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ORIGINAL ARTICLE

Everolimus plus early tacrolimus minimization: a phase III, randomized, open-label, multicentre trial in renal transplantation

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Summary

There is increasing interest in tacrolimus-minimization regimens. ASSET was an open-label, randomized, 12-month study of everolimus plus tacrolimus in *de-novo* renal-transplant recipients. Everolimus trough targets were 3–8 ng/ml throughout the study. Tacrolimus trough targets were 4–7 ng/ml during the first 3 months and 1.5–3 ng/ml (n = 107) or 4–7 ng/ml (n = 117) from Month 4. All patients received basiliximab induction and corticosteroids. The primary objective was to demonstrate superior estimated glomerular filtration rate (eGFR; MDRD-4) at Month 12 in the tacrolimus 1.5–3 ng/ml versus the 4–7 ng/ml group. Secondary endpoints included incidence of biopsy-proven acute rejection (BPAR; Months 4–12) and serious adverse events (SAEs; Months 0–12). Statistical significance was not achieved for the primary endpoint (mean eGFR: 57.1 vs. 51.7 ml/min/1.73 m^2), potentially due to overlapping of achieved tacrolimus exposure levels (Month 12 mean ± SD, tacrolimus 1.5–3 ng/ml: 3.4 ± 1.4; tacrolimus 4–7 ng/ml: 5.5 ± 2.0 ng/ml). BPAR (months 4–12) and SAE rates were comparable between groups (2.7% vs. 1.1% and 58.7% vs. 51.3%; respectively). Everolimus-facilitated tacrolimus minimization, to levels lower than previously investigated, achieved good renal function, low BPAR and graft-loss rates, and an acceptable safety profile in renal transplantation over 12 months although statistically superior renal function of the 1.5–3 ng/ml tacrolimus group was not achieved. (ClinicalTrials.gov: NCT00369161) is registered at http://www.clinicaltrials.gov.

Introduction

Although standard immunosuppression regimens achieve low rates of rejection in the first year after transplantation, long-term renal-transplant survival rates remain relatively poor [1,2]. Risk factors for chronic allograft nephropathy, one of the major causes of graft loss [3], include calcineurin inhibitor (CNI) nephrotoxicity, donor

age, graft quality and co-morbidities [2]. In addition, patients treated with CNIs have an increased risk of developing cardiovascular events and new-onset diabetes mellitus (NODM) [4–6]. These consequences may be averted by early CNI minimization or elimination. Findings from previous studies indicate that cyclosporine (CsA) minimization or elimination strategies using mycophenolate mofetil or the mammalian target of rapamycin inhibitors (mTOR inhibitors)/proliferation signal inhibitors (PSIs), everolimus and sirolimus, have the potential to reduce CsA-associated toxicities and preserve renal function without compromising immunosuppressive efficacy [7–14]. Such strategies may ultimately facilitate improved long-term renal-allograft survival and patient outcomes.

Due to the number of patients currently treated with tacrolimus in clinical practice, there is an increasing interest in regimens that allow tacrolimus minimization. Data from a pilot study demonstrated that everolimus plus tacrolimus (tacrolimus target troughs: 4–7 and 8–11 ng/ml) achieved good efficacy, renal function and an acceptable safety profile over 6 months [15]. ASSET, a 12-month, multicentre, randomized study was designed to investigate the potential of everolimus in allowing minimization of tacrolimus exposure to levels lower than previously assessed (target trough 1.5–3 ng/ml).

Materials and methods

Study design

ASSET was a randomized, open-label, 12-month trial of *de-novo* renal-transplant recipients who received everolimus plus tacrolimus (two target levels), basiliximab and corticosteroids and was performed in 36 centres across 13 countries. This study (NCT00369161) was designed and implemented in accordance with the International Conference on Harmonisation Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations and with the ethical principles laid down in the Declaration of Helsinki. Enrolment commenced in June 2006 and the study completed in December 2008.

Study population

All patients provided written informed consent prior to enrolment and received their first dose of tacrolimus within 24 h of graft reperfusion. Donors were 10–65 years old, either deceased or living, and unrelated or non-HLA-identical related to the recipient. Major exclusion criteria included patients who had received a previous organ transplant, multiple organ transplants, donation after cardiac death, cold ischaemic time ≥30 h, donor-specific transfusions, an A-B-O-incompatible or T-cell cross-match-positive transplant, and patients with panel-reactive-antibody levels ≥50%. All cytomegalovirus (CMV)-negative patients who received an organ from a CMV-positive donor, and any patient who received antibody treatment for an acute-rejection episode, received CMV prophylaxis.

Immunosuppression

All patients received tacrolimus (Prograf®; Astellas, Tokyo, Japan) plus everolimus (Certican®; Novartis, Basel, Switzerland) in combination with basiliximab induction (Simulect®; Novartis) and corticosteroids. In the first 3 months all patients received tacrolimus at daily doses in order to achieve target trough levels between 4 and 7 ng/ml (first dose: 0.1 mg/kg/day). If patients experienced delayed graft function, tacrolimus administration could be delayed for ≤14 days. Biopsy-proven acute rejection (BPAR) therapy was administered according to local practice.

The everolimus starting dose and trough target used in this study (1.5 mg b.i.d; 3–8 ng/ml) were derived from previous trials [15,16]. A central laboratory analysed study drug levels using liquid chromatography/coupled mass spectrometry. On Day 1, patients were assigned a unique patient number and randomized 1:1 by an independent voice-recognition system to either further reduce tacrolimus exposure (trough target: 1.5–3 ng/ml) or to continue tacrolimus at a trough target of 4–7 ng/ml. Each investigator received treatment-allocation cards with the randomization group information covered by a label. At the end of Month 3, the patient's randomization group was revealed and their tacrolimus-dosing regimen was amended accordingly.

