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ORIGINAL ARTICLE

Renal function, efficacy and safety postconversion from twice- to once-daily tacrolimus in stable liver recipients: an open-label multicenter study

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Conflicts of interest
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Summary

This multicenter, open-label, phase III study assessed renal function, safety, and efficacy in stable adult liver transplant recipients converted from tacrolimus twice-daily (BID) to once-daily (QD). Patients received tacrolimus BID for 6 weeks before conversion to tacrolimus QD (1:1 [mg:mg] total daily dose basis) for 12 weeks. Primary endpoint: change in steady state creatinine clearance (CrCl) between treatment phases. Of 112 patients enrolled, 98 were converted to QD dosing (full analysis set [FAS]). Mean (SD) tacrolimus dose was 3.7 (1.7) mg/day during BID and at conversion, and 3.9 (1.8) mg/day at Week 12. 74.5% of patients required no dose adjustment on conversion (FAS). Mean tacrolimus whole blood trough levels were at the lower end of the recommended range during tacrolimus BID and QD; the difference between mean steady-state trough levels was statistically significant (7.5 ng/ml vs. 6.5 ng/ml; $P < 0.0001$). Following conversion, mean tacrolimus trough levels were reduced by 15% (about 1 ng/ml) without any cases of acute rejection, remained stable during the remainder of the study, and were more consistent, showing reduced between- and within-patient variability in trough levels. Renal function remained stable, demonstrating noninferiority of tacrolimus QD versus BID (relative difference in mean calculated CrCl −0.1% [±6.3%]). Patient and graft survival were 100%. Adverse events incidence was low during both treatment phases.

Introduction

The immunosuppressive agent tacrolimus is available as an immediate-release formulation administered twice daily for the prevention and treatment of allograft rejection in liver, kidney, and heart transplantation (Prograf® [tacrolimus twice daily; BID]; Astellas Pharma Europe Limited, Middlesex, UK). A prolonged-release formulation of tacrolimus has also been developed (Advagraf® [tacrolimus once daily; QD]; Astellas Pharma Europe Limited, UK) to provide more consistent exposure and a convenient once-daily dosing, which may improve long-term patient outcomes.

Nonadherence to post-transplant immunosuppression in liver transplant recipients has been identified as a significant cause of late rejection and death in liver transplant recipients [1–3]. Nonadherence may result in blood trough levels of the immunosuppressive agent falling below the therapeutic threshold or causing variable exposure [4]. For calcineurin inhibitors, which have a narrow therapeutic window, this is especially important as these fluctuations can lead to graft rejection and loss [5]. Treatment adherence levels decline over time after transplantation [6], so strategies to improve adherence, such as reduced dosing frequency [7], are important in the long term. A simplified regimen of once-daily dosing could

help transplant patients to maintain long-term adherence to immunosuppression and improve consistency of exposure [8,9].

In previous studies, tacrolimus QD has been shown to be therapeutically equivalent to tacrolimus BID in *de novo* liver transplant recipients [10–12]. The conversion of stable liver transplant recipients from tacrolimus BID to tacrolimus QD on a 1:1 (mg:mg) total daily dose basis was investigated in a previous double-crossover study [13]. The relationship between area under the curve and tacrolimus blood trough levels was the same for both tacrolimus BID and tacrolimus QD, hence the same target trough levels are appropriate for both formulations [13]. Steady-state exposure of tacrolimus QD was equivalent to the BID formulation after conversion on a mg:mg total daily dose basis, but within-patient variability in exposure was reduced. Approximately 80% of patients in that study required no dose adjustment after conversion.

As renal failure is a significant cause of morbidity and mortality among liver transplant recipients [14–16], maintaining good renal function in liver transplant recipients should be an important consideration in improving outcomes post-transplantation. Previous studies in *de novo* and conversion liver transplant recipients have shown good renal function with tacrolimus QD [10,12,13].

The objective of this study was to assess renal function, safety, and efficacy in stable adult liver transplant recipients converted from tacrolimus BID to a tacrolimus QD-based immunosuppressive regimen on a 1:1 (mg-for-mg) total daily dose basis.

Patients and methods

Study design

This was a multicenter, open-label, single-sequence, crossover, phase IIIb study designed to assess the safety and efficacy of an immunosuppressive regimen based on tacrolimus QD in stable liver transplant subjects (NCT00384202). At enrollment (Week −6), eligible patients continued to receive tacrolimus BID as the study medication for 6 weeks. Visits during the tacrolimus BID phase were scheduled at Weeks −6, −4, −2, and Day −1 (Fig. 1). At Day 1, eligible patents were converted to tacrolimus QD on a 1:1 (mg:mg) total daily dose basis. Patients then received tacrolimus QD treatment (administered in the morning) for 12 weeks. Visits during the tacrolimus QD phase were scheduled at Day 1, and at Weeks 1, 2, 4, 6, 8, 10, and 12.

