Received: 25 October 2018 | Revised: 4 December 2018 | Accepted: 27 December 2018

DOI: 10.1111/ajt.15251

ORIGINAL ARTICLE

AJT

A randomized trial of everolimus-based quadruple therapy vs standard triple therapy early after lung transplantation

Jens Gottlieb[1,2] | **Claus Neurohr**[3] | **Joachim Müller-Quernheim**[4] | **Hubert Wirtz**[5] | **Bjoern Sill**[6] | **Heinrike Wilkens**[7] | **Vasiliki Bessa**[8] | **Christoph Knosalla**[9] | **Martina Porstner**[10] | **Carmen Capusan**[10] | **Martin Strüber**[11]

[1]Department of Respiratory Medicine, Hannover Medical School, Hannover, Germany

[2]Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH), Member of the German Center for Lung Research (DZL), Hannover, Germany

[3]Grosshadern Medical Clinic and Policlinic V, LMU Munich University Hospital, Munich, Germany

[4]Pulmonology Medical Clinic, Freiburg University Hospital, Freiburg, Germany

[5]Internal Medicine/Department of Pneumology, Leipzig University Hospital, Leipzig, Germany

[6]Department of Cardiovascular Surgery, Hamburg-Eppendorf University Hospital, Hamburg, Germany

[7]Internal Medicine V, University Hospital of Saarland, Homburg Saar, Germany

[8]Department of Pneumology, Ruhrlandklinik, West German Center for Lung Transplantation, University Hospital Essen, University Duisburg-Essen, Essen, Germany

[9]Department of Cardiothoracic and Vascular Surgery, German Heart Institute Berlin, DZHK (German Center for Cardiovascular Research), Berlin, Germany

[10]Novartis Pharma GmbH, Nuremberg, Germany

[11]Department of Cardiothoracic Surgery, Newark Beth Israel Medical Center, Children's Hospital of New Jersey, Newark, New Jersey

Correspondence
Jens Gottlieb
Email: Gottlieb.Jens@mh-hannover.de

Funding information
The study was funded by Novartis Pharma GmbH, Nuremberg, Germany

Calcineurin inhibitor (CNI) therapy after lung transplantation increases risk of kidney failure. Early everolimus-based quadruple low CNI immunosuppression may improve renal function without compromising efficacy or safety. A prospective, randomized, open-label, 12-month multicenter trial was conducted at 8 German sites. Patients 3-18 months after lung transplantation were randomized (1:1), stratified by baseline estimated glomerular filtration rate (eGFR). In the quadruple low CNI regimen, patients received everolimus (target trough level 3-5 ng/mL) with reduced CNI (tacrolimus 3-5 ng/mL or cyclosporine 25-75 ng/mL) and a cell cycle inhibitor plus prednisone. In the standard triple CNI regimen, patients received tacrolimus (target trough level >5 ng/mL) or cyclosporine (>100 ng/mL) and a cell cycle inhibitor plus prednisone. Of the 180 patients screened, 130 were randomized: 67 in the quadruple low CNI group and 63 in the standard triple CNI group. The primary endpoint (eGFR after 12 months) demonstrated superiority of the quadruple low CNI regimen: 64.5 mL/min vs

Abbreviations: ANCOVA, analysis of covariance; BOS, bronchiolitis obliterans syndrome; BPAR, biopsy-proven acute rejection; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; CNI, calcineurin inhibitor; CsA, cyclosporine; EC-MPS, enteric-coated mycophenolate sodium; eGFR, estimated GFR; FEV_1, forced expiratory volume in 1 second; GFR, glomerular filtration rate; ITT, intention-to-treat; LOCF, last observation carried forward; LS, least square; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; SD, standard deviation; SF-36, Short Form Health Survey.

54.6 mL/min for the standard triple group (least squares mean, analysis of covariance; $P < .001$). Key efficacy parameters (biopsy-proven acute rejection, chronic lung allograft dysfunction, and death) and safety endpoints were similar between both groups. Quadruple low CNI immunosuppression early after lung transplantation was demonstrated to be efficacious and safe. Clinical trials registry: ClinicalTrials.gov NCT01404325.

KEYWORDS

clinical research/practice, cyclosporine A (CsA), everolimus, immunosuppressant - calcineurin inhibitor, immunosuppressant - mechanistic target of rapamycin, immunosuppressant - mechanistic target of rapamycin (mTOR), immunosuppression/immune modulation, lung transplantation/pulmonology, rejection, tacrolimus

1 | INTRODUCTION

The number of lung transplants has been rising steadily over the past decades.[1] Advances in donor management and preservation methods have expanded the donor pool and, increasingly, elderly patients with comorbidities are undergoing transplantation.[1,2] Long-term outcomes, however, remain inferior compared to other forms of solid organ transplantation. Fifty-six percent of lung transplant recipients survive more than 5 years,[1] with chronic lung allograft dysfunction (CLAD) and infections being the leading causes of death.[1]

Lung transplant recipients are at high risk of graft rejection, necessitating a more intensive immunosuppressive therapy relative to other solid organ transplants.[3] Maintenance therapy typically consists of a calcineurin inhibitor (CNI), a cell cycle inhibitor, and corticosteroids.[4] Chronic CNI therapy, however, is frequently associated with nephrotoxicity[5] and is an independent predictor for a decline in glomerular filtration rate (GFR) after lung transplantation.[6] This effect is dose dependent.[7,8] End-stage kidney disease including the need for renal transplantation affects almost 5% of the recipients within the first 5 years.[9] End-stage kidney disease adversely affects mortality. In large epidemiological studies, an estimated glomerular filtration rate (eGFR) <60 mL/min per 1.73 m^2 was independently associated with higher rates of cardiovascular deaths, overall mortality, and hospitalizations.[10,11] These effects were more pronounced in patients with lower GFR.

Maintaining immunosuppressive efficacy and preserving long-term renal function has prompted interest in the use of mammalian target of rapamycin (mTOR) inhibitors to minimize CNI exposure. Additionally, consistent with evidence from renal[12,13] and heart[14] transplantation, the risk of cytomegalovirus (CMV) infections is reduced in lung transplant patients receiving an mTOR inhibitor–based immunosuppressive protocol.[15-17]

Everolimus in conjunction with reduced-exposure CNI therapy maintains immunosuppressive efficacy in de novo renal,[18] liver,[19] and heart[20] transplant patients. In lung transplantation, 2 randomized trials of everolimus with reduced CNI exposure, initiated early posttransplant, have shown a lower rate of acute rejection than standard CNI-based triple therapy in comparison to mycophenolic acid (MPA) formulations without affecting the incidence of CLAD.[15,21] In terms of renal function, de novo use of everolimus with moderately reduced CNI exposure does not appear to influence posttransplant renal deterioration after lung transplantation.[15,21] In contrast, a moderate improvement in renal function was observed in long-term thoracic transplant recipients, including lung transplanted patients, who were switched to add-on everolimus with low-dose CNI therapy late after transplantation.[22,23]

The aim of the current study was to demonstrate that everolimus with low CNI exposure in a quadruple immunosuppression regimen is superior to a standard triple CNI regimen in terms of renal function, as assessed by eGFR, in patients with impaired kidney function early after lung transplantation. The study included patients with mild-to-moderate impairment of kidney function and excluded those with severe impairment in whom an improvement in function was less likely.