All patients received 20 mg basiliximab 2 h prior to transplantation and on Day 4 post-transplantation. Intravenous prednisone (or equivalent) was administered either pre- or intra-operatively according to centre practice. All patients received 20 mg of oral prednisone (or equivalent) on Day 1 and a minimum dose of 5 mg/day; steroid regimens were consistent within each centre.

Primary and secondary endpoints

The primary objective was to evaluate whether superior renal function, assessed by estimated glomerular filtration rate [eGFR; modification of diet in renal disease (MDRD) 4-variable formula], was achieved in the tacrolimus 1.5–3 ng/ml vs. the 4–7 ng/ml group at Month 12. The main secondary objective was to evaluate the non-inferiority of BPAR rates from Months 4 to 12 between the two groups (non-inferiority margin = 8%). Other secondary endpoints assessed at Month 12 included: the incidence of composite efficacy failure (BPAR, graft loss, death or lost

to follow-up); the incidence of each of the components of composite efficacy failure; renal function, as measured by serum creatinine and creatinine clearance (Cockcroft–Gault formula); and renal function, assessed by mean eGFR (MDRD-4) from Months 4–12. Safety endpoints included the incidence of adverse events (AEs) and serious AEs (SAEs) over 12 months, and the incidence of NODM from Months 0–12 and 4–12. NODM was defined as patients who were non-diabetic before transplantation, received glucose-lowering treatment for ≥30 days, and had a random plasma-glucose value of ≥11.1 mM and two fasting plasma glucose values of ≥7 mM or a plasma glucose value (2-hour oral glucose tolerance test) ≥11.1 mM post-transplantation.

Statistical methods

Demographic and background information was summarized using frequency counts (percentages) for categorical variables, and descriptive statistics of mean, standard deviation, median, minimum and maximum for continuous variables. All efficacy analyses and summaries of baseline characteristics were conducted according to the intention-to-treat (ITT) principle and included all randomized patients who received at least one dose of study drug. As the primary analysis, eGFR values at Month 12 were compared between the two treatment groups using a *t*-test at the one-sided significance level of 0.025. As eGFR values were not available for the patients who died or experienced graft loss, this analysis was performed on the modified ITT population (mITT): all ITT patients who had eGFR values at Month 12 including data reported after study-drug discontinuation. As the main secondary analysis, the incidence rates of BPAR from Month 4 to Month 12 were compared to demonstrate that tacrolimus 1.5–3 ng/ml is not inferior to 4–7 ng/ml with non-inferiority margin of 8% at the one-sided significance level of 0.025. All safety data over the 12 months were analysed for the safety population: all patients who received ≥1 dose of study drug and had ≥1 post-baseline safety assessment.

Results

Patient disposition and demographics

In total, 109 and 119 patients were randomized to tacrolimus 1.5–3 ng/ml or 4–7 ng/ml, respectively (four patients from one site were excluded from the ITT population for efficacy analysis due to administrative problems leading to unreliable data entries but were included in the Safety population). Patient and donor demographics and transplant background characteristics and retention rates at Month 12 are displayed in Table 1 and Fig. 1. More patients in the tacrolimus 4–7 ng/ml were undergoing transplantation owing to hypertension/nephrosclerosis compared with the tacrolimus 1.5–3 ng/ml group; more patients in the tacrolimus 1.5–3 ng/ml group were undergoing transplantation owing to polycystic disease compared with the tacrolimus 4–7 ng/ml group.

Table 1. Patient and donor demographics and transplant background characteristics (intention-to-treat population).

	Evrl + tac 1.5–3 ng/ml (*n* = 107)	Evrl + tac 4–7 ng/ml (*n* = 117)	*P*-value
Patient demographics			
Age, mean years (±SD)	44.6 (12.8)	46.9 (12.1)	0.192*
Male, *n* (%)	59 (55.1)	69 (59.0)	0.591†
Race, *n* (%)			
Caucasian	89 (83.2)	98 (83.8)	1.000†,‡
Black	6 (5.6)	1 (0.9)	
Oriental	0 (0.0)	2 (1.7)	
Other	12 (11.2)	16 (13.7)	
Primary disease leading to transplantation, *n* (%)			
Hypertension/nephrosclerosis	8 (7.5)	20 (17.1)	0.042†
Glomerulonephritis/glomerular disease	27 (25.2)	27 (23.1)	0.756†
Diabetes mellitus	7 (6.5)	11 (9.4)	0.471†
Polycystic disease	21 (19.6)	11 (9.4)	0.036†
Unknown	19 (17.8)	21 (17.9)	1.000†
Number of HLA mismatches at Loci DR, *n* (%)			0.615§
1	57 (53.3)	63 (53.8)	
2	18 (16.8)	22 (18.8)	
CMV positive¶, *n* (%)	75 (70.1)	79 (67.5)	0.773†
PRA**, mean (±SD)	1.8 (6.0)	1.4 (5.3)	0.139*
Donor demographics			
Age, mean years (±SD)	44.7 (12.3)	47.3 (11.4)	0.135*
Male, *n* (%)	69 (64.5)	61 (52.1)	0.078†
Donor characteristics, *n* (%)			0.668††
Deceased heart beating	77 (72.0)	83 (70.9)	
Living related	20 (18.7)	19 (16.2)	
Living unrelated	10 (9.3)	15 (12.8)	

*Wilcoxon rank-sum test.
†Fisher's exact test.
‡Caucasian versus other groups.
§Mantel-Haenszel test.
¶Data were missing for one patient in each treatment group.
**most recent evaluation prior to study commencement.
††Chi-square test.
SD, standard deviation; HLA, human leukocyte antigen; CMV, cytomegalovirus; PRA, panel reactive antibody.

