduction on Day 42, tacrolimus trough levels were lower versus Arm 1 from the next trough level measurement at Day 56, but remained higher than the targeted 4–5 ng/ml. As there were 12 days between the 25% dose reduction (Day 42) and the next trough measurement, and taking assay variability into consideration, clinicians may have felt that they could not target the trough level to 4–5 ng/ml with confidence without more frequent trough measurements. Potentially ambiguous instruction on the appropriate procedure in relation to mean trough levels of prolonged-release tacrolimus in the protocol could also have played a role. In Arm 2, sirolimus mean whole-blood trough levels generally remained within the target range, although the mean sirolimus dose was >1 mg throughout the study. Sirolimus trough levels for patients who discontinued due to AEs were within the target range, although generally lower than the overall Arm 2 study population. Mean tacrolimus trough levels for these patients were above target levels at EOS and higher than the overall Arm 2 study population.

Table 4. Most commonly reported postrandomization adverse events (≥5% in either treatment arm) from Day 28 to Week 52 (ITT).

Adverse event	Arm 1: prolonged-release tacrolimus + MMF (n = 362)	Arm 2: prolonged-release tacrolimus + sirolimus (n = 368)
Overall, *n* (%)	307 (84.8)	309 (84.0)
Diarrhea	47 (13.0)	36 (9.8)
Leukopenia	47 (13.0)	9 (2.4)
Cytomegalovirus infection	43 (11.9)	14 (3.8)
Edema peripheral	42 (11.6)	66 (17.9)
Escherichia UTI	39 (10.8)	24 (6.5)
Blood creatinine increased	33 (9.1)	32 (8.7)
UTI bacterial	33 (9.1)	18 (4.9)
Nasopharyngitis	30 (8.3)	29 (7.9)
Tremor	28 (7.7)	26 (7.1)
Cough	24 (6.6)	14 (3.8)
Hypertension	21 (5.8)	22 (6.0)
Renal impairment	21 (5.8)	17 (4.6)
Dyslipidemia	20 (5.5)	22 (6.0)
UTI	20 (5.5)	21 (5.7)
UTI enterococcal	19 (5.2)	26 (7.1)
Anemia	18 (5.0)	22 (6.0)
Kidney transplant rejection	18 (5.0)	17 (4.6)
Diabetes mellitus	14 (3.9)	25 (6.8)
Hypercholesterolemia	13 (3.6)	22 (6.0)
Hyperlipidemia	12 (3.3)	24 (6.5)
Proteinuria	6 (1.7)	22 (6.0)

All patients received oral MMF until Day 27. For patients randomized to Arm 2 only, MMF was discontinued and sirolimus was initiated on Day 28 and continued throughout the study. ITT, intent to treat; MMF, mycophenolate mofetil; UTI, urinary tract infection.

The primary endpoint of renal function (GFR, measured by iohexol clearance) and secondary renal function measures eGFR (MDRD4 and CKD-EPI) and CrCl (Cockcroft–Gault) were comparable between arms at Week 52, indicating that a lower dose prolonged-release tacrolimus/sirolimus-based regimen did not reduce renal function impairment compared with a standard prolonged-release tacrolimus/MMF-based regimen. Compared with earlier studies showing increased nephrotoxicity with calcineurin inhibitor plus sirolimus-based regimens [24], this study confirmed no increase in toxicity with prolonged-release tacrolimus in combination with low-dose sirolimus, which is encouraging for clinical practice. Studies carried out previously using

twice-daily, immediate-release tacrolimus reported similar renal function with tacrolimus/MMF and low-dose tacrolimus/sirolimus-based regimens [19,22].

Iohexol clearance is considered a gold standard for the measurement of GFR in the evaluation of renal function, and this was the first large, randomized study to use iohexol clearance as a primary endpoint in kidney transplantation. Accurate monitoring of renal function following transplantation is essential [25], and although iohexol clearance is considered one of the most accurate methods of assessing renal function, measuring GFR is more complex and time consuming than estimating GFR. For this reason, previous studies evaluating immunosuppressive regimens have relied on estimates of GFR or CrCl to monitor renal function [13,21,26]. The accuracy of GFR estimation methods varies and may be affected by characteristics such as ethnic group, body mass index, and stage of renal failure [27,28], making estimated renal function data compared between studies less reliable.