2 | METHODS

2.1 | Study design and conduct

The Efficacy of Everolimus in Combination with Specific Standard Immunosuppressive Regimen Lung Transplant Recipients (4EVERLUNG) study was performed at 8 lung transplant centers in Germany between February 2012 (first patient in) and January 2017 (last patient out). The study was conducted in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice and the ethical principles laid down in the Declaration of Helsinki. The study protocol was approved by the institutional review board or ethics committee at each center and was registered at clinicaltrials.gov (NCT01404325). All patients provided written informed consent.

In this 12-month, prospective, open-label study, patients with mild-to-moderate renal dysfunction (eGFR ≥50 and ≤90 mL/min per 1.73 m^2 according to the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] formula) were randomized at 3-18 months after lung transplantation to a regimen of everolimus with reduced CNI exposure (quadruple low CNI) or to standard CNI therapy (standard triple CNI). All patients in both groups received a cell cycle inhibitor and steroids. The study design is summarized in Figure 1.

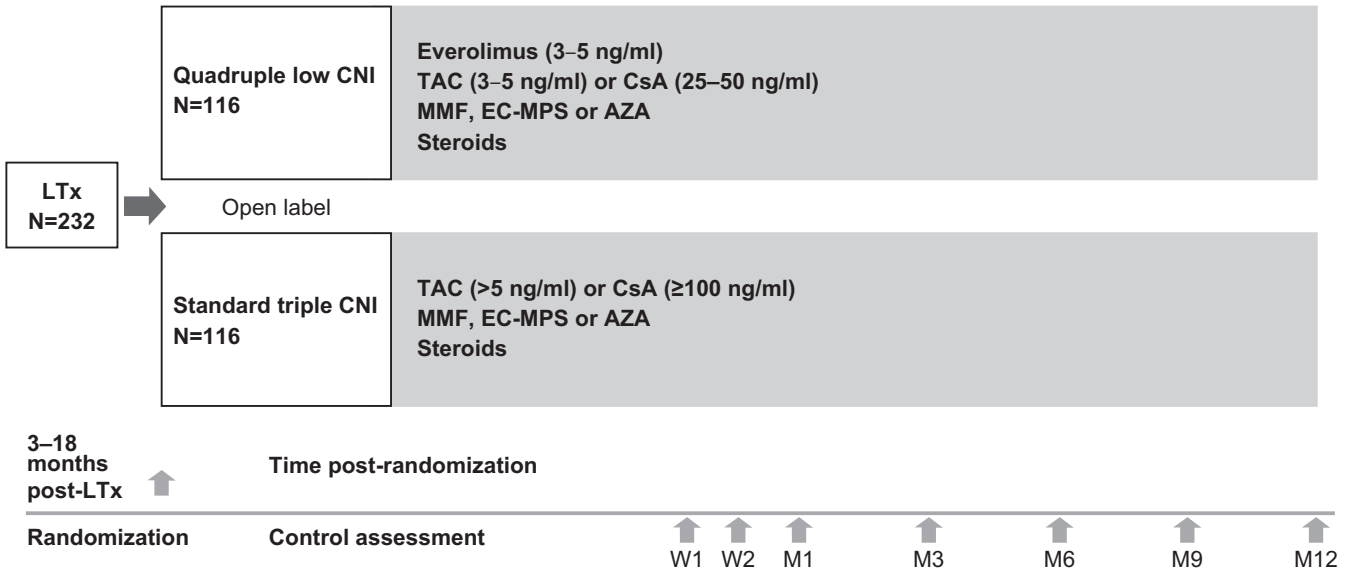


FIGURE 1 Study design. AZA, azathioprine; CNI, calcineurin inhibitor; EC-MPS, enteric-coated mycophenolate sodium; MMF, mycophenolate mofetil; CsA, cyclosporine; TAC, tacrolimus; LTx, lung transplantation; W, week; M, month

2.2 | Eligibility criteria

The study population comprised adults (>18 years) who had received a lung transplant from a deceased donor 3-18 months prior to study entry. All patients were required to have mild-or-moderate renal impairment, defined as eGFR ≥50 and ≤90 mL/min per 1.73 m^2 (CKD-EPI), prior to inclusion confirmed by 1 measurement prior to screening (maximum 42 days), a second measurement at screening, and a third measurement at baseline (ie, the randomization visit). Any 1 of these 3 measurements in the range ≥50 and ≤90 mL/min per 1.73 m^2 qualified patients for study entry. All 3 measurements had to be in the range ≥40 and ≤100 mL/min per 1.73 m^2.

Patients were also required to be receiving CNI therapy, a cell cycle inhibitor, and steroids at the time of randomization. Key exclusion criteria were bronchiolitis obliterans syndrome (BOS) grade >1 at time of randomization, ≥2 episodes of antibody-treated acute rejection or ≥1 steroid-sensitive episode of acute rejection in the 3 months prior to randomization, urinary protein excretion >1 g/24 hours at randomization, a history of thrombotic microangiopathy, and significant cytopenia, as per protocol-specified definitions (see Table S1 for detailed exclusion criteria).

2.3 | Randomization and treatment

Randomization (1:1 ratio) was performed centrally. The randomization list was produced by an independent statistician not otherwise involved in the study using a validated system that automated the random assignment of treatment arms to randomization numbers in the specified ratio. Investigators were notified of the randomization group by fax. Randomization was stratified by eGFR at the baseline visit, ie, at randomization (40 to 60 mL/min per 1.73 m^2, >60 to 75 mL/min per 1.73 m^2, and >75 to 100 mL/min per 1.73 m^2).

In patients randomized to the quadruple low CNI regimen, everolimus was initiated within 24 hours after randomization to target a trough concentration of 3-5 ng/mL. Immediate reduction of the current CNI dose by 50% was recommended, to target a tacrolimus trough concentration of 3-5 ng/mL or a cyclosporine (CsA) trough concentration of 25-75 ng/mL in conjunction with mycophenolate mofetil (MMF, maximum dose 2000 mg/d) or enteric-coated mycophenolate sodium (EC-MPS, maximum dose 1440 mg/d) or azathioprine (2 mg/kg with leukocyte count maintained at 5000/μL), and corticosteroids (prednisone equivalent ≤0.15 mg/kg) were to be given from randomization to the end of the study (Figure 1).