Medication exposure

From Day 1 to Month 3, mean tacrolimus exposure was generally within the pre-specified target range (4–7 ng/ml) for both groups. From Months 4 to 12, mean tacrolimus exposure was within the target range for the 4–7 ng/ml

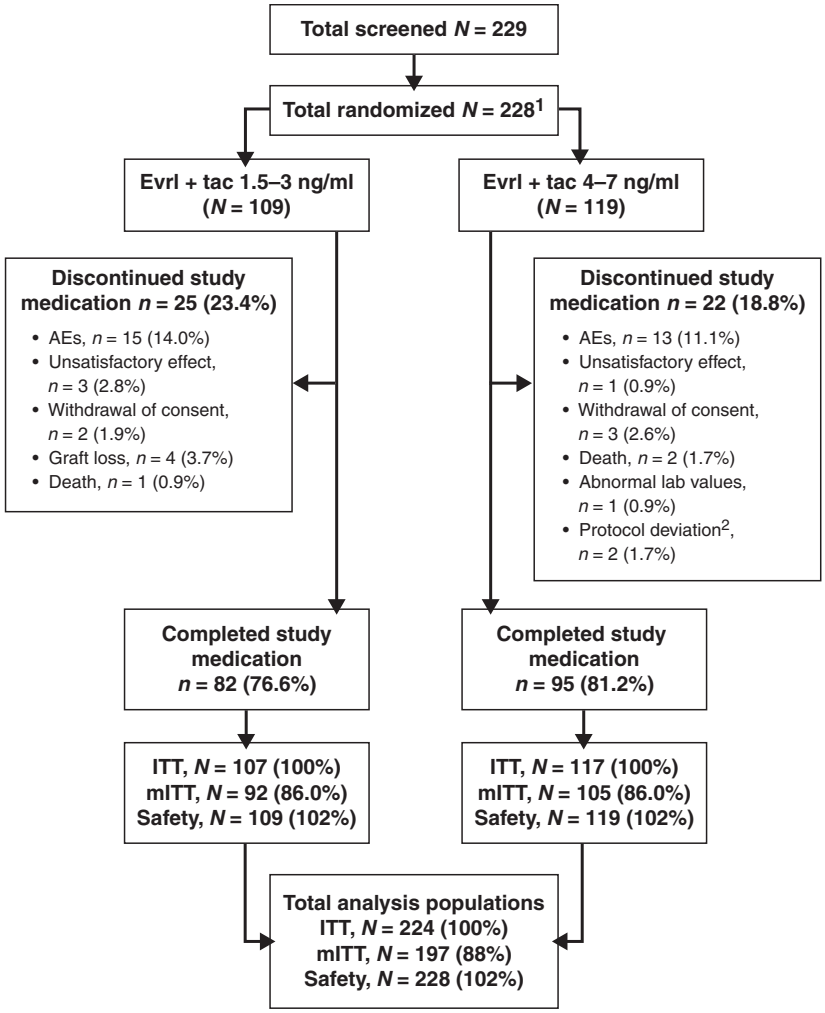

Figure 1 Patient disposition. Percentages are calculated using the ITT population as the denominator; ITT population included all randomized patients who received at least one dose of study drug; mITT population included all patients who had eGFR values at Month 12 including data reported after study-drug discontinuation; safety population included all patients who received at least one dose of study drug and had at least one post-baseline safety assessment; [1]four patients were excluded from the ITT population due to administrative problems leading to unreliable data entries; however, these patients were included in the safety population; [2]one patient did not present for study visits and one patient stopped everolimus treatment for 10 days due to elevated urea and creatinine levels. AE, adverse event; ITT, intention-to-treat; mITT, modified intention-to-treat.

group but above the range for the 1.5–3 ng/ml group (Fig. 2a). Mean ± SD exposure at Month 12 was 5.5 ± 2.0 for the tacrolimus 4–7 ng/ml group and 3.4 ± 1.4 ng/ml for the 1.5–3 ng/ml group. In total, 44.3% of patients in the tacrolimus 1.5–3 ng/ml group and 69.8% in the 4–7 ng/ml group had tacrolimus trough concentrations within their respective target ranges at Month 12.

The mean everolimus dose was comparable between groups throughout the 12-month study period (Fig. 3). Mean ± SD everolimus exposure was within target range (3–8 ng/ml) at all visits and generally comparable between groups (5.8 ± 2.6 in the tacrolimus 1.5–3 ng/ml group vs. 5.5 ± 2.7 ng/ml in the tacrolimus 4–7 ng/ml group at Month 12 ($P = 0.233$); Fig. 2b). Mean steroid dose was generally higher in the tacrolimus 1.5–3 ng/ml group throughout the study with the greatest difference between the groups at Month 4 (Fig. 4). From Months 4–12, mean steroid doses ranged from 0.8–3.0 mg/kg/day in the tacrolimus 1.5–3 ng/ml group and 0.3–0.6 mg/kg/day in the 4–7 ng/ml group.

Renal function results

At Month 12, mean eGFR was higher in the tacrolimus 1.5–3 ng/ml group versus the 4–7 ng/ml group (57.1 ± 19.5 vs. 51.7 ± 20 ml/min/1.73 m^2, respectively; treatment difference: 5.3 ml/min/1.73 m^2; 95% CI: −0.2, 10.9; Fig. 5) although statistical significance was not achieved ($P = 0.0299$) at the level of 0.025. A *post-hoc* ANOVA of the eGFR (MDRD) difference at Month 12 adjusting for the eGFR (MDRD) value at Month 3 (start of different treatment regimens) as a sensitivity analysis yielded similar results ($P = 0.0445$).

