ORIGINAL ARTICLE

Long-term outcomes of thoracic transplant recipients following conversion to everolimus with reduced calcineurin inhibitor in a multicenter, open-label, randomized trial

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SUMMARY

The NOCTET study randomized 282 patients ≥1 year after heart or lung transplantation to continue conventional calcineurin inhibitor (CNI) therapy or to start everolimus with reduced-exposure CNI. Last follow-up, at ≥5 years postrandomization (mean: 5.6 years) was attended by 72/140 everolimus patients (51.4%) and 91/142 controls (64.1%). Mean measured GFR remained stable in the everolimus group from randomization (51.3 ml/min) to last visit (51.4 ml/min) but decreased in controls (from 50.5 ml/min to 45.3 ml/min) and was significantly higher with everolimus at last follow-up ($P = 0.004$). The least squares mean (SE) change from randomization was −1.5 (1.7)ml/min with everolimus versus −7.2 (1.7)ml/min for controls (difference: 5.7 [95% CI 1.7; 9.6]ml/min; $P = 0.006$). The difference was accounted for by heart transplant patients (difference: 6.9 [95% 2.3; 11.5]ml/min; $P = 0.004$). Lung transplant patients showed no between-group difference at last follow-up. Rates of rejection, death, and major cardiac events were similar between groups, as was graft function. Pneumonia was more frequent with everolimus (18.3% vs. 6.4%). In conclusion, introducing everolimus in maintenance heart transplant patients, with reduced CNI, achieves a significant improvement in renal function which is maintained for at least 5 years, but an early renal benefit in lung transplant patients was lost. Long-term immunosuppressive efficacy was maintained.

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Introduction

The prevalence and impact of renal dysfunction after heart transplantation are well documented, with a progressive decline in renal function eventually leading to end-stage renal failure in approximately one in ten recipients [1,3]. Poor renal function is also significantly associated with increased risk of all-cause and cardiac mortality after heart transplantation [4]. Although less widely reported, lung transplant patients also experience a high rate of end-stage renal disease [5]. The etiology of renal deterioration after thoracic transplantation is complex, with related comorbidities such as pretransplant renal dysfunction [5] playing a role as well as conventional risk factors such as diabetes [6,7] and older age [6]. One of the few modifiable factors that could potentially ameliorate nephron loss over time is reducing long-term exposure to calcineurin inhibitor (CNI) therapy. Use of the mammalian target of rapamycin (mTOR) inhibitor everolimus to reduce CNI exposure in *de novo* heart transplant patients has been investigated in several trials [8–10]. Results have consistently shown that immunosuppressive efficacy is maintained compared with conventional cyclosporine (CsA)-based regimens [8–10] with an associated reduction in cardiac allograft vasculopathy [8], although predefined renal endpoints have not been met [6, 8]. Randomized trials of conversion from tacrolimus-based immunosuppression to everolimus are lacking. There has been one randomized study of everolimus in *de novo* lung transplant patients, with 3 years' follow-up, which found that everolimus with reduced CsA achieved similar rates of survival and rejection compared with conventional CsA therapy in the intention-to-treat (ITT) analysis [11]. Both treatment groups showed substantial renal impairment at 3 years (mean serum creatinine: 160 μmol vs. 152 μmol). In maintenance thoracic patients, however, only two randomized trials of everolimus with reduced CNI (CsA) have been performed: one small study in 34 patients [12] and the large Nordic Certican Trial in Heart and Lung Transplantation (NOCTET) trial [13].

In the NOCTET study, 282 heart or lung transplant patients at least 1 year post-transplant were randomized to continue their CNI-based regimen or to start everolimus with reduced-exposure CNI [13]. After 12 [13] and 24 [14] months, renal function was significantly higher in the everolimus cohort. To determine whether this effect is sustained long term, patients were followed for a minimum of 5 years after randomization. This report presents data on renal function, graft function, and safety outcomes at the final follow-up visit.

Patients and methods

Study design

NOCTET was a 12-month, open-label, multicenter, randomized study performed at 10 transplant centers in Denmark, Norway, and Sweden [13]. Patients who completed the 12-month study were invited to enter the follow-up phase which concluded at month 24 postrandomization. All patients who participated in the follow-up phase and who attended an annual clinic visit at 5, 6, 7, or 8 years after randomization were asked to participate in a long-term follow-up analysis.

Eligibility

Patients aged >18 years were eligible for inclusion in the core if they had undergone heart or lung transplantation at least 1 year previously and were receiving either CsA or tacrolimus at the time of entry to the NOCTET study. All patients were required to have a measured or calculated GFR ≥20 ml/min/1.73 m^2 and <90 ml/min/1.73 m^2; that is, patients with very poor

(CKD stage 1) or normal renal function were excluded. Full eligibility criteria have been published previously [13].