In patients randomized to the standard triple CNI arm, triple immunosuppression was continued as per center practice, with either a tacrolimus trough concentration >5 ng/mL or a CsA trough concentration ≥100 ng/mL (≥50 ng/mL at 2 years posttransplant), with MMF, EC-MPS, or azathioprine without specified doses and steroids (prednisone equivalent ≤0.2 mg/kg).

Prophylactic treatment for CMV was at the discretion of the investigator. Lifelong prophylactic treatment for *Pneumocystis carinii* pneumonia with trimethoprim/sulfamethoxazole was recommended; aerosolized pentamidine or dapsone could be administered to patients unable to tolerate oral trimethoprim/sulfamethoxazole.

2.4 | Evaluation and definitions

The primary endpoint was renal function at month 12 after randomization, as assessed by GFR estimated by the CKD-EPI formula.[24] Secondary endpoints are shown in Table S2. Exercise capacity was assessed by the 6-minute walk test. Quality of life was assessed using the Short Form Health Survey (SF-36), where higher scores on a scale of 1-100 indicate better quality of life. Both were measured at randomization and 12 months thereafter. CLAD was defined as persistent

decline of forced expiratory volume in 1 second (FEV_1) below 81% of baseline.[25] Acute rejection was defined as the presence of at least 1 symptom (dyspnea, fever, or malaise) or finding (infiltrates, decrease of FEV_1 ≥10% compared to previous measurement, pleural effusion, biopsy ≥A0) combined with exclusion of new infective agents and reversibility by rescue immunosuppression. When diagnosing acute rejection, other possible causes of pulmonary dysfunction such as infections had to be excluded and reversibility in response to standard treatment had to be proven. Biopsy-proven acute rejection (BPAR) was defined as acute rejection with biopsy grade >A0. Treatment-emergent adverse events are reported, ie, adverse events that started at the date of randomization or subsequently, up to the end of the study period (excluding events occurring ≥7 days after study drug discontinuation). Where no new causative organism was identified in cases of infection, the source was reported as "not specified." CMV infection as an adverse event was defined at the discretion of the investigator.

2.5 | Statistical analysis

A sample size of 116 randomized patients in each group, allowing for a dropout rate of 20%, was calculated to have 80% power to detect a difference in mean eGFR of 7 mL/min per 1.73 m^2 between groups at month 12 based on a SD of 15 mL/min per 1.73 m^2 and using a *t* test with a 5% 2-sided significance level. A difference of 7 mL/min per 1.73 m^2 was considered appropriate based on evidence from the Nordic Certican Trial in Heart and Lung Transplantation (NOCTET) study,[22] allowing for the shorter time from transplantation expected in the current trial compared to that in the NOCTET study population.[22] Due to slow recruitment, enrollment was stopped in December 2015 when 130 patients had been randomized. Post hoc, the power to detect a between-group difference for the primary endpoint was calculated to be 62%. No interim analysis was performed at that time point.

The primary endpoint was evaluated using analysis of covariance (ANCOVA) with treatment and center as factors, and eGFR at randomization as covariate. Unadjusted values and adjusted means (least square [LS] means) are presented with 2-sided 95% confidence intervals (CIs) and a 2-sided *P* value. If a patient discontinued from the study prematurely after randomization, missing data were imputed via a multiple imputation procedure. As a sensitivity analysis, the primary analysis was repeated (1) with missing values imputed by the last observation carried forward (LOCF) method using the last available postbaseline value and (2) in the per protocol population based on unadjusted values.

The intention-to-treat (ITT) population comprised all randomized patients who received at least 1 dose of study drug and provided a valid eGFR value at randomization. The per protocol population comprised all ITT patients without major protocol deviations. The safety population comprised all patients who received at least 1 dose of study drug and underwent at least 1 postrandomization safety assessment.

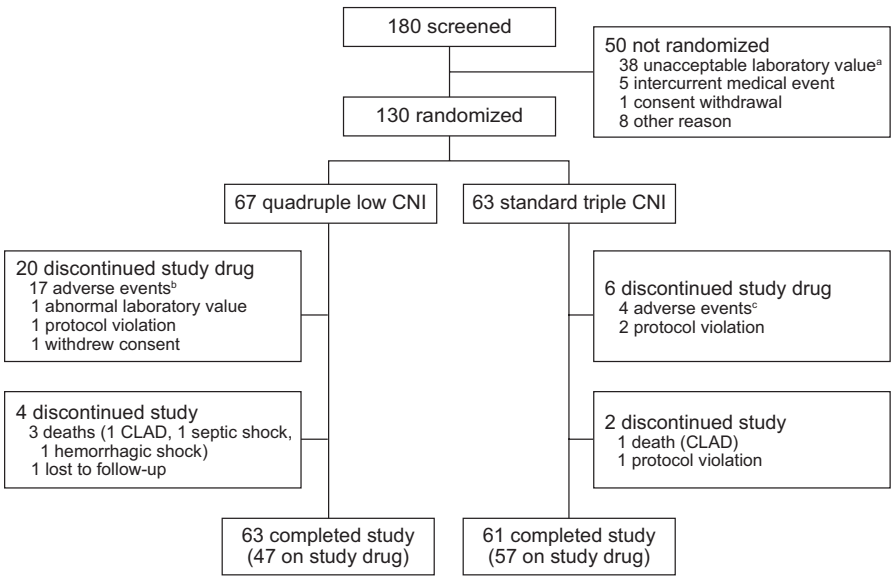
2.6 | Role of the funding source

The funders of the study (Novartis Pharma GmbH) contributed to the study design and coordinated data collection, and reviewed drafts of the manuscript for factual accuracy. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3 | RESULTS

3.1 | Study population

Of the 180 screened patients, 130 were randomized (Figure 2). Sixty-seven patients were randomized to the quadruple low CNI



[a]20 abnormal eGFR, 14 low eGFR, 4 leukopenia
[b]4 transplant rejection, 3 leukopenia, 10 other
[c]1 transplant rejection/pulmonary function decline, 2 hematological abnormality, 1 worsening of general condition

FIGURE 2 CONSORT diagram. CLAD, chronic lung allograft dysfunction; CNI, calcineurin inhibitor; CONSORT, Consolidated Standards of Reporting Trials; eGFR, estimated glomerular filtration rate

group: 63 of these (94%) completed the study and 47 (70%) were still on study drug at the end of the study. Of the 63 patients randomized to standard triple CNI therapy, 61 (97%) completed the study, with 57 (90%) remaining on study drug. All 130 randomized patients were included in the ITT and safety populations, while the per protocol population included 89 patients (37 quadruple low CNI therapy, 52 standard triple CNI therapy). The most frequent reasons for exclusion from the per protocol population were discontinuation of study drug before the month 12 visit (20 quadruple low CNI therapy, 6 standard triple CNI therapy) and study drug exposure outside target range on ≥2 consecutive study visits (10 quadruple low CNI therapy, 3 standard triple CNI therapy).