The other renal function parameters were consistent with the primary renal function results: at Month 12, mean serum creatinine level was 1.44 ± 0.509 for the tacrolimus 1.5–3 ng/ml group vs. 1.60 ± 0.711 mg/dl for

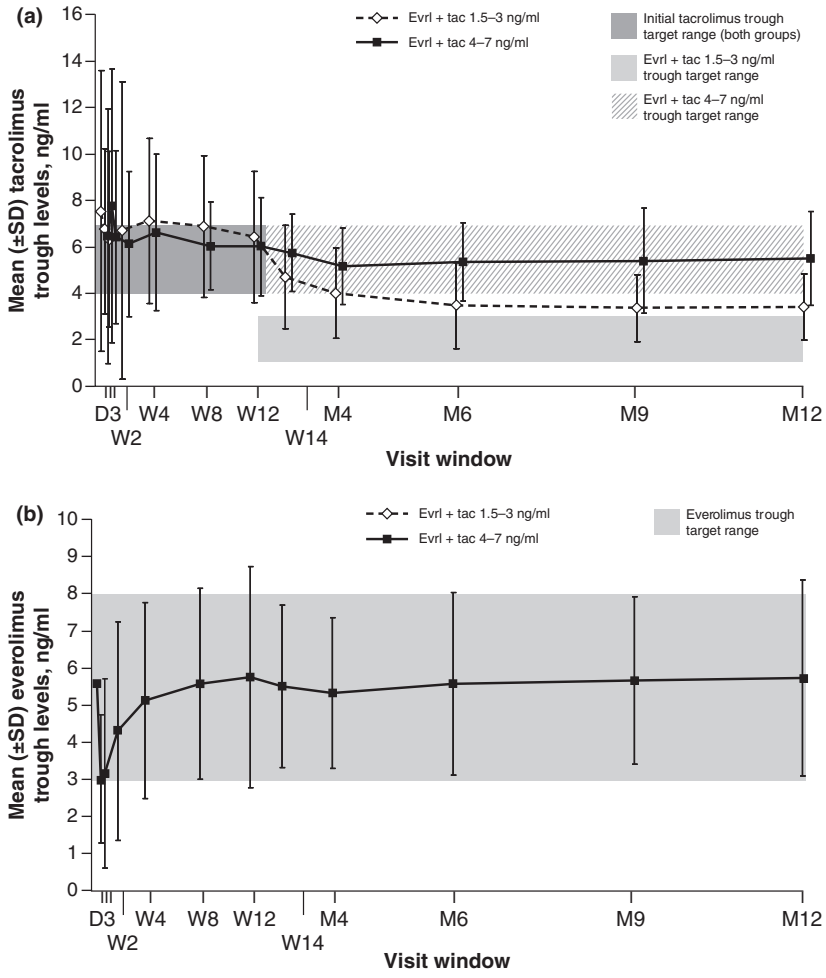

Figure 2 Mean study-drug trough levels by visit window and treatment group over 12 months (safety population). (a) Tacrolimus, (b) Everolimus. (a) At Month 12, 44.3% in the 1.5–3 ng/ml group and 69.8% of patients in the 4–7 ng/ml group had tacrolimus trough levels within the pre-specified target ranges; mean values are joined by a horizontal line; the vertical lines connect the mean value with ± standard deviation at each time point. (b) At Month 12, 79.7% in the 1.5–3 ng/ml group and 86.5% of patients in the 4–7 ng/ml group had everolimus trough levels within the pre-specified target range; target range 3–8 ng/ml; mean values are joined by a horizontal line; the vertical lines connect the mean value with ± standard deviation at each time point. D, day; W, week; M, month.

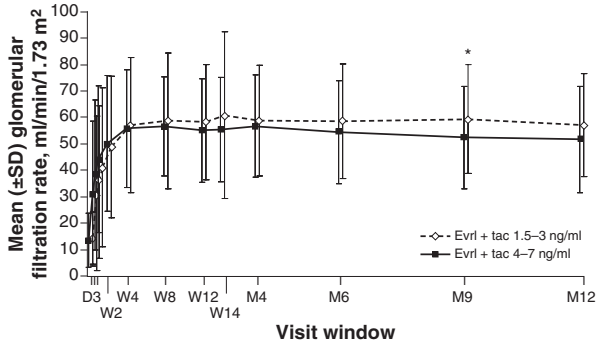

Figure 3 Mean everolimus dose levels by visit window and treatment group over 12 months (safety population). D, day; W, week; M, month.

the tacrolimus 4–7 ng/ml group ($P = 0.0447$). Mean (±SD) creatinine clearance (Cockcroft–Gault formula) at Month 12 was 67.1 ± 23.0 vs. 61.1 ± 19.7 ml/min ($P = 0.0253$), a difference of 6.0 ml/min in favour of the tacrolimus 1.5–3 ng/ml group.