Intervention

Randomization was performed centrally by a computer-based automated system. In the everolimus group, the starting dose of everolimus was 0.75–1.5 mg b.i.d., adjusted to target a trough level 3–8 ng/ml, and the CNI dose was adjusted to target a CsA trough level <75 ng/ml or a tacrolimus trough level <4 ng/ml. In CsA-treated patients receiving mycophenolic acid (MPA), the MPA dose was reduced as necessary to maintain the same MPA level as before CsA dose reduction. In the control arm, the CNI-based immunosuppressive regimen remained unchanged and was administered as per local practice. After the core 12-month study, the immunosuppressive regimen was at the discretion of the investigator.

Data collection

At the last follow-up visit, GFR was measured using Cr-ethylenediamine tetraacetic acid (Cr-EDTA) clearance or an equivalent method. Other data collection included immunosuppressive therapy and concomitant medication; results from routine echocardiographic recording in heart transplant patients (left ventricular end systolic dimension [LVESD] [cm], left ventricular dimension [LVEDD] [cm], left ventricular ejection fraction [LVEF] [%]); results from routine spirometric recording in lung transplant patients (forced expiratory volume in 1 second [FEV1] [L/s and %], forced vital capacity [FVC] [L and %]); physical examination; vital signs; laboratory data; and adverse events occurring between month 24 and the last follow-up visit.

Statistical analysis

Analyses were based on the last routine visit attended by each patient at 5 years postrandomization or later. The primary efficacy variable, the change in measured GFR (mGFR) from randomization to month 12, was reassessed at the last follow-up visit. Comparisons between the everolimus and control groups for the change in mGFR from randomization to the last visit were performed using analysis of covariance (ANCOVA) with treatment, center, and adjunctive therapy at randomization (MPA or azathioprine) as factors, and baseline mGFR and age as covariates. All analyses were two-sided, performed at the 5% level of significance.

The safety population included all patients who entered the follow-up phase. The ITT population was defined as patients in the safety population who completed the 24-month study visit and provided at least one measurement for mGFR or lung or cardiac structure or function at the last follow-up visit. The per-protocol population comprised all ITT patients who were on randomized treatment at the last follow-up visit.

Results

Patient population

Of the 282 patients who were randomized in the core study, 221 patients attended the 24-month study visit. In total, 176 of these 221 patients entered the long-term follow-up and formed the long-term safety population. A last visit at ≥5 years postrandomization was completed by 163 patients (the ITT population), comprising 125 heart transplant patients and 38 lung transplant patients (Fig. 1).

The mean time from randomization to the follow-up visit was 2077 days (5.7 years) in the everolimus group and 2013 days (5.5 years) in the control group (mean: 5.6 years overall). Median follow-up time was 5.6 years and 5.1 years in the everolimus and control arms, respectively. The demographics of the two treatment groups were comparable both overall (Table 1) and within the heart and lung transplant subpopulations (Table S1).

Immunosuppression and concomitant medication

At month 24, 93.1% (67/72) of patients randomized to everolimus were still receiving everolimus, and all patients were still on CNI therapy (11 had switched from CsA to tacrolimus). By last follow-up, 91.0% (61/72) of patients remained on everolimus. Of the six patients who discontinued everolimus, four remained on CsA and two switched from CsA to tacrolimus. Everolimus trough levels were stable at last follow-up (mean: 4.4 ng/ml) (Table 1). In the control arm, nine patients (9.9%) had switched from CsA to tacrolimus by month 24, and 13 patients (14.3%) were receiving everolimus therapy by last follow-up. The mean trough level of CsA was 46% lower in the everolimus versus controls at month 24 (60 ng/ml vs. 111 ng/ml) and 36% lower at last follow-up (60 ng/ml vs. 94 ng/ml) (Table 1). In the control arm, there was a 20% decrease