The study was conducted in accordance with the Declaration of Helsinki. Independent Ethics Committees from each study center granted approval for the study prior to implementation. Written informed consent was obtained from each patient. The date of first enrollment was October 2006 and the date of last evaluation was November 2007. This study was conducted at 13 clinical sites in Europe (Poland, France, United Kingdom, Republic of Ireland, Spain, and Germany).

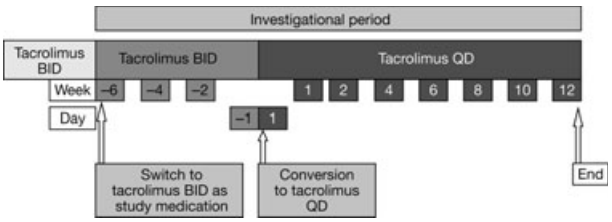

Figure 1 Study design.

Study population

Male and female patients (age ≥18 years) were eligible to enter the study if they had received a liver transplant at least 12 months prior to enrollment, were clinically stable in the opinion of the investigator, were being treated with tacrolimus BID, and their tacrolimus BID dose had been unchanged for a minimum of 12 weeks prior to enrollment. Whole blood tacrolimus trough levels must have been in the range 5–15 ng/ml. The immunosuppressive regimen must also have remained unchanged in the previous 12 weeks.

Patients were excluded from participation if they had a previous organ transplant other than a liver; an acute rejection (AR) episode within 12 weeks prior to enrollment, or an AR episode within the 24 weeks prior to enrollment that required anti-lymphocyte antibody therapy; the diagnosis of new onset malignancy after transplantation (with the exception of basal cell or squamous cell carcinoma of the skin that had been successfully treated); a known allergy to the study drug or any of its components; any unstable medical condition that would interfere with the study objectives in the opinion of the investigator; any form of substance abuse, psychiatric disorder or condition which, in the opinion of the investigator, would complicate communication with the investigator; participated in another clinical trial, taken an investigational drug, received prohibited concomitant therapy, or received prohibited concomitant therapy within 28 days prior to enrollment; proteinuria >2 g/24 h; calculated creatinine clearance (CrCl) <40 ml/min (Cockcroft–Gault formula); total bilirubin ≥50 μmol/l; elevated serum glutamic pyruvic transaminase (SGPT)/alanine aminotransferase (ALT) or serum glutamic oxaloacetic transaminase (SGOT)/aspartate aminotransferase(AST) levels ≥3 times the upper value of the normal range for the investigational site. Patients were also

excluded if they were a breast-feeding mother; HIV positive; or unlikely to comply with the visits scheduled in the protocol.

Patients were eligible for entry into the tacrolimus QD phase if, during the tacrolimus BID phase, there had been no change in tacrolimus BID dose, concomitant immunosuppressant doses had remained unchanged, and tacrolimus whole blood trough levels had stayed within the recommended range of 5–15 ng/ml. Patients were excluded from entering the tacrolimus QD phase if, during the tacrolimus BID-treatment phase, they had experienced AR, calculated CrCl <40 ml/min (Cockcroft–Gault), total bilirubin ≥50 μmol/l or elevated SGPT/ALT or SGOT/AST levels ≥3 times the upper value of the investigational site.

Drug administration

The study medications were tacrolimus BID (Prograf®; Astellas Pharma Europe Limited), administered twice daily in the morning and evening, and tacrolimus QD (Advagraf®; Astellas Pharma Europe Limited) administered once daily in the morning. Tacrolimus BID and QD capsules were swallowed with fluid, on an empty stomach or at least 1 h before or 2–3 h after a meal.

During the tacrolimus QD phase, tacrolimus dose modifications were to be made only if indicated by clinical signs or if whole blood trough levels deviated >20% from the mean levels during the tacrolimus BID phase. Tacrolimus whole blood trough levels were monitored locally by microparticle enzyme immunoassay (MEIA; IMX®), enzyme multiplied immunoassay technique (EMIT), or high-performance liquid chromatography (HPLC)–mass spectroscopy (MS)/MS analyses. Blood samples (2 ml) were taken before the morning dose of tacrolimus at all scheduled visits and as clinically indicated.