Efficacy results

There were 75 and 93 patients at risk of BPAR at Month 4 in the tacrolimus 1.5–3 ng/ml and 4–7 ng/ml groups, respectively. The BPAR rate from Month 4 to 12 was 2.7% in the tacrolimus 1.5–3 ng/ml and 1.1% in the tacrolimus 4–7 ng/ml group, respectively (treatment difference: 1.6%; 95% CI: −2.6%, 5.8%). Therefore a difference of 5.8% can be ruled out which is lower than the prespecified non-inferiority margin of 8% (P-value = 0.0014 non-inferiority test). Over the total treatment period of 12 months, the rates of BPAR and graft loss were higher in the tacrolimus 1.5–3 ng/ml group. These differences were primarily observed prior to Month 4 (Table 2) with the majority of BPAR events occurring during the first 2–3 weeks (data not shown). The reasons for graft loss were: thrombotic microangiopathy, necrosis, bleeding of the kidney with possible infected arterial anastomoses, immunosuppression withdrawal (one patient each), and technical issues and acute rejection (two patients each) in the tacrolimus 1.5–3 ng/ml group, and acute rejection

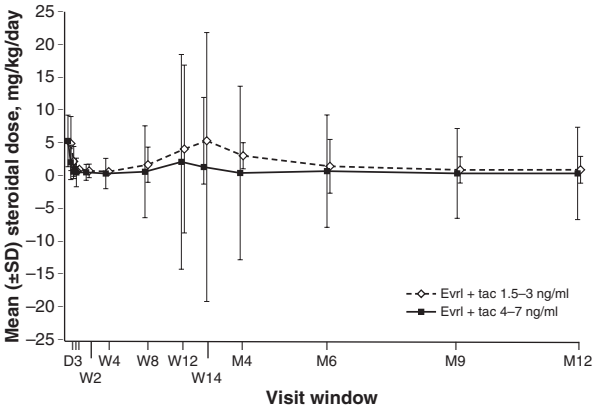

Figure 4 Mean prednisolone (or equivalent) dose by visit window and treatment group (intent-to-treat population). Data are mean prednisolone or prednisolone-equivalent dose of immunosuppressive corticosteroid medication; error bars represent the standard deviation at each time point; regardless of reason, zero doses were used in calculations for periods of temporary interruption to study medication. D, day; W, week; M, month.

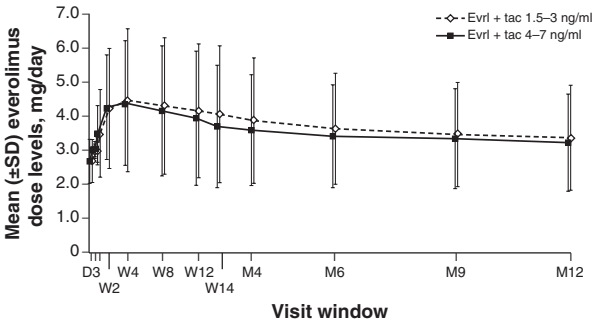

Figure 5 Mean estimated glomerular filtration rate (Modification of Diet in Renal Disease four-variable formula) by visit window and treatment group (modified intention-to-treat population). Error bars represent the standard deviation at each time point. As eGFR values are not available for patients who died or experienced graft loss, this analysis was performed on a modified ITT population (mITT); all patients who had eGFR values at Month 12, including data reported after study-drug discontinuation; *$P = 0.082$ compared with everolimus + tacrolimus 4–7 ng/ml using a 0.025% statistical significance level, calculated by a two-sample *t*-test. eGFR, estimated glomerular filtration rate; D, day; W, week; M, month.

and chronic rejection (one patient each) in the 4–7 ng/ml group.

Over the 12-month study period, the majority of BPARs were mild in severity with seven patients versus one patient experiencing BPAR of Banff Grade IIA in the tacrolimus 1.5–3 ng/ml group and the 4–7 ng/ml group, respectively, and one patient in each group experiencing a BPAR of Banff Grade IIB. A total of 20.6% vs. 11.1% of patients received steroid treatment for acute rejection

Table 2. Summary of efficacy outcomes by treatment group over 12 months of treatment and from Months 4 to 12 (intention-to-treat population).

	Evrl + tac 1.5–3 ng/ml (n = 107)	Evrl + tac 4–7 ng/ml (n = 117)	Difference in event rate (95% CI)
Overall efficacy analyses (12 months of treatment)			
Efficacy failure*	29 (27.1)	14 (12.0)	15.1 (4.9, 25.4)†
BPAR	20 (18.7)	9 (7.7)	11.0 (2.2, 19.8)‡
Death or graft loss	10 (9.3)	5 (4.3)	5.1 (−1.5, 11.7)
Graft loss	8 (7.5)	2 (1.7)	5.8 (0.3, 11.3)
Death	3 (2.8)	3 (2.6)	0.2 (−4.0, 4.5)
Lost to follow up	4 (3.7)	1 (0.9)	2.9 (−1.1, 6.8)
Months 4 to 12 efficacy analyses			
Efficacy failure*	5 (6.7)	4 (4.3)	2.4 (−4.6, 9.4)
BPAR	2 (2.7)	1 (1.1)	1.6 (−2.6, 5.8)§,¶
Death or graft loss	3 (4.0)	2 (2.2)	1.8 (−3.5, 7.2)
Graft loss	1 (1.3)	1 (1.1)	0.3 (−3.1, 3.6)
Death	2 (2.7)	1 (1.1)	1.6 (−2.6, 5.8)
Lost to follow up	0 (0.0)	1 (1.1)	−1.1 (−3.2, 1.0)

*Efficacy failure was defined as at least one of: BPAR, graft loss, death or lost to follow up.

†$P = 0.006$, Cochran-Mantel-Haenzel general association test stratified for centre.

‡$P = 0.0138$, Cochran-Mantel-Haenzel general association test stratified for centre.

§Non-inferior $P = 0.0014$.

¶$P = 0.1653$, Cochran-Mantel-Haenzel general association test stratified for centre.

CI, confidence interval; BPAR, biopsy-proven acute rejection.

while five BPARs required antibody therapy (three patients in the tacrolimus 1.5–3 ng/ml group versus two in the tacrolimus 4–7 ng/ml group). All BPARs that occurred after tacrolimus-dose modification (end of Month 3 onwards) were mild in severity and resolved with steroid treatment.