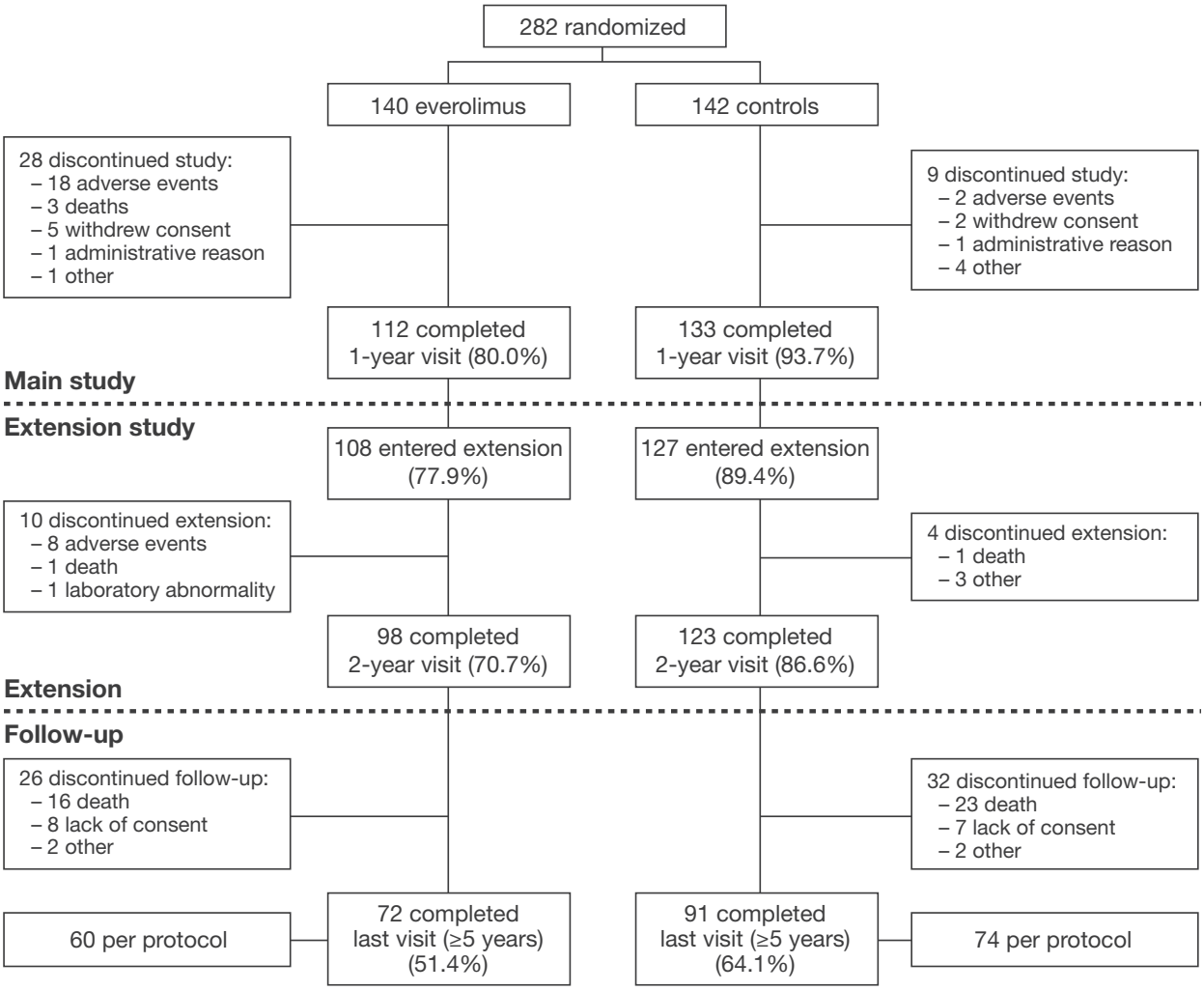

Figure 1 Patient disposition.

in mean CsA trough concentration from randomization (139 ng/ml) to month 24 (111 ng/ml), with a further 15% decrease to last follow-up (94 ng/ml). A similar reduction in mean CsA level was observed in the control patients who remained on the randomized drug regimen (141 ng/ml at baseline, 112 ng/ml at month 24, 98 ng/ml at month 24). Tacrolimus levels were also substantially lower in the everolimus arm (49% at month 24 and 29% at last follow-up), but patient numbers were low. The mean (SD) mycophenolate mofetil dose at last follow-up was lower in the everolimus group (1330 [589] mg/day; $n = 52$) versus the control arm (1968 [657] mg/day; $n = 55$). Mean steroid dose was similar for the everolimus cohort (5.5 [2.0] mg/day; $n = 51$) and the control group (5.5 [1.9] mg/day; $n = 62$). Immunosuppression by treatment group is shown separately for heart and lung transplant patients in Supplementary Table 1.

Renal function

Measured GFR was similar in the everolimus group (mean: 51.3 ml/min) and control group (mean: 50.5 ml/min) at the point of randomization in the NOCTET trial. Values increased in the everolimus arm at months 12 and 24, declining subsequently such that at last follow-up mean mGFR had returned to a level identical to that seen at randomization (51.4 ml/min) (Table 2, Fig. 2a). In contrast, mean mGFR decreased in the control arm from 12 months onwards, with a mean of 45.3 ml/min at last follow-up (Table 2, Fig. 2a). When the primary endpoint, change in mGFR from randomization, was analyzed at last follow-up, the least squares (LS) mean (SE) change was −1.5 (1.7) ml/min in the everolimus group ($n = 68$) versus −7.2 (1.7) ml/min in the control arm, a difference of 5.7 (95% CI: 1.7; 9.6) ml/

Table 1. Patient characteristics (ITT population).