In the subgroup analysis of the primary variable, a significant interaction effect of treatment-by-donor age (<50 vs. ≥50 years) was observed, although the overall interaction effect of treatment-by-donor age as a continuous variable did not reach statistical significance. When the analysis was performed separately for each donor age group, mean GFR at Week 52 was significantly higher in Arm 2 versus Arm 1 in only the subgroup of patients with donor age <50 years. This is in line with results from a previous study that reported better outcomes with a tacrolimus/sirolimus-based regimen for patients receiving donor kidneys without preexisting interstitial fibrosis and tubular atrophy (generally younger donor age). However, for patients receiving donor kidneys with interstitial fibrosis and tubular atrophy (generally older donor age), improved outcomes were reported with a tacrolimus/MMF-based regimen [20]. These subgroup analyses were a secondary comparison of the primary variable and not adjusted for multiple testing and so should be interpreted with caution; however, the data suggest that specific patient subgroups could potentially benefit from receiving different prolonged-release tacrolimus-based immunosuppressive regimens.

The incidence of the composite endpoint of efficacy failure was significantly higher in Arm 2 versus Arm 1. This difference was driven by a higher incidence of postrandomization withdrawal due to AEs in Arm 2, as incidence of graft loss was comparable between arms. These findings are consistent with other studies that suggest the clinical usage of sirolimus could be limited by tolerability issues [16]. However, it should be noted that although more patients were withdrawn due to AEs in Arm 2, the number of AEs reported was comparable between arms. Results from this study showed similar postrandomization rates of graft and patient survival, AR and BCAR to previously reported studies using prolonged-release tacrolimus [13,29].

A similar number of serious treatment-emergent AEs thought to be related to the study drugs were reported in both arms; however, a significantly higher number of patients (more than twice as many) withdrew permanently from the study due to adverse events in Arm 2 versus Arm 1. A lower incidence of CMV infection was reported in the sirolimus-treated Arm 2 versus Arm 1. This is in line with a previous study, which showed that the rate of CMV infection was higher in patients treated with a low-dose tacrolimus regimen versus a sirolimus regimen [21].

This study had a number of limitations. A tacrolimus plus sirolimus immunosuppressive regimen is not as widely used as the tacrolimus plus MMF regimen. Physicians in this open-label study may have felt less confident in managing patients on this combination, which might have impacted the incidence of patients in Arm 2 who were withdrawn early from the study, in particular, those withdrawn due to AEs. The higher withdrawal rate in Arm 2 versus Arm 1 should be taken into consideration when interpreting the primary analysis. In addition, whole-blood tacrolimus trough levels remained higher than target in Arm 2 from Day 42 to the EOS.

Overall, data from both arms of this study confirm that once-daily, prolonged-release tacrolimus-based immunosuppression is efficacious and has an acceptable tolerability profile over 52 weeks of treatment in *de novo* kidney transplant recipients when used in combination with MMF or delayed-initiation sirolimus. Renal function measured by iohexol clearance was comparable between arms; however, we cannot exclude that the tacrolimus trough levels that remained higher than the target in Arm 2 (in combination with sirolimus) could have been responsible for renal toxicity. While the subgroup analysis suggests that specific groups of patients could benefit from receiving a prolonged-release tacrolimus plus sirolimus regimen (e.g., recipients with a younger donor), additional studies would need to be performed to confirm these findings. However, given the lower incidence of intolerable AEs leading to patient discontinuation from this study, prolonged-release tacrolimus plus MMF may be an improved treatment regimen versus lower dose,

prolonged-release tacrolimus plus sirolimus, although the latter regimen provides a viable alternative.

Authorship

All authors were involved with the interpretation of data and critically reviewing the manuscript at each stage of development. OR, FL, MC, LR, CM, FC and BC were also involved in the data acquisition. RO was involved in the study conception, study design, and data acquisition. MC was involved in the study design and data acquisition. RL was involved with the study conception and data acquisition. GK was involved in the study conception, study design, and data analysis. MB was involved in the study conception and study design. Contributors who did not fulfill authorship criteria are acknowledged.

Funding

This Phase IV study was sponsored by Astellas Pharma. Editorial support was funded by Astellas Pharma.