A *post-hoc* analysis was conducted to assess the relationship between study drug exposure and the occurrence of BPAR. For patients with a rejection within the first 30 days, trough levels on or prior to rejection date were averaged. For patients without a rejection within the first 30 days, trough levels by Day 30 were averaged. Median trough levels of tacrolimus and everolimus in the tacrolimus 1.5–3 ng/ml group were slightly lower in patients with BPAR versus those without a BPAR [tacrolimus: 4.94 ng/ml (range 1.75–13.0; n = 14) vs. 6.50 ng/ml (range 2.52–17.10; n = 93) and everolimus: 2.80 ng/ml (range 1.45–7.80; n = 11) vs. 3.53 ng/ml (range 1.17–8.73; n = 93), respectively]. Median trough levels in the tacrolimus 4–7 ng/ml group were more comparable in patients with versus without a BPAR [tacrolimus: 6.25 ng/ml (range 4.75–7.0; n = 5) vs. 6.26 ng/ml (range 2.48–21.9; n = 112) and everolimus: 3.20 ng/ml (range 2.55–4.23; n = 5) vs. 3.59 ng/ml (range 1.30–7.55; n = 112)]. No

relationship was identified between either everolimus or tacrolimus levels and the occurrence of BPAR.

Safety results

The incidence of AEs was comparable between groups and the majority were mild-to-moderate in severity (Table 3). Acne was more frequently reported in the tacrolimus 1.5–3 ng/ml group versus the 4–7 ng/ml group while hypercholesterolaemia, hypertriglyceridaemia, increase in serum creatinine and insomnia were more frequently reported in the tacrolimus 4–7 ng/ml group. In both groups, bacterial infections were most commonly reported (bacterial: 39.4% vs. 35.3%; viral: 9.2% vs. 10.9% and fungal: 5.9% vs. 7.3% in the tacrolimus 1.5–3 ng/ml group versus the tacrolimus 4–7 ng/ml group, respectively). The overall incidence of CMV and BK virus infection in this study was low (CMV: 1.8% vs. 2.8% and BK: 4.2% vs. 0.8% in the tacrolimus 1.5–3 ng/ml group versus the 4–7 ng/ml group, respectively). The reason for the higher incidence of BK virus infection in the tacrolimus 1.5–3 ng/ml group is unclear.

The incidence of SAEs and discontinuations due to SAEs was marginally higher in the tacrolimus 1.5–3 ng/ml group versus the 4–7 ng/ml group (SAEs: 58.7% vs. 51.3%; discontinuations: 11.9% vs. 6.7%, respectively). There were no SAEs that predominantly contributed to these differences and the incidence of serious infections was similar in both groups (tacrolimus 1.5–3 ng/ml: 24.8% vs. 4–7 ng/ml: 26.9%). Over this 12-month study, 8.3% of patients in the tacrolimus 1.5–3 ng/ml group vs. 7.6% in the 4–7 ng/ml group required study-drug adjustments or interruptions due to SAEs. Only one neoplasm was reported (tacrolimus 4–7 ng/ml group).

Three deaths occurred in each group. Four of these deaths, two in each group, occurred during Months 4–12. In the tacrolimus 1.5–3 ng/ml group, deaths were due to acute pancreatitis (Day 72), viral encephalitis (Day 359) and bleeding after a nephrectomy (Day 175). In the tacrolimus 4–7 ng/ml group, deaths were due to haemolytic uremic syndrome (Day 19), invasive aspergillosis (Day 239) and sepsis caused by pneumonia (Day 198). The deaths that occurred due to viral encephalitis, haemolytic uremic syndrome and invasive aspergillosis were considered by the investigator to be related to the study drug.

There was a similar, low rate of tissue-regeneration complications in the two groups (lymphocele: 7.3% vs. 10.9%, wound complication: 4.6% vs. 5.9%, wound dehiscence: 3.7% vs. 1.7% and incisional hernia: 1.8% vs. 2.5%, for the tacrolimus 1.5–3 ng/ml group versus the tacrolimus 4–7 ng/ml group, respectively).

At Month 12, the mean (range) urinary-protein concentrations in the tacrolimus 1.5–3 ng/ml group and the 4–7 ng/ml group were 0.24 (0.00–3.49) and 0.27 g/l (0.00–4.15), respectively. Proteinuria reported as an AE is presented in Table 3. Vital signs and laboratory abnormalities were generally comparable between groups. The

Table 3. Summary of adverse events (including infections) in ≥10% of patients in either treatment group (safety population).