	Everolimus (n = 72)		Control (n = 91)	
Recipient age (years), mean (SD)	56.2 (9.8)		55.4 (10.4)	
Female recipient, n (%)	19 (26)		23 (25)	
Body mass index (kg/m^2), mean (SD)	28.3 (4.9)		26.0 (5.5)	
History of diabetes, n (%)	8 (11.1)		13 (14.3)	
History of hypertension	52 (72.2)		67 (73.6)	
Smoking status				
Current smoker	0 (0)		5 (5.6)	
Former smoker	33 (48.5)		42 (47.2)	
Never smoked	35 (51.5)		42 (47.2)	
Missing	4		2	
Donor age (years), mean (SD)	39.4 (14.9)		37.7 (15.2)	
Time from randomization to follow-up visit (days)				
Mean (SD)	2077 (302)		2013 (271)	
Median (range)	2057 (1837, 2209)		1869 (1816, 2186)	
Immunosuppression	*n (%)*	*Mean (SD)*	*n (%)*	*Mean (SD)*
Everolimus (ng/ml), mean (SD)				
Month 24	67 (93.1)	4.4 (1.6)	0	–
Last visit	61 (84.7)	4.4 (1.7)	13 (14.3)	5.2 (2.4)
CsA (ng/ml), mean (SD)				
Month 24	61 (84.7)	60 (50)	82	111 (40)
Last visit	50 (69.4)	60 (32)	71	94 (32)
Tacrolimus (ng/ml), mean (SD)				
Month 24	11 (15.3)	4.5 (1.5)	9 (9.9)	8.8 (3.6)
Last visit	10 (13.9)	5.1 (1.6)	10 (11.0)	7.2 (2.0)

mGFR, measured glomerular filtration rate; CsA, cyclosporine.

Data on drug trough concentration and dose are shown only for patients who remained on treatment.

min (P = 0.006). The difference at last follow-up was accounted for by improved preservation of renal function in heart transplant patients under everolimus (Table 2, Fig. 2b). Lung transplant recipients had lower mean mGFR at randomization than heart transplant patients and a similar decline in mGFR was observed in both treatment groups by last follow-up (Table 2, Fig. 2c).

The change in mGFR from randomization to last follow-up was also assessed in the per-protocol population with data available at both time points (everolimus: 60, controls: 74). The LS mean (SE) change was −2.1 (2.0) ml/min under everolimus versus −6.6 (1.9) ml/min in the control group (difference: 4.6 [95% CI −0.1,9.2] ml/min, P = 0.054).

Efficacy

Three patients experienced rejection between month 24 and last follow-up: one heart transplant patient in the everolimus cohort and two heart transplant patients in the control arm. No cases required antibody therapy or led to graft loss.

During the period from month 24 to last follow-up, there were 16 deaths in the everolimus group. The most frequent reasons were malignancy (4), myocardial infarction (3), and pulmonary embolism (2). One lung transplant patient in the everolimus cohort was retransplanted following graft loss due to lung fibrosis and chronic rejection. There were 23 deaths in the control arm; no single cause of death was reported in more than one patient except for multi-organ failure, which occurred in two patients.

In the subpopulation of heart transplant recipients, LVESD remained normal and unchanged from randomization to last follow-up in both treatment groups, but LVEDD decreased significantly in the control arm (−0.3 [95% CI −0.4, −0.1; P = 0.001 versus baseline [LS mean values]) (Table 3). The difference in the change from baseline for both LVESD and LVEDD was significantly different between the everolimus group and controls (P = 0.002 and P = 0.003, respectively) (Table 3). LVEF decreased significantly in both treatment groups from randomization to last follow-up. The LS mean change was −7.4 (95% CI: −9.4; −5.5% for everolimus and −8.3 (95% CI: −10.0; −6.7% for controls)

Table 2. Measured GFR (mGFR, ml/min) and change in mGFR from randomization (ITT population).