Patient demographics and clinical characteristics at the time of randomization were well matched between the 2 treatment groups other than a slightly higher rate of hypertension in the standard triple CNI arm (Table 1). Prior to randomization, 13 patients (19%) in the quadruple low CNI group and 16 patients (25%) in the standard triple CNI group had experienced acute rejection before inclusion. The median time between transplantation and randomization was 10.6 months in the quadruple low CNI group and 8.7 months in the standard triple CNI group. At randomization, the mean (SD) unadjusted eGFR was 65.7 (11.5) mL/min per 1.73 m^2 in the quadruple low CNI group and 67.3 (11.7) mL/min per 1.73 m^2 in the standard triple CNI group (P = .437).

3.2 | Immunosuppression

In the quadruple low CNI group, the mean (SD) everolimus level was 4.2 (1.4) ng/mL at month 1 and 4.3 (1.1) ng/mL at month 12 (Figure S1A). The proportion of patients with an everolimus concentration below target was 5% to 15% between months 1 and 12 in the quadruple low CNI group. In this group, 45 patients were receiving tacrolimus and 22 were receiving CsA at randomization. Mean tacrolimus exposure from month 1 to month 12 was 5.4 (1.9) ng/mL at month 12 (Figure S1B); between 47% and 56% of the patients were within target range and 18%–27% of the patients exceeded 5 ng/mL at any given study visit. Mean CsA levels were within target range at most time points in the quadruple low CNI group (Figure S1C); between 76% and 94% of the patients were within target range and 6%-24% were above target range at any given study visit. The majority of patients (63/67 [94%]) were receiving MMF. The mean (SD) dose of MMF from month 1 to the end of the study was 1472 (666) mg/d, with 48% receiving a mean dose of <2 g over this period in the quadruple group.

In the standard triple CNI group, 45 patients were receiving tacrolimus and 18 patients were receiving CsA at randomization. The mean tacrolimus level was ≈10 ng/mL throughout the study (Figure S1B), with between 98% and 100% of patients above the minimum target threshold at any given study visit. The mean CsA level was ≈105 ng/mL (Figure S1C), with 50%-77% of the patients above the minimum target threshold at any given study visit. In the standard triple CNI group, 68 patients (92%) received MMF. The mean (SD) dose of MMF from month 1 to the end of the study was 1472 (666) mg/d in the standard triple group, with 52% of the patients receiving a mean dose of <2 g or over the study period.

3.3 | Renal function

The primary endpoint, adjusted mean of eGFR at month 12 postrandomization, was 64.5 mL/min per 1.73 m^2 (95% CI 59.4; 69.6) in the quadruple low CNI group and 54.6 mL/min per 1.73 m^2 (95% CI 49.5; 59.7) in the standard triple CNI group with a difference of 9.9 mL/min per 1.73 m^2 (95% CI 5.3; 14.5), P < .001 (LS mean values, ANCOVA). A significant between-group difference was also seen when the analysis was confirmed using the LOCF method or in the per protocol population (Table 2).

In the standard triple CNI group there was a gradual decrease in eGFR over the 12 months after randomization (Figure 3). In contrast, mean eGFR increased in the quadruple low CNI group within the first week after randomization, becoming significantly higher than in the standard triple CNI group by month 1 after randomization and remaining so thereafter (Figure 3). At month 12 postrandomization, the mean (SD) unadjusted eGFR was 68.6 (16.3) mL/min per 1.73 m^2 in the quadruple low CNI group vs 61.2 (14.3) mL/min per 1.73 m^2 in the standard triple CNI group (P = .006) (LOCF). Similar results were seen when eGFR was calculated using the Modification of Diet in Renal Disease or Cockcroft-Gault method, but the between-group difference was nonsignificant at the month 12 visit when eGFR was calculated by the cystatin C–based Hoek's formula (P = .184) (Table 2, Table S3).

In a post hoc analysis, the primary endpoint was calculated according to baseline eGFR. For patients with baseline eGFR in the range 40-60 mL/min per 1.73 m^2, the between-group difference was 15.4 mL/min per 1.73 m^2 (95% CI 5.8; 25.0) in favor of the quadruple low CNI group (P = .003); for patients with baseline eGFR in the range 61-75 mL/min per 1.73 m^2, the difference was 6.6 mL/min per 1.73 m^2 (95% CI −1.2; 14.3) (P = .097); and for patients with baseline eGFR in the range 76-100 mL/min per 1.73 m^2 the difference was 6.1 mL/min per 1.73 m^2 (95% CI −3.3; 15.6) (P = .194) (LS mean values, ANCOVA).

Between baseline and month 12, eGFR improved by >10 mL/min per 1.73 m^2 in 30% (20/67) and 3% (2/63) of the patients in the quadruple low CNI and standard triple CNI groups, respectively (P < .001). The proportion of patients in whom eGFR declined by >10 mL/min per 1.73 m^2 was 24% (16/67) in the quadruple low CNI group and 38% (24/63) in the standard triple CNI group (P = .090). No patient required renal replacement therapy during the study.

Mean (SD) total protein at month 12 was similar in both treatment groups: 66.8 (5.0) g/L in the quadruple low CNI group and 66.9 (4.6) g/L in the standard triple CNI group. New-onset proteinuria was not reported for patients in the quadruple low CNI group, and for 1 patient in the standard triple CNI group.

3.4 | Immunosuppressive efficacy

By month 12, 10 patients had experienced 11 episodes of clinically suspected acute rejection during treatment in each group (P = .811),

TABLE 1 Baseline characteristics (ITT/safety population)