	Evrl + tac 1.5–3 ng/ml (*n* = 109)	Evrl + tac 4–7 ng/ml (*n* = 119)
Any AE	107 (98.2)	117 (98.3)
SAEs	64 (58.7)	61 (51.3)
AEs leading to study drug discontinuation	19 (17.4)	12 (10.1)
Most frequently reported AEs (≥10% of patients in any treatment group)*		
Metabolism and nutrition disorders	84 (77.1)	101 (84.9)
Hypercholesterolaemia	23 (21.1)	31 (26.1)
Dyslipidaemia	14 (12.8)	21 (17.6)
Hyperlipidaemia	15 (13.8)	19 (16.0)
Hypokalaemia	17 (15.6)	16 (13.4)
Diabetes mellitus	14 (12.8)	18 (15.1)
Hyperglycaemia	15 (13.8)	14 (11.8)
Hyperkalaemia	14 (12.8)	11 (9.2)
Hypertriglyceridaemia	6 (5.5)	15 (12.6)
Infections and infestations	73 (67.0)	80 (67.2)
Urinary tract infection	36 (33.0)	42 (35.3)
Gastrointestinal disorders	48 (44.0)	56 (47.1)
Constipation	15 (13.8)	22 (18.5)
Diarrhoea	17 (15.6)	19 (16.0)
General disorders and administration-site conditions	47 (43.1)	56 (47.1)
Peripheral oedema	26 (23.9)	28 (23.5)
Pyrexia	13 (11.9)	19 (16.0)
Oedema	10 (9.2)	13 (10.9)
Injury, poisoning and procedural complications	46 (42.2)	41 (34.5)
Complications of transplanted kidney	20 (18.3)	17 (14.3)
Procedural pain	12 (11.0)	12 (10.1)
Vascular disorders	36 (33.0)	43 (36.1)
Hypertension	19 (17.4)	19 (16.0)
Lymphocoele	8 (7.3)	13 (10.9)
Renal and urinary disorders	39 (35.8)	35 (29.4)
Proteinuria	12 (11.0)	9 (7.6)
Blood and lymphatic system disorders	37 (33.9)	35 (29.4)
Anaemia	30 (27.5)	28 (23.5)
Investigations	23 (21.1)	36 (30.3)
Blood creatinine increased	12 (11.0)	19 (16.0)
Skin and subcutaneous tissue disorders	31 (28.4)	20 (16.8)
Acne	15 (13.8)	6 (5.0)
Nervous system disorders	21 (19.3)	20 (16.8)
Headache	11 (10.1)	9 (7.6)
Psychiatric disorders	17 (15.6)	25 (21.0)
Insomnia	12 (11.0)	20 (16.8)

*By primary system organ class and preferred term; data are investigator reported AEs; values are *n* (%).
AE, adverse event; SAE, serious AE.

mean (range) total cholesterol concentration was 5.64 (3.20–10.0) vs. 5.57 (3.60–10.60) mM and the mean (range) total triglyceride concentration was 2.26 (0.60–7.50) vs. 2.49 (0.60–6.90) mM in the tacrolimus 1.5–3 ng/ml group versus the 4–7 ng/ml group, respectively. A similar proportion of patients in each group received angiotensin-converting enzyme inhibitors (35.8% vs. 36.1%, respectively), and angiotensin II antagonists (13.8% vs. 15.1%). The proportion of patients who received lipid-lowering agents was also comparable between groups (HMG-CoA reductase inhibitors: 16.5% in the tacrolimus 1.5–3 ng/ml group vs. 17.6% in the tacrolimus 4–7 ng/ml group, respectively).

Adverse events commonly associated with calcineurin-inhibitor treatment

The incidence of insomnia was numerically lower in the tacrolimus 1.5–3 ng/ml group versus the tacrolimus 4–7 ng/ml group (11% vs. 16.8%, respectively) while the incidence of tremor, hyperglycaemia and alopecia was comparable between groups (tremor: 5.5% vs. 2.5%; hyperglycaemia: 13.8% vs. 11.8%; alopecia: 0.9% vs. 0.8%). The rate of NODM from Months 0–12 and from Months 4–12 was numerically lower in the tacrolimus 1.5–3 ng/ml group [Months 0–12: 17.8% vs. 20.5%, respectively (95% CI for difference in event rates: −13.0, 7.5); Months 4–12: 2.7% vs. 8.6%, respectively (−12.7, 0.8; $P = 0.086$)].

Discussion

Data from this study build upon a previously published 6-month pilot study [15], which demonstrated that everolimus-facilitated tacrolimus minimization (to target trough levels of 4–7 and 8–11 ng/ml) was efficacious with good renal function and an acceptable safety profile in *de-novo* renal-transplant recipients. The initial 3 months of the ASSET study confirmed the pilot study's findings, and from Months 4 to 12 demonstrated that everolimus plus tacrolimus, at even lower levels than previously investigated, achieved good renal function, low rates of BPAR and graft loss, and an acceptable safety profile.

The primary objective of the ASSET study – to demonstrate superiority of mean eGFR (MDRD-4) at Month 12 for the tacrolimus 1.5–3 ng/ml group versus the tacrolimus 4–7 ng/ml group – was not achieved, although there was a treatment difference of 5.3 ml/min/1.73 m^2 in eGFR at Month 12. As a high proportion of patients in the tacrolimus 1.5–3 ng/ml group had tacrolimus levels above the target range, the smaller-than-hypothesized difference in renal function between the two groups may be attributable to the relatively small separation of the immunosuppression regimens that were actually employed. In addition, the study was powered to have 80% probability of detecting a treatment difference in GFR at Month 12 of 7 ml/min under the assumption of common standard deviation of 17.3 ml/min, but the observed treatment difference and standard deviations were 5.34 ml/min and 19.77 ml/min, respectively. Although the study did not demonstrate superior renal function in the tacrolimus 1.5–3 ng/ml group versus the tacrolimus 4–7 ng/ml group these results build upon data from a previously published study, which showed that an everolimus-facilitated CsA-minimization regimen had comparable efficacy, safety and improved renal function over 1 year compared with MPA plus standard CsA [14].

The ASSET study met its main secondary objective: a difference of more than 8% in BPAR rates from Months 4 to 12 between the two groups was ruled out. However, the overall incidence of BPAR during the 12-month study period was higher in the tacrolimus 1.5–3 ng/ml group, which was largely attributable to the higher rate reported prior to tacrolimus minimization in the 1.5–3 ng/ml group. As the baseline characteristics between the treatment groups were generally comparable and the treatment regimens were identical during this period, there is no apparent reason for the higher incidence of BPAR in the tacrolimus 1.5–3 ng/ml group versus the tacrolimus 4–7 ng/ml group. It is possible that this higher BPAR rate had subsequent deleterious effects on the GFR, potentially providing an additional explanation for the fact that superior renal function was not demonstrated in the tacrolimus 1.5–3 ng/ml group. The BPAR rates over the 12-month study period in this study (18.7% in the tacrolimus 1.5–3 ng/ml group vs. 7.7% in the tacrolimus 4–7 ng/ml group) were generally comparable to a previous study that investigated early minimization of tacrolimus in everolimus-treated patients (14% in both treatment groups) [15]. Throughout the study, BPARs were mainly of mild severity and resolved with steroid treatment; these factors are consistent with good long-term graft survival and patient outcomes [17,18]. As the efficacy of the treatment regimens was similar in the tacrolimus 1.5–3 ng/ml group versus the 4–7 ng/ml group, these results indicate that everolimus provides potent immunosuppression, allowing early tacrolimus minimization to ∼3.4 ng/ml without compromising efficacy.