	Everolimus				Controls						
	Rdn	12 months	24 months	Last follow-up	Rdn	12 months	24 months	Last follow-up	Month 12	Month 24	Last follow-up
Observed values, mean (SD)									*P* values*		
All	51.3 (14.7) (*n* = 72)	57.4 (14.8) (*n* = 72)	56.5 (15.7) (*n* = 72)	51.4 (18.8) (*n* = 70)	50.5 (13.1) (*n* = 91)	49.8 (15.9) (*n* = 90)	48.5 (14.0) (*n* = 90)	45.3 (16.1) (*n* = 87)	**<0.001**	**<0.001**	**0.004**
Heart transplants	52.8 (14.5) (*n* = 54)	60.1 (14.7) (*n* = 54)	58.4 (16.2) (*n* = 54)	54.2 (19.2) (*n* = 53)	51.3 (13.0) (*n* = 71)	50.6 (15.5) (*n* = 71)	49.6 (13.4) (*n* = 70)	46.1 (16.4) (*n* = 67)	**<0.001**	**<0.001**	**0.002**
Lung transplants	46.5 (14.4) (*n* = 18)	49.6 (12.5) (*n* = 18)	50.7 (13.1) (*n* = 18)	42.4 (14.5) (*n* = 17)	47.8 (13.4) (*n* = 20)	46.7 (17.5) (*n* = 19)	44.8 (15.7) (*n* = 20)	42.9 (14.7) (*n* = 20)	0.110	**0.010**	0.916
Change from randomization (ANCOVA), LS mean (SE)									Difference (95% CI), *P* value†		
All	–	−5.4 (1.3)	−4.3 (1.3)	−1.5 (1.7)	–	−2.3 (1.3)	−4.2 (1.3)	−7.2 (1.7)	7.7 (4.7;10.8) <0.001	8.5 (5.5, 11.6) <0.001	5.7 (1.7; 9.6) 0.006
Heart transplants	–	−7.0 (1.5)	−5.3 (1.5)	−0.3 (2.0)	–	−0.9 (1.4)	−2.6 (1.4)	−6.6 (1.9)	7.9 (4.6; 11.2) <0.001	7.9 (4.7;11.2) <0.001)	6.9 (2.3; 11.5) 0.004
Lung transplants	–	−3.1 (2.9)	−3.7 (2.8)	−5.0 (2.7)	–	−3.7 (3.3)	−7.2 (3.1)	−5.4 (3.0)	6.9 (−1.7; 15.4) 0.111	10.9 (2.8; 19.1) 0.010	0.4 _(−7.4; 8.3) 0.916

*P value for everolimus vs controls.

†P value for difference between groups, calculated for change in mGFR using ANCOVA model with treatment and center as factors and randomization value as covariates for change, and calculated for change in mGFR according to time post-transplant using covariance analysis (i.e., interaction between treatment group and time post-transplant) Rnd, randomization; LS, least squares. Significant *P* values are shown in bold.

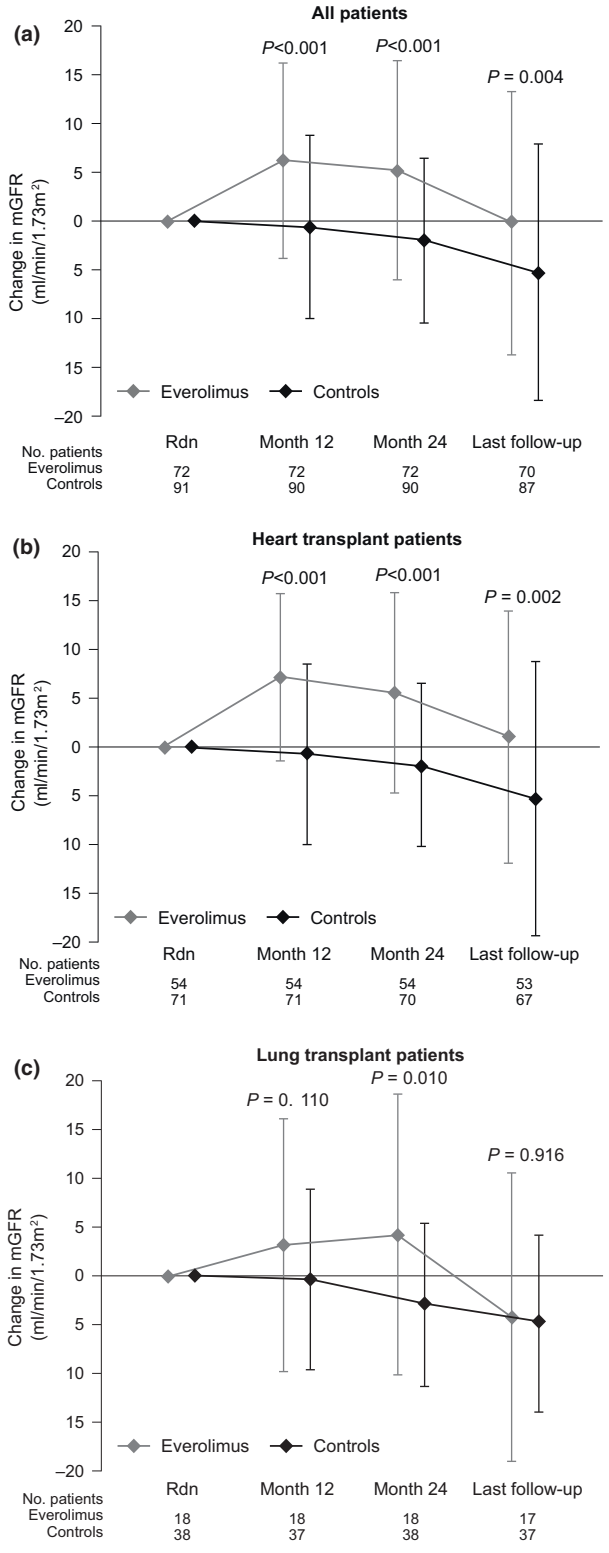

Figure 2 Change in measured GFR (mGFR) from randomization to months 12 and 24 and last follow-up for (a) all patients, (b) heart transplant recipients, (c) lung transplant recipients (ITT population). Rnd, randomization.