	Quadruple low CNI (N = 67)	Standard triple CNI (N = 63)
Age (y), median [25th, 75th quartiles]	58 [49, 61]	56 [50, 60]
Sex (male/female), n (%)	40 (60)/27 (40)	41 (65)/22 (35)
Body mass index, mean ± SD	24 ± 4	25 ± 3
Blood pressure (mm Hg), mean ± SD	135/80	135/80
White, n (%)	67 (100)	61 (97)
Concurrent disease at time of transplantation, n (%)		
Diabetes mellitus	5 (7)	3 (5)
Hypertension	24 (36)	30 (48)
Primary diagnosis, n (%)		
COPD/emphysema	28 (42)	27 (43)
Pulmonary fibrosis	18 (27)	18 (29)
Cystic fibrosis	8 (12)	5 (8)
Other	13 (19)	13 (21)
eGFR category, n (%)		
40-60 mL/min per 1.73 m^2	23 (34)	17 (27)
61-75 mL/min per 1.73 m^2	29 (43)	29 (46)
76-100 mL/min per 1.73 m^2	15 (22)	17 (27)
eGFR (CKD-EPI), mL/min per 1.73 m^2		
Mean ± SD	66 ± 12	67 ± 12
Median [25th, 75th quartiles]	64 [57, 72]	65 [58, 77]
CMV risk category, n (%)		
High (D+/R-)	17 (25)	15 (24)
Intermediate (D+/R+, D-/R+)	37 (55)	31 (49)
Low (D-/R-)	13 (19)	15 (24)
Time posttransplant at randomization, mo		
Median [25th, 75th quartiles]	10.9 [6.5, 14.4]	8.7 [6.5, 12.4]
Prior acute rejection episodes n (%)	13 (19)	16 (25)
Donor age (y)		
Median [25th, 75th quartiles]	52 [42, 60]	50 [39, 56]
Cold ischemia time (h), mean ± SD	8.6 ± 2.5	9.0 ± 2.6
Transplant procedure, n (%)		
Unilateral left	3 (4.5)	2 (3.2)
Unilateral right	3 (4.5)	3 (4.8)
Bilateral	61 (91.0)	58 (92.1)
Immunosuppression, n (%)		
Cyclosporine	22 (33)	18 (29)
Tacrolimus	45 (67)	45 (71)
Mycophenolate mofetil	63 (94)	58 (92)
Enteric-coated mycophenolate sodium	2 (3)	1 (2)
Azathioprine	2 (3)	4 (6)
Steroids (prednisone)	67 (100)	63 (100)

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CMV, cytomegalovirus; CNI, calcineurin inhibitor; COPD, chronic obstructive pulmonary disease; D, donor; ITT, intention-to-treat; R, recipient; SD, standard deviation.

including 6 episodes of BPAR (n = 5 grade A1, n = 1 grade A2) in the quadruple low CNI group and 4 episodes of BPAR (all grade A1) in the standard triple CNI group (P = .670). In total, 45 and 41 biopsies (P = .738) were performed in the quadruple low CNI and standard triple CNI groups. The majority of biopsy results (77% in the quadruple low CNI group, 76% in the standard triple CNI group) were A0, similar in both groups (P = .956).

New-onset CLAD developed in 5 patients (7%) in the quadruple low CNI group and 9 patients in the standard triple CNI group (14%) by month 12 (P = .197).

One patient in each group died due to CLAD (log rank P = .897). Two additional patients died in the quadruple low CNI group, due to septic shock (day 239) and hemorrhagic shock (day 209). There was no difference in mortality between the groups (log rank P = .251). All deaths occurred >30 days after study drug discontinuation.

3.5 | Exercise capacity and quality of life

The mean (SD) distance in the 6-minute walk test in the quadruple low CNI group and standard triple CNI groups, respectively, was 475 (80) m vs 492 (92) m at baseline, and 484 (104) m vs 496 (104) at month 12 (P = .785).

At month 12, the mean (SD) physical component summary score of the SF-36 was 47.2 (8.8) vs 47.7 (7.1) in the quadruple low CNI and standard triple CNI groups, respectively (P = .734), while the mean (SD) mental component summary score was 49.7 (11.8) vs 53.8 (9.1) (P = .032).

3.6 | Safety

Almost all patients experienced at least 1 adverse event (quadruple low CNI 99%, standard triple CNI 97%; P = .611), with acne (P = .002) and peripheral edema (P = .027) occurring more frequently in the quadruple low CNI arm (Table 3). Adverse events with a suspected relation to study drug occurred in 47 patients (70%) in the quadruple low CNI group and in 40 patients (64%) in the standard triple CNI group (P = .459), with acne again more frequent in the quadruple low CNI group (P = .009) (Table 3). Serious adverse events were reported in 29

TABLE 2 Renal endpoints at month 12 postrandomization

ANOVA analysis: eGFR (CKD-EPI), mL/min per 1.73 m^2	Quadruple low CNI LS mean (95% CI)	Standard triple CNI LS mean (95% CI)	Difference LS mean (95% CI)	*P* value
ITT population, multiple imputation	64.5 (59.4; 69.6)	54.6 (49.5; 59.7)	9.9 (5.3; 14.5)	<.001
ITT population, LOCF	64.4 (59.1; 69.7)	55.4 (50.1; 60.7)	9.1 (4.3; 13.8)	<.001
Per protocol population[a]	72.1 (65.9; 78.2)	60.1 (55.0; 65.2)	12.0 (6.5; 17.5)	<.001
Unadjusted eGFR[b], mL/min per 1.73 m^2	**Mean (SD)**	**Mean (SD)**	***P* value**	
CKD-EPI formula[24]	68.6 (16.3)	61.2 (14.3)	.006	
Hoek's formula[26c]	60.8 (14.2)	57.5 (14.1)	.184	
MDRD formula[27]	67.0 (14.8)	60.5 (14.4)	.033	
Cockcroft-Gault formula[28]	76.2 (20.6)	68.4 (17.8)	.023	

ANOVA, analysis of variance; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CNI, calcineurin inhibitor; eGFR, estimated GFR; ITT, intention-to-treat; LOCF, last observation carried forward; LS, least squares; MDRD, Modification of Diet in Renal Disease; SD, standard deviation.

[a]Quadruple low CNI, n = 37; standard triple CNI, n = 52.

[b]Last observation carried forward (LOCF) method.

[c]Hoek's formula calculates GFR = −4.32 + 80.35/cystatin C (mg/L).

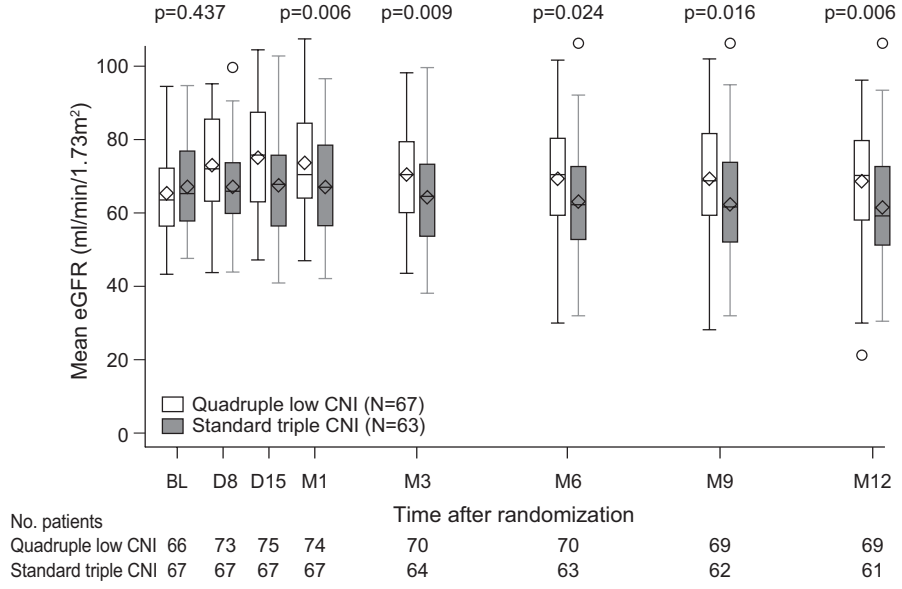


FIGURE 3 Mean unadjusted eGFR, CKD-EPI according to treatment group (last observation carried forward method). Vertical bars indicate standard deviation. BL, baseline; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; D, day; M, month