Conflicts of interest

The authors of this manuscript have conflict of interests to disclose as described by *Transplant International*: L. Rostaing has received honoraria from Novartis, Veloxis, Astellas, Amgen, LSB, Fresenius, Genzyme, and BMS, and has acted as a scientific advisor for Novartis, Astellas, and Genzyme and a consultant for Veloxis; R. Oberbauer has participated in advisory boards for Astellas, Amgen, and Sandoz, has received honoraria from Abbott, Amgen, BMS, Fresenius, and Pfizer, and has received research funding and support for travel from Fresenius; M. Christiaans has received honoraria and travel support from Astellas, attended an Astellas-sponsored advisory board, received an unrestricted grant for an investigator study, and received honoraria for development of an educational presentation from Astellas; R. Langer has received honoraria from Astellas, Novartis, Roche, and Teva; B. Charpentier has participated in studies sponsored by Astellas; M. Brown is employed by Astellas Pharma Inc; G. Kazeem is a consultant statistician working on behalf of Astellas Pharma Europe Ltd, and he has also received support for travel from Astellas; F. Lehner has received honoraria, grants, and support for travel from Novartis, Astellas, Pfizer, and Roche and has participated in studies sponsored by Astellas; O. Rummo, M. Carmellini, C. Mousson, and F. Citterio have no conflict of interests in relation to this publication. This Phase IV study was sponsored by Astellas Pharma, the manufacturer of prolonged-release tacrolimus (Advagraf). Under the direction of the authors, Nina C. Kennard and Amy MacLucas from iS LifeScience drafted the initial version of the manuscript and provided editorial support throughout its development.

Acknowledgements

The authors would like to thank Nina C. Kennard and Amy MacLucas from iS LifeScience for providing editorial support.

APPENDIX 1

Participating centers

Australia: Munish Heer, Wai Lim; *Austria*: Ferdinand Mühlbacher, Johann Pratschke, Rainer Oberbauer; *Belarus*: Oleg O. Rummo; *Belgium*: Cathérine Bonvoisin, Dirk Kuypers; *Czech Republic*: Jarmila Dedochova, Milan Kuman; *France*: Yann Le Meur, Bernard Charpentier, Patrice Deteix, Luc Frimat, Philippe Lang, Yvon Lebranchu, Christophe Legendre, Christiane Mousson, Lionel Rostaing, Pierre Francois Westeel; *Germany*: Frank Lehner, Peter Schenker, Karl-Friedrich Hilgers, Oliver Witzke, Ingeborg Hauser, Volker Kliem, Martin Zeier, Ulrich Kunzendorf, Michael Bartels (previously Sven Jonas), Markus Guba, Heiner H. Wolters; *Hong Kong*: Tak-Mao Chan; *Hungary*: Robert M. Langer; *Italy*: Mario Carmellini, Franco Citterio, Flavia Caputo (previously Vito Sparacino); the *Netherlands*: Maarten Christiaans; *Poland*: Michal Nowicki, Maciej Glyda; *Republic of Korea (South Korea)*: Yu Seun Kim, Jongwon Ha, Chan Duck Kim, Duck Jong Han, Yeong Hoon Kim; *Russia*: Dmitry Perlin, Alexander Sal'mayer, Sergey B. Semchenko, Igor Nesterenko; *Spain*: Antonio Franco, Josep Grinyó, Ainhoa Inza, Juan Carlos Ruiz; *Taiwan*: Yang-Jen Chiang, Po-Chang Lee; *Turkey*: Gultekin Suleymanlar, Sinasi Sevmis, Serdar Kacar.