($P < 0.001$ versus randomization in both groups). The change from randomization to last follow-up was similar in both groups (Table 3). Mean heart rate remained stable in both groups between randomization (everolimus: 83 bpm, controls: 84 bpm) and last follow-up (82 bpm and 81 bpm, respectively). Mean blood pressure also remained stable (everolimus: 138/85 mmHg at randomization versus 137/85 mmHg at last follow-up; controls: 138/86 mmHg vs. 137/86 mmHg).

Among the lung transplant recipients, there was a significant decrease within both treatment groups in FVC from randomization to last follow-up (LS mean change: −0.3 L in both groups; $P = 0.009$ for everolimus, $P = 0.025$ for controls) (Table 3). FEV_1 also decreased significantly from randomization (LS mean change: −0.3 L/s in both arms; $P = 0.001$ for everolimus, $P = 0.002$ for controls). The changes from randomization to last follow-up were similar between treatment groups for both parameters (Table 3).

Safety and tolerability

Between month 24 and last follow-up, 85.4% (70/82) patients in the everolimus group and 77.7% (73/94) patients in the control group experienced one or more adverse events (Table 4). Adverse events are described separately for the heart and lung transplant subpopulations in Table S2. Infections were reported in 52.4% and 29.8% of patients in the everolimus and control arms, respectively. The difference in rates of infection was accounted for by the heart transplant subpopulation (35 and 19 infections, respectively); occurrence of infection was similar in both groups in the lung transplant cohort (19 in the everolimus-treated patients, 18 in controls). Pneumonia occurred more frequently as an adverse event (18.3% vs. 6.4%) and as a serious adverse event (9.8% vs. 3.2%) under everolimus compared with the control arm. The rate of pneumonia as an adverse event in the everolimus versus control arms was 13.1% (8/61) vs. 5.5% (4/73) in the heart transplant cohort and 33.3% (7/21) vs. 9.5% (2/21) in the lung transplant cohort. No case was classified as interstitial pneumonia. Type 2 diabetes was reported as an adverse event in one patient in each group, with inadequate control of diabetes reported in one additional patient in the control group. Mean (SD) values for HbA1c were similar (everolimus: 6.3 [0.9]%, controls: 6.4 [1.4]%). Proteinuria was reported in three everolimus-treated patients and one control patient (3.7% and 1.1%, respectively).

Table 3. Change in cardiac and lung function parameters from randomization to last follow-up (ITT population).

	Change from randomization (ANCOVA) LS mean (95% CI)		
	Everolimus *P* value*	Controls *P* value*	Difference *P* value†
Heart transplants			
LVEF (%)	−7.4 (−9.4, −5.5) (n = 31) $P < 0.001$	−8.3 (−10.0, −6.7) (n = 46) $P < 0.001$	0.9 (−1.4, 3.2) $P = 0.449$
LVESD (cm)	0.3 (−0.0, 0.6) n = 26 $P = 0.087$	−0.2 (−0.5, 0.2) (n = 37) $P = 0.296$	0.5 (0.2, 0.7) $P = 0.002$
LVEDD (cm)	−0.0 (−0.1, 0.2) (n = 41) $P = 0.739$	−0.3 (−0.4, −0.1) (n = 59) $P = 0.001$	0.3 (0.1, 0.5) $P = 0.003$
Lung transplants			
FEV_1 (L/s)	−0.3 (−0.5, −0.1) (n = 16) $P = 0.001$	−0.3 (−0.5, −0.1) (n = 20) $P = 0.002$	0.0 (−0.2, 0.2) $P = 0.950$
FVC (L)	−0.3 (−0.5, −0.1) (n = 16) $P = 0.009$	−0.3 (−0.5, −0.0) (n = 20) $P = 0.025$	−0.0 (−0.3, 0.3) $P = 0.887$

LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; FEV, forced expiratory volume in 1 s; FVC, forced vital capacity; BP =blood pressure.

Values shown are mean (SD).

**P* values for change from baseline.

†*P* values for between-group differences in change from baseline to last follow-up by ANCOVA including strata, site and treatment as factors, and baseline and age as covariates.

Table 4. Adverse events reported between month 24 and last follow-up (long-term safety population).