The safety profiles of the two groups were generally comparable and AEs were manageable. Although SAEs were marginally higher in the tacrolimus 1.5–3 ng/ml group versus the 4–7 ng/ml group there were no specific SAEs that predominantly contributed to these differences, and a comparable proportion of patients in each group required study-drug adjustments or interruptions due to SAEs. Infection rates were also higher in the tacrolimus

1.5–3 ng/ml group although the rates of infections and serious infections in both groups were lower than in previously published studies of everolimus plus standard-exposure CNIs [12,19,20]. Partially due to the CMV prophylaxis employed, and consistent with previous everolimus studies [12,21–23], the incidence of CMV infection was low in this study. Indeed, when an everolimus plus tacrolimus regimen was compared with an MMF plus tacrolimus regimen in a randomized, controlled trial, the incidence of CMV infection was significantly lower in the everolimus group [12]. Similar CMV infection rates to those reported for the everolimus regimens in the ASSET study have previously been reported with sirolimus plus CNI regimens, including tacrolimus [24,25]. With the exception of insomnia, the incidence of AEs commonly attributed to CNI exposure was generally comparable between groups. Treatment regimens that include standard-dose tacrolimus in renal-transplant recipients have previously been associated with an increased risk of NODM [26]. In contrast, a low incidence of NODM with everolimus-facilitated tacrolimus minimization was reported in the ASSET study. Also of interest, the incidence of diarrhoea in the ASSET study was lower compared with clinical trials of patients treated with MMF plus tacrolimus [27,28]. This is noteworthy as diarrhoea may lead to drug-dose reductions, reduced efficacy and an increased risk of acute rejection [29,30]. Finally, it is important to acknowledge the higher incidence of acne in the tacrolimus 1.5–3 ng/ml group versus the 4–7 ng/ml group, which is potentially attributable to the higher mean dose of corticosteroids in this group throughout the study and the higher rate of steroid treatment for acute rejection.

Several methodological factors should be taken into account in the interpretation of this study. Most importantly, the mean tacrolimus trough concentration was above the target level (an infringement of the study protocol) in the tacrolimus 1.5–3 ng/ml group from Month 4 onwards, meaning that the tacrolimus levels in the two groups overlapped. The two treatment groups had more similar immunosuppressive regimens, therefore, than specified in the study protocol which makes it difficult to make definitive conclusions regarding the two regimens. These data suggest that there is ongoing reluctance to reduce CNIs even in the presence of everolimus. In addition, the investigators chose to administer higher doses of steroids in patients randomized to the tacrolimus 1.5–3 ng/ml group, as demonstrated by the higher mean doses of corticosteroids in the tacrolimus 1.5–3 ng/ml group versus the tacrolimus 4–7 ng/ml group throughout the study. Secondly, the patients in this study were at immunological low risk and extrapolation of these results to other transplant populations should be conducted with caution. Thirdly, the primary endpoint analyses were based on the mITT population and did not include patients who experienced graft loss or died. Finally, as the purpose of the study was to explore the extent of tacrolimus minimization that could be achieved using everolimus without impacting on patient outcomes and not to make comparisons to another regimen, a control arm was not included.

In conclusion, these data concur with previous findings suggesting that everolimus in combination with early and substantial tacrolimus minimization (~3.4 ng/ml), basiliximab induction therapy, and low-dose corticosteroids achieves low BPAR and graft-loss rates, good renal function and an acceptable safety profile. Results are awaited from a further study of everolimus-facilitated tacrolimus minimization [31].

Authorship

RML: study design, writing of the paper and performance of the study, and has received honoraria from Novartis. RH: study design, writing of the paper and performance of the study, and has received consultancy fees from Novartis and grant/research support from Novartis, Astellas, Roche and Wyeth. SV: study design, writing of the paper and performance of the study, and has received honoraria from Novartis, Astellas and Wyeth. MC: study design, writing of the paper and performance of the study, and has received grants from Novartis and grant/research support from Roche and Astellas. HTS: study design, writing of the paper and performance of the study, and has received grant/research support from Novartis, Astellas, Janssen-Cilag, Bristol-Myers Squibb and Pfizer, payment for scientific advice from Novartis and honoraria from Novartis, Astellas, Janssen-Cilag, Bristol-Myers Squibb and Wyeth. KC: study design, writing of the paper and performance of the study, and reports no conflicts of interest. EC: study design, writing of the paper and performance of the study, and has provided an expert testimony for Novartis. LR: study design, writing of the paper and performance of the study and has received honoraria from Novartis, Astellas, BMS and Genzyme and acted as an adviser at advisory boards for Novartis and Astellas. MV: study design, writing of the paper and performance of the study, and has a financial relationship with Novartis. UM, BU and GJ: study design, writing of the paper and are employees of Novartis. GD: study design, writing of the paper, participated in the data analyses, and is an employee of Novartis. JP: study design, writing of the paper, performance of the study, and has received honoraria from Novartis.

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