TABLE 3 Adverse events, infections, and hospitalizations (safety population)

	Quadruple low CNI (n = 67)	Standard triple CNI (n = 63)	*P* value
Any adverse event, n (%)	66 (99)	61 (97)	.611
Any adverse event with a suspected relation to study drug, n (%)	47 (70)	40 (64)	.459
Any serious adverse event, n (%)	29 (43)	22 (35)	.372
Any fatal serious adverse event, n (%)	0	0	—
Any adverse event leading to study drug discontinuation, n (%)	17 (25)	0 (0)	<.001
Any adverse event requiring dose adjustment, n (%)	24 (36)	35 (56)	.034
Selected adverse events occurring in ≥5% of patients in either treatment group, n (%)			
Leukopenia	15 (22)	20 (32)	.242
Nasopharyngitis	17 (25)	17 (27)	.845
Peripheral edema	22 (33)	10 (16)	.027
Decreased FEV_1	15 (22)	13 (21)	.834
Acne	12	1	.002
CMV infection	10 (15)	14 (22)	.367
Diarrhea	9 (13)	12 (19)	.477
Nausea	9 (13)	10 (16)	.805
Respiratory tract infection	9 (13)	8 (13)	1.000
Infections – no. of events			
Specified	41	38	—
Not specified	33	41	—
Bacterial infection	14	9	—
Viral infection	21	28	—
Fungal infection	6	1	—
(Prolonged) hospitalization – no. of events, n	91	65	.520
(Prolonged) hospitalization – no. of patients with events (%)	24 (36)	19 (30)	.577

CMV, cytomegalovirus; CNI, calcineurin inhibitor; FEV_1, forced expiratory volume in 1 second.

quadruple low CNI patients and 22 standard triple CNI patients (43% vs 35%, *P* = .372), the most common being decreased FEV (quadruple low CNI 13%, standard triple CNI 8%; *P* = .400). No serious adverse event showed a significantly different incidence between treatment groups.

Adverse events led to study drug discontinuation in 17 patients (25%) in the quadruple low CNI group (graft rejection 4, leukopenia 3, and 1 case each of various other adverse events including abdominal pain, acne, CMV infection, chest pain, and decline in graft function). No patient who continued the standard triple CNI regimen after randomization stopped the study drug due to adverse events (*P* < .001 vs quadruple low CNI therapy). Adverse events led to dose adjustment or temporary interruption of study drug in 28 patients (41.8%) in the quadruple low CNI group and 29 patients (46.0%) in the standard triple group.

Infectious episodes occurred in 44 patients in the quadruple low CNI group (66%) and in 46 patients (73%) in the standard triple CNI group. Of the 153 infectious episodes reported, the source organism was determined in 79 cases, the most frequent being viral infections (Table 3). The most commonly reported infections were nasopharyngitis (25% and 27% of the patients in the quadruple low CNI group and standard triple CNI groups, respectively), CMV infection (15% and 22%), respiratory tract infection (13% and 13%), pneumonia (8% and 5%), and urinary tract infection (6% and 5%). CMV was reported as a serious adverse event in 1 patient in the quadruple low CNI group and in 3 patients in the standard triple CNI group, while pneumonia was reported as a serious adverse event in 3 patients in each group. BK virus infection was newly detected in 1 patient in the standard triple CNI group and no patient in the quadruple low CNI group.

There were more new or prolonged hospitalization episodes in the quadruple low CNI group, but these occurred in a comparable proportion of patients in both groups (Table 3).

4 | DISCUSSION

In this randomized trial, renal function at 1 year was significantly higher in maintenance lung transplant patients who were converted

early to a quadruple protocol containing everolimus with reduced CNI compared to a standard-dose CNI-based regimen, while immunosuppressive efficacy was maintained.

An epidemiological study of >1.2 million patients with a mean age of 52 years has demonstrated impaired survival in patients with eGFR lower than 60 mL/min per 1.73 m^2 with an adjusted hazard ratio of 1.2. This effect was related not only to cardiovascular death but also to hospitalizations, and was more pronounced in advanced renal insufficiency with a hazard ratio of 3.2 for patients with eGFR between 15 and 29 mL/min per 1.73 m^2.[10] While chronic lung allograft dysfunction and infections are the main causes of death after lung transplantation, improved survival in recent years has meant that late death due to cardiovascular comorbidities is becoming more important. Although the 4EVERLUNG study was not powered to detect survival differences or the incidence of end-stage kidney disease, the difference in kidney function based on these large epidemiological studies may be regarded as clinically significant.

The study selectively recruited patients with mild-or-moderate renal dysfunction, since the potential to improve renal function was considered necessary to justify conversion to the quadruple regimen. The most marked benefit, however, was observed in the subpopulation with baseline eGFR in the range 40-60 mL/min per 1.73 m^2. Most previous randomized trials of everolimus with reduced CNI exposure initiated early after lung transplantation did not specify criteria for renal function at study entry, and no advantage for kidney function was noted for everolimus-based immunosuppression.[15,21] In the randomized NOCTET study, a small improvement in measured GFR using everolimus with reduced CNI was observed after 1 and 2 years in long-term thoracic transplant patients.[22,23] In that trial, patients were required to have a minimal baseline measured GFR of 20 mL/min, and the mean baseline eGFR was only 43 mL/min per 1.73 m^2 in the cohort of lung transplant recipients[22] compared to 65 mL/min per 1.73 m^2 in the quadruple low CNI cohort of our study. In the current trial the quadruple low CNI patients were included much earlier, at an average of 10.8 months after lung transplantation, in contrast to 52 months in the NOCTET trial. This window seems appropriate to minimize the time-dependent development of irreversible CNI-related kidney damage.[29]

The everolimus target range (3-5 ng/mL) in the 4EVERLUNG trial was lower than in other recent trials of lung transplant recipients, most of which used a range of 3-8 ng/mL.[15,22] In contrast to all but the NOCTET study, the everolimus group received quadruple therapy, including a cell cycle inhibitor (almost always MMF) and steroids to ensure immunosuppressive efficacy with reduced CNI exposure with limited side effects. This was confirmed by the fact that adverse events were similar to those with standard triple therapy within 12 months of treatment.