	Everolimus (n = 82)	Control (n = 94)
At least one adverse event, *n* (%)	70 (85.4)	73 (77.7)
Adverse events occurring in ≥5% of patients in either group		
Bronchitis	10 (12.2)	9 (9.6)
Pneumonia	15 (18.3)	6 (6.4)
Urinary tract infection	4 (4.9)	3 (3.2)
Diarrhea	8 (9.8)	9 (9.6)
Squamous cell carcinoma	2 (2.4)	6 (6.4)
Basal cell carcinoma	8 (9.8)	5 (5.3)
Peripheral edema	10 (12.2)	11 (11.7)
Obliterative bronchiolitis	3 (3.7)	5 (5.3)
Rash	2 (2.4)	6 (6.4)
Hyperuricemia	7 (8.5)	7 (7.4)
Hypertension	5 (6.1)	2 (2.1)
Percutaneous coronary intervention	5 (6.1)	3 (3.2)

One or more serious adverse event was reported in 41.5% (34/82) of everolimus-treated patients versus 34.0% (32/94) of control patients, with the difference largely due to the variation in incidence of pneumonia. During the period from month 24 to last follow-up, 14 and 16 major cardiac adverse events were reported in the everolimus and control groups, respectively. The most frequent events were percutaneous coronary intervention (everolimus: 5, controls: 3), nonspecified arrhythmias (everolimus: 3, controls: 3), and atrial flutter (everolimus: 1, controls: 4). Malignancies were reported in nine and seven patients, respectively, in the everolimus and control groups.

Laboratory and hematology parameters were similar between treatment groups at last follow-up other than serum creatinine, which was significantly lower in the everolimus arm (mean [SD]: 125 [39] μmol/l vs. 141 [44] μmol/l (Table S3). Mean (SD) triglyceride level was 2.2 [2.0] mmol/l vs. 1.8 (0.9) mmol/l ($P = 0.092$) in the everolimus and control groups, respectively, at last follow-up, and urea levels were 10.8 (4.9) mol/l vs. 12.1 (4.4) mmol/l ($P = 0.074$).

Discussion

These results represent the longest follow-up data to date for maintenance thoracic transplant patients randomized to start everolimus, with CNI reduction, or to continue conventional CNI-based therapy. At a minimum of five years postrandomization, patients receiving everolimus with reduced CNI continued to show significantly improved renal function versus the control group, with a mean difference in change from baseline of ~6 ml/min. This advantage, however, was restricted to heart transplant patients: The higher mGFR observed in everolimus-treated lung transplant recipients at month 24 was subsequently lost, with no difference between treatment groups at last follow-up. Heart and lung graft function was similar in the everolimus and control groups.

The CsA trough concentration was 36% lower in the everolimus group versus controls at the last visit, a somewhat smaller difference than at 1 year (~50%) [13]

or 2 years (~46%) [14]. It is possible that those patients who discontinued everolimus had required relatively high CNI exposure prior to starting everolimus such that the difference narrowed after they stopped everolimus therapy. Nevertheless, reducing the blood concentration by approximately a third after starting everolimus appears adequate to maintain an improvement in renal function versus standard-exposure CNI, presumably due to less CNI-related nephrotoxicity over time.

The literature includes only one other randomized trial of everolimus with reduced CNI in maintenance thoracic transplant patients. This was the SHIRAKISS study of 34 heart patients (mean: 2.5 years post-transplant) with progressive renal function, who received everolimus with CsA exposure similar to that of the current trial (~60 ng/ml) [12]. Renal function, based on creatinine clearance, remained stable after starting everolimus during a 3-year follow-up period, consistent with our own results [12,15]. A striking observation was that mGFR was virtually identical in the everolimus and control arms of the lung transplant subpopulation at last follow-up and that the significantly higher mGFR values at month 24 were lost. In contrast, the between-group difference was largely unchanged in the heart transplant subpopulation between month 24 and last follow-up. There is no clear physiological explanation for this difference. Mean GFR was lower in lung transplant patients compared with heart transplant patients at randomization and at all points thereafter, raising the question of whether they are more vulnerable to CNI-related nephrotoxicity and other insults, regardless of a reduction in CNI exposure. No rejection episodes occurred in lung transplant recipients during the follow-up phase, so additional immunosuppression to manage rejection cannot account for the difference. As only 17 patients on everolimus survived to the long-term follow-up visit (i.e., $\geq$5 years), any additional statistical analysis is not meaningful. The increased mortality rate in lung transplant recipients compared with heart transplant patients is in accordance with published experience [16].

When renal function was assessed in the per-protocol population, which only included patients who remained on the randomized study drug, the change in renal function showed little difference to the overall cohort. Patient numbers were not, however, markedly different between the two populations.

By the time the study population entered the long-term follow-up phase, patients were a mean of 8.3 years after heart transplantation and 6.3 years after lung transplantation, and all patients were a minimum of 3 years post-transplant. As would be expected in this maintenance cohort, graft rejection was rare in both treatment groups, with no discernible difference in risk, as observed in this cohort previously at one [13] and two [14] years. Mortality was high, but this is not unusual in a population of this type [16] with a high level of disease-related and concomitant morbidities such as diabetes, and there was no indication of drug-related deaths in either group.