REFERENCES

1. Hardinger KL, Brennan DC. Novel immunosuppressive agents in kidney transplantation. *World J Transplant* 2013; **3**: 68.
2. Mayer AD. Chronic rejection and graft half-life: five-year follow-up of the European Tacrolimus Multicenter Renal Study. *Transplant Proc* 2002; **34**: 1491.
3. Gondos A, Döhler B, Brenner H, Opelz G. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. *Transplantation* 2013; **95**: 267.
4. Chapman JR, O'Connell PJ, Nankivell BJ. Chronic renal allograft dysfunction. *J Am Soc Nephrol* 2005; **16**: 3015.
5. El-Zoghby ZM, Stegall MD, Lager DJ, *et al.* Identifying specific causes of kidney allograft loss. *Am J Transplant* 2009; **9**: 527.
6. Spasovski G, Masin-Spasovska J. How to improve the survival of the kidney transplant – is it only the pharmaceutical management? *Expert Opin Pharmacother* 2014; **15**: 5.
7. Borra LCP, Roodnat JI, Kal JA, Mathot RA, Weimar W, Van Gelder T. High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term outcome after kidney transplantation. *Nephrol Dial Transplant* 2010; **25**: 2757.
8. Isakova T, Xie H, Messinger S, *et al.* Inhibitors of mTOR and risks of allograft failure and mortality in kidney transplantation. *Am J Transplant* 2013; **13**: 100.
9. Sellarés J, de Freitas DG, Mengel M, *et al.* Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* 2012; **12**: 388.
10. Halloran P. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; **351**: 2715.
11. Rostaing L, Cantarovich D, Mourad G, *et al.* Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. *Transplantation* 2005; **79**: 807.
12. Shihab F, Christians U, Smith L, Wellen JR, Kaplan B. Focus on mTOR inhibitors and tacrolimus in renal transplantation: pharmacokinetics, exposure–response relationships, and clinical outcomes. *Transpl Immunol* 2014; **31**: 22.
13. Albano L, Banas B, Klempnauer J, *et al.* OSAKA trial: a randomized, controlled trial comparing tacrolimus QD and BD in kidney transplantation. *Transplantation* 2013; **96**: 897.
14. van Hooff JP, Squifflet J-P, Wlodarczyk Z, Vanrenterghem Y, Paczek L. A prospective randomized multicenter study of tacrolimus in combination with sirolimus in renal-transplant recipients. *Transplantation* 2003; **75**: 1934.
15. Gelens MACJ, Christiaans MHL, van Heurn ELW, van den Berg-Loonen EPM, Peutz-Kootstra CJ, van Hooff JP. High rejection rate during calcineurin inhibitor-free and early steroid withdrawal immunosuppression in renal transplantation. *Transplantation* 2006; **82**: 1221.
16. Vitko S, Wlodarczyk Z, Kyllönen L, *et al.* Tacrolimus combined with two different dosages of sirolimus in kidney transplantation: results of a multicenter study. *Am J Transplant* 2006; **6**: 531.
17. Mendez R, Gonwa T, Yang HC, Weinstein S, Jensik S, Steinberg S. A prospective, randomized trial of tacrolimus in combination with sirolimus or mycophenolate mofetil in kidney transplantation: results at 1 year. *Transplantation* 2005; **80**: 303.
18. Halleck F, Duerr M, Waiser J, *et al.* An evaluation of sirolimus in renal transplantation. *Expert Opin Drug Metab Toxicol* 2012; **8**: 1337.
19. Lo A, Egidi MF, Gaber LW, *et al.* Observations regarding the use of sirolimus and tacrolimus in high-risk cadaveric renal transplantation. *Clin Transplant* 2004; **18**: 53.
20. Gelens MA, Steegh FM, van Hooff JP, *et al.* Immunosuppressive regimen and interstitial fibrosis and tubules atrophy at 12 months postrenal transplant. *Clin J Am Soc Nephrol* 2012; **7**: 1010.
21. Ekberg H, Tedesco-Silva H, Demirbas A, *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
22. Russ GR, Campbell S, Chadban S, *et al.* Reduced and standard target concentration tacrolimus with sirolimus in renal allograft recipients. *Transplant Proc* 2003; **35**: 115.
23. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; **33**(Suppl. 1): S11.
24. Oberbauer R, Segoloni G, Campistol JM, *et al.* Early cyclosporine withdrawal from a sirolimus-based regimen results in better renal allograft survival and renal function at 48 months after transplantation. *Transpl Int* 2005; **18**: 22.
25. Marcén R, Dal Canton A. Glomerular filtration rate: utility for assessing long-term renal allograft outcomes in kidney allograft recipients. *J Nephrol* 2013; **26**: 1009.
26. Bechstein W, Paczek L. A comparative, randomized trial of concentration-controlled sirolimus combined with reduced-dose tacrolimus or standard-dose tacrolimus in renal allograft. *Transplant Proc* 2013; **45**: 2133.
27. Buron F, Hadj-Aissa A, Dubourg L. Estimating glomerular filtration rate in kidney transplant recipients: performance over time of four creatinine-based formulas. *Transplantation* 2011; **92**: 1005.
28. Earley A, Miskulin D, Lamb E, Levey A, Uhlig K. Estimating equations for glomerular filtration rate in the era of creatinine standardization: a systematic review. *Ann Intern Med* 2012; **156**: 785.
29. Krämer BK, Charpentier B, Bäckman L, *et al.* Tacrolimus once daily (ADVAGRAF) versus twice daily (PROGRAF) in de novo renal transplantation: a randomized phase III study. *Am J Transplant* 2010; **10**: 2632.