The incidence and severity of acute rejection showed no relevant differences between treatment groups in our trial. Numerically, more patients in the standard triple CNI group developed new-onset CLAD by 12 months than in the quadruple low CNI group, but the study was not powered for this endpoint. Published evidence regarding the effect of everolimus-based therapy on the risk of CLAD has not shown a clear benefit. Two recent randomized trials did not observe an overall reduction in CLAD among patients who remained on everolimus therapy for up to 3 years compared to those given mycophenolate,[15,21] although 1 of the studies reported a lower incidence of CLAD for everolimus vs MMF in a per protocol analysis.[15]

We did not detect signals of overimmunosuppression with regard to infections within the 1-year time frame of this trial. In contrast to all other trials of everolimus in lung transplantation,[15,22,30] the rates of adverse events or serious adverse events were similar between treatment groups in the 4EVERLUNG study. Use of the quadruple protocol also had the potential to increase specific side effects. The known hematological effects of MPA[31,32] may have promoted leukopenia and other hematological abnormalities, although MMF doses were not different between treatment arms. Differences in the pattern of adverse events (for example, the higher rates of peripheral edema and acne) may have accounted for the small but significant reduction in the SF-36 mental component summary score in patients randomized to the quadruple therapy group.

There were numerically fewer cases of CMV infection in the quadruple low CNI arm, compatible with significantly fewer CMV infections in 2 randomized everolimus trials.[15,21] Discontinuation due to adverse events was more frequent in the quadruple low CNI group. Only 2 adverse events (graft rejection and leukopenia) led to discontinuation in more than 1 patient. Given the fact that overall rejections and leukopenia were not different between groups, there was no clear pattern of everolimus-related events leading to a change in regimen. It seems possible that in this open-label study there was a tendency to revert to standard therapy rather than to try and manage side effects under the novel everolimus-based regimen. In other recent randomized trials of immunosuppression following lung transplantation, the proportion of patients not on study treatment after 3 years has ranged from 35% to 55%.[15,21,33] The 29% rate of discontinuation of the quadruple regimen in the 1-year 4EVERLUNG study was comparable to these numbers, and reflects the limitation of open-label studies and the tendency of investigators towards reverting to conventional treatment when adverse events occur.

The sample size was smaller than planned. Slow recruitment required early termination of recruitment, such that the expected population of 232 patients was not achieved and a post hoc calculation indicated that the randomized population of 130 offered only 62% power to detect a significant between-group difference in the primary endpoint. Additionally, certain limitations in terms of the study design and implementation should be considered. First, like most randomized trials of immunosuppression in solid organ transplant recipients, it was an open-label study. This is explained by the fact that most immunosuppressants (including everolimus) are narrow therapeutic index drugs, and an open-label trial permitted concentration-controlled dosing of the study drugs. Second, tacrolimus levels were slightly above target in the quadruple arm, which might have underscored the renal protective effect in the quadruple low group. Third, direct measurement of GFR is ideal but challenging in daily practice, so GFR was estimated using the CKD-EPI equation, which is considered appropriate in the setting of lung transplantation.[34]

GFR formulae for estimation may have limitations in overweight or underweight patients. Lastly, it should be stressed that the study selected patients with baseline eGFR in the range 50-90 mL/min per 1.73 m^2, and who were 3-18 months posttransplant, and these findings cannot necessarily be extrapolated to other types of patients.

In conclusion, despite being underpowered, this randomized trial demonstrated that renal function in lung transplant patients was significantly higher 1 year after early conversion to everolimus with reduced CNI exposure than with standard triple CNI therapy. Immunosuppressive efficacy was comparable using these regimens, both of which included MPA (or azathioprine) and steroids. Based on these findings, introduction of everolimus to support reduced CNI exposure can be considered in lung transplant patients with mild-to-moderate renal dysfunction.

ACKNOWLEDGMENTS

The authors would like to thank Dr Christian Sieder for providing biostatistical support, and to C. Dunstall for writing services, funded by Novartis Pharma GmbH. The authors also gratefully acknowledge the 4EVERLUNG Study Investigators and other key contributors: Christine Andres; Gertrud Armbruster; Sandra Arnold; Markus Barten; Jürgen Barton; Christina Baum; Elisabeth Becker; Heidrun Behmer; Christiane Berger; Patricia Berger; Angela Bergner; Vasiliki Bessa; Martin Bettmann; Benjamin-Alexander Bollmann; Francesco Tommaso Bonella; Julia Brust; Felix Ceelen; Nora Drick; Angela Englert; Björn Frye; Anne-Kathrin Funkat; Katharina Gawenda; Jens Gottlieb; Mark Greer; Linda Haesler; Matthias Hecker; Angela Henning; Heidrun Villena Hermoza; Ina Hettich; Susanne Hoyer; Marco Idzko; Christopher Jürgens; Christina Keller; Dagmar Kemper; Bjoern Elmar Kleibrink; Nikolaus Kneidinger; Christoph Knosalla; Angelika Krill; Sven Lehmann; Christian Lensch; Sophia Maron; Konstantin Mayer; Wolfram Meschede; Anna Lassia Meyer; Christina Mihm; Christiane Moelgen; Moritz Alexander Mohadjer; Joachim Müller-Quernheim; Claus Neurohr; Janine Nowack; Christine Oelschner; Antje Prasse; Thomas Rabis; Jessica Rademacher; Felix Ringshausen; Bianca Rink; Cornelia Ruback; Stephanie Schmidt; Tanja Schmies; Kristin Schulz; Robby Schurzmann; Martina Seibert; Nasanin Shegeftfard; Bjoern Sill; Bibiane Söllner; Urte Sommerwerck; Nicole Sprathoff; Katharina Steffen; Martin Strüber; Hendrik Suhling; Sivagurunathan Sutharsan; Monika Teichmann; Ina Wagner; Alexandra Wald; Elke Wenzel; Bettina Weingard; Heinrike Wilkens; Hubert Wirtz; Christian Witt; and Nadine Zmudzinski. The study was funded by Novartis Pharma GmbH, Nuremberg, Germany

DISCLOSURE

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. J. Gottlieb has received research support, honorarium for advisory, and speaker's fees from Novartis Pharma Germany. C. Neurohr has received research support from Novartis Pharma Germany. J. Müller-Quernheim has received grant/research support from Novartis Pharma Germany. H. Wirtz has received honoraria for advisory boards and speaker fees from Novartis. H. Wilkens has received speaker's fees from Novartis. C. Knosalla has received research support from Novartis Pharma Germany. M. Porstner and C. Capusan are employees of Novartis Pharma Germany. The other authors have no conflicts of interest to disclose.