Safety observations revealed nothing unexpected based on previous experience with everolimus. There was a higher rate of pneumonia with everolimus versus controls, as observed during the first year post-transplant in the current study [13], which was concentrated in the lung transplant subpopulation, but there were no cases of interstitial pneumonia and no pneumonia-related deaths. Approximately half of all cases of pneumonia reported as adverse events were graded as serious adverse events in both treatment groups, suggesting a similar severity. It is feasible that adjunctive therapy with MPA as well as low-exposure CNI in the everolimus-treated group may have contributed to the development of pneumonia and other infections. One small retrospective study has shown that starting everolimus with reduced-exposure CNI but without MPA can maintain immunosuppressive efficacy [17], an approach which merits further investigation. No other notable safety differences were observed between groups, including the incidence and type of major adverse cardiac events, although there was a nonsignificant trend to higher triglyceride levels in the everolimus cohort.

The randomized, multicenter study design of NOCTET was robust and had the merit of assessing renal function by direct measurement of GFR instead of by estimated values. It was open label, as is usually necessary in transplant populations due to the requirement for concentration-controlled dosing. The major limitation of this follow-up dataset, however, is the risk of bias due to incomplete patient follow-up. Only 51.4% of patients randomized to everolimus were followed to the last visit (with 43.6% [61/140] still receiving everolimus), and 64.1% of patients in the CNI group. Data collection from patients who did not attend the final follow-up was not possible as patient consent was not provided. *Post hoc,* we performed a statistical analysis of the probability for patients in either the everolimus or control arm continuing to the final follow-up visit, using an explorative logistic regression with mGFR at 24 months, treatment, and the interaction between mGFR and treatment included as factors. This showed

no difference between the probability of continuation between groups ($P = 0.231$); that is, any effect of renal function at month 24 on the likelihood of long-term follow-up was similar between the two groups. Discontinuations in the everolimus arm were predominantly due to adverse events; subsequently death was the major reason for discontinuation. While neither reason would seem likely to skew renal function in either group, this cannot be discounted. A proportion of patients also switched from CsA to tacrolimus, but the rate of switching was broadly similar between groups. One notable feature was the decrease in CsA exposure in the control arm during the core study and subsequently, with approximately 30% lower levels at the final follow-up visit compared with randomization. The between-group difference in renal function is likely to have been diminished by this decrease in CsA exposure in this cohort of long-term patients. It should also be noted that the majority of patients were receiving CsA, not tacrolimus. A meta-analysis of randomized trials has not shown any difference in renal dysfunction between the two CNI agents [18], with one randomized trial confirming this similarity even after ten years' follow-up [19], but conflicting data exist [20] and the results observed here do not necessarily apply to tacrolimus-treated patients. We did not systematically document urinary protein at the follow-up visit, which with hindsight is regrettable in view of concerns about mTOR inhibitor-related proteinuria [21]. While the reported rate of proteinuria as an adverse event was low, this is not as reliable as laboratory data. We are also aware that combined analysis of heart and lung transplant patients is a potential limitation. Lastly, the eligibility criteria excluded patients with CKD stage 1 or normal renal function, and results cannot be extrapolated to these patient types.

In conclusion, converting maintenance heart transplant patients with mild-to-moderate renal dysfunction to everolimus with low-exposure CNI achieves a significant improvement in renal function versus conventional CNI therapy which is sustained for at least 5 years. Introduction of everolimus was not associated with any loss of long-term immunosuppressive efficacy, although late pneumonia was more frequent. The decline in lung function in the lung transplant recipients, and in cardiac function in the heart transplant patients, was as expected in these patient groups, and no differences were observed between the treatment groups. In the lung transplant subpopulation, the renal benefit observed for everolimus with low-exposure CNI at one and 2 years was lost at the last follow-up.

Author contributions

Lars Gullestad, Hans Eiskjaer, Gerdt C Riise, Dag Solbu, and Martin Iversen designed the study. Lars Gullestad, Hans Eiskjaer, Gerdt C Riise, Kjartan Krason, Einar Gude, Øystein Bjørtuft, Martin Iversen, Hans Henrik Schultz, Finn Gustafsson, Göran Dellgren, Göran Rådegran, Lennart Hansson, and Kjell Jansson undertook the study and collected data. Lars Gullestad, Hans Eiskjaer, Gerdt C Riise, and Dag Solbu developed the manuscript, following which it was reviewed and approved by all authors. All authors approved the last version and had full access to the data. Lars Gullestad, Hans Eiskjaer, Gerdt C Riise, and Martin Iversen had the final responsibility for the decision to submit this manuscript for publication.

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Conflicts of interest

The authors have no conflicts of interest to declare other than Dag Solbu who is an employee of Novartis Scandinavia.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Patient characteristics by organ type (ITT population).

Table S2. Adverse events reported between month 24 and last follow-up by organ type (long-term safety population).

Table S3. Laboratory and hematology values at month 24 and last follow-up (long-term safety population).

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