REFERENCES

1. Chambers DC, Yusen RD, Cherikh WS, et al. The Registry of the International Society for Heart and Lung Transplantation: thirty-fourth Adult Lung And Heart-Lung Transplantation Report-2017; Focus Theme: allograft ischemic time. *J Heart Lung Transplant*. 2017;36(10):1047-1059.
2. Munshi L, Keshavjee S, Cypel M. Donor management and lung preservation for lung transplantation. *Lancet Respir Med*. 2013;1(4):318-328.
3. Afshar K. Future direction of immunosuppression in lung transplantation. *Curr Opin Organ Transplant*. 2014;19(6):583-590.
4. Scheffert JL, Raza K. Immunosuppression in lung transplantation. *J Thorac Dis*. 2014;6(8):1039-1053.
5. Nankivell BJ, P'Ng CH, O'Connell PJ, Chapman JR. Calcineurin inhibitor nephrotoxicity through the lens of longitudinal histology: comparison of cyclosporine and tacrolimus eras. *Transplantation*. 2016;100(8):1723-1731.
6. Barraclough K, Menahem SA, Bailey M, Thomson NM. Predictors of decline in renal function after lung transplantation. *J Heart Lung Transplant*. 2006;25(12):1431-1435.
7. Hornum M, Burton CM, Iversen M, Hovind P, Hilsted L, Feldt-Rasmussen B. Decline in 51Cr-labelled EDTA measured glomerular filtration rate following lung transplantation. *Nephrol Dial Transplant*. 2007;22(12):3616-3622.
8. Mason DP, Solovera-Rozas M, Feng J, et al. Dialysis after lung transplantation: prevalence, risk factors and outcome. *J Heart Lung Transplant*. 2007;26(11):1155-1162.
9. Hellemons ME, Bakker SJ, Postmus D, et al. Incidence of impaired renal function after lung transplantation. *J Heart Lung Transplant*. 2012;31(3):238-243.
10. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004;351(13):1296-1305.
11. Carrero JJ, Hecking M, Chesnaye NC, Jager KJ. Sex and gender disparities in the epidemiology and outcomes of chronic kidney disease. *Nat Rev Nephrol*. 2018;14(3):151-164.
12. Tedesco-Silva H, Felipe C, Ferreira A, et al. Reduced incidence of cytomegalovirus infection in kidney transplant recipients receiving everolimus and reduced tacrolimus doses. *Am J Transplant*. 2015;15(10):2655-2664.
13. Brennan DC, Legendre C, Patel D, et al. Cytomegalovirus incidence between everolimus versus mycophenolate in de novo renal transplants: pooled analysis of three clinical trials. *Am J Transplant*. 2011;11(11):2453-2462.
14. Kobashigawa J, Ross H, Bara C, et al. Everolimus is associated with a reduced incidence of cytomegalovirus infection following de novo cardiac transplantation. *Transpl Infect Dis*. 2013;15(2):150-162.
15. Strueber M, Warnecke G, Fuge J, et al. Everolimus versus mycophenolate mofetil de novo after lung transplantation: a prospective, randomized, open-label trial. *Am J Transplant*. 2016;16(11):3171-3180.
16. Rittà M, Costa C, Solidoro P, et al. Everolimus-based immunosuppressive regimens in lung transplant recipients: impact on CMV infection. *Antiviral Res*. 2015;113:19-26.
17. Ghassemieh B, Ahya VN, Baz MA, et al. Decreased incidence of cytomegalovirus infection with sirolimus in a post hoc randomized,

multicenter study in lung transplantation. *J Heart Lung Transplant*. 2013;32(7):701-706.
18. Tedesco Silva H Jr, Cibrik D, Johnston T, et al. Everolimus plus reduced-exposure CsA versus mycophenolic acid plus standard-exposure CsA in renal-transplant recipients. *Am J Transplant*. 2010;10(6):1401-1413.
19. De Simone P, Nevens F, De Carlis L, et al. Everolimus with reduced tacrolimus improves renal function in de novo liver transplant recipients: a randomized controlled trial. *Am J Transplant*. 2012;12(11):3008-3020.
20. Eisen HJ, Kobashigawa J, Starling RC, et al. Everolimus versus mycophenolate mofetil in heart transplantation: a randomized, multicenter trial. *Am J Transplant*. 2013;13(5):1203-1216.
21. Glanville AR, Aboyoun C, Klepetko W, et al. European and Australian Investigators in Lung Transplantation. Three-year results of an investigator-driven multicenter, international, randomized open label de novo trial to prevent BOS after lung transplantation. *J Heart Lung Transplant*. 2015;34(1):16-25.
22. Gullestad L, Iversen M, Mortensen SA, et al. Everolimus with reduced calcineurin inhibitor in thoracic transplant recipients with renal dysfunction: a multicenter, randomized trial. *Transplantation*. 2010;8(7):864-872.
23. Gullestad L, Mortensen S-A, Eiskjær H, et al. Two-year outcomes in thoracic transplant recipients after conversion to everolimus with reduced calcineurin inhibitor within a multicenter, open-label, randomized trial. *Transplantation*. 2010;90(12):1581-1589.
24. 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(9):604-612.
25. Verleden GM, Raghu G, Meyer KC, Glanville AR, Corris P. A new classification system for chronic lung allograft dysfunction. *J Heart Lung Transplant*. 2014;33(2):127-133.
26. Hoek FJ, Kemperman FA, Krediet RT. A comparison between cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate. *Nephrol Dial Transplant*. 2003;18(10):2024-2031.
27. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med*. 1999;130(6):461-470.
28. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron*. 1976;16(1):31-41.
29. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD. Calcineurin inhibitor nephrotoxicity: longitudinal assessment by protocol histology. *Transplantation*. 2004;78(4):557-565.
30. Snell GI, Valentine VG, Vitulo P, et al.; RAD B159 Study Group. Everolimus versus azathioprine in maintenance lung transplant recipients: an international, randomized, double-blind clinical trial. *Am J Transplant*. 2006;6(1):169-177.
31. Borrows R, Chusney G, Loucaidou M, et al. Mycophenolic acid 12-h trough level monitoring in renal transplantation: association with acute rejection and toxicity. *Am J Transplant*. 2006;6(1):121-128.
32. Boudjema K, Camus C, Saliba F, et al. Reduced-dose tacrolimus with mycophenolate mofetil vs. standard-dose tacrolimus in liver transplantation: a randomized study. *Am J Transplant*. 2011;11(5):965-976.
33. Treede H, Glanville AR, Klepetko W, et al.; European and Australian Investigators in Lung Transplantation. Tacrolimus and cyclosporine have differential effects on the risk of development of bronchiolitis obliterans syndrome: results of a prospective, randomized international trial in lung transplantation. *J Heart Lung Transplant*. 2012;31(8):797-804.
34. Degen DA, Janardan J, Barraclough KA, et al. Predictive performance of different kidney function estimation equations in lung transplant patients. *Clin Biochem*. 2017;50(7–8):385-393.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Gottlieb J, Neurohr C, Müller-Quernheim J, et al. A randomized trial of everolimus-based quadruple therapy vs standard triple therapy early after lung transplantation. *Am J Transplant*. 2019;19:1759–1769. https://doi.org/10.1111/ajt.15251