Diagnosis and classification of rejection

Liver biopsy was performed for suspected AR (indicated by symptoms, signs, and liver function tests) as soon as possible after onset of clinical/laboratory signs and symptoms and prior to any treatment for rejection, and was evaluated by a local histopathologist. If the rejection episode occurred during the tacrolimus BID-treatment phase, the patient was to be withdrawn from the study.

Endpoints

The primary safety endpoint was relative difference in mean CrCl (calculated by Cockcroft–Gault method) at steady state during the tacrolimus BID-treatment phase (Week −6 to Day −1) compared to the tacrolimus QD phase (Week 6 to Week 12) in the per-protocol set (PPS).

The secondary endpoints were incidence of AR and biopsy-proven AR (BPAR), adverse events (AEs), serious AEs, patient and graft survival, and changes in blood pressure (change in 24-h mean arterial blood pressure from Day −1 [tacrolimus BID-treatment phase] to Week 12 [tacrolimus QD-treatment phase]), glycosylated hemoglobin (HbA_{1c}), and liver function (SGOT/AST, SGPT/ALT, and total bilirubin) after conversion to tacrolimus QD (between Day −1 and Week 12) in the full analysis set (FAS). Vital signs (heart rate, blood pressure, and body weight) were assessed at every scheduled study visit.

Populations for analysis

The FAS (i.e. intent-to-treat population) included all patients who received at least one dose of study medication in each treatment phase (i.e. at least one dose of tacrolimus BID and one dose of tacrolimus QD) with sufficient recorded data for deriving the primary variable at least once during each treatment phase.

The PPS included all patients in the FAS who had no major protocol violations and who contributed sufficient recorded data for deriving the primary endpoint at least once during the steady-state phase for each treatment (Week −6 to Day −1 for tacrolimus BID, and Week 6 to Week 12 for tacrolimus QD). Major protocol violations included: no CrCl measurement obtained during tacrolimus BID and/or tacrolimus QD treatment phases; major violation of inclusion or exclusion criteria; noncompliance with tacrolimus switch and dose adjustment rules; use of forbidden concomitant medication for ≥7 consecutive days; or change of immunosuppressive regimen (combination of medication) for ≥7 consecutive days.

The safety analysis set included all participants who received at least one dose of study medication (i.e. tacrolimus BID or QD) and for whom any data are reported after first dose of study medication.

Assuming 20% of patients would be excluded from the PPS, approximately 125 patients were planned for enrollment to reach a power greater than 90% for assessing noninferiority of tacrolimus QD. The noninferiority margin was predefined as 10% of the reference mean (tacrolimus BID), which was considered to be clinically meaningful.

Statistical methods

Analysis of the primary endpoint, change in mean calculated CrCl (Cockcroft–Gault formula) during the steady-state phase of each treatment (Week −6 to Day −1 for tacrolimus BID and Week 6 to Week 12 for tacrolimus

QD), was carried out for the PPS. The two treatment arms were compared for noninferiority, with tacrolimus BID as the reference treatment, and with a noninferiority margin of −10% of the mean CrCl for tacrolimus BID. Mean CrCl during the steady-state phase was defined as mean of the calculated CrCl at Weeks −6, −4, and −2, and Day −1 for the tacrolimus BID-treatment phase, and mean of the calculated CrCl at Weeks 6, 8, 10, and 12 for the tacrolimus QD treatment phase. If there was no recording of the primary variable during the tacrolimus QD steady-state phase, the last recorded value in Weeks 1–4 was used for performing the comparison.

The comparison of CrCl was based on the lower limit of the two-sided 95% confidence interval (CI) (corresponding to a one-sided significance level of 2.5%) for the relative difference of population means ([CrCl during the tacrolimus QD phase – CrCl during the tacrolimus BID phase]/CrCl during the tacrolimus BID phase), which should lie above the acceptance limit of −10% of the tacrolimus BID mean for concluding noninferiority. Analysis was performed using an appropriate analysis of variance for repeated measurements that included the data from scheduled visits from Week −6 to Day −1 and Week 6 to Week 12. The primary analysis was repeated in the FAS to assess the robustness of the results of the primary analysis. For secondary endpoints, Kaplan–Meier analyses were conducted for the time to first AR and time to first BPAR (FAS).

The AEs were coded using the Medical Dictionary for Regulatory Activities version 6.1 and summarized by primary system organ class, high-level term, and preferred term. Patient and graft survival were summarized for the safety analysis set by overall frequencies and Kaplan–Meier estimates for time to death and time to graft loss were conducted as appropriate. Changes in ambulatory blood pressure, HbA_{1c}, total bilirubin, SGPT/ALT, and SGOT/AST from Day −1 to Week 12 were summarized for the FAS and analyzed using the one sample *t*-test.

Additional analyses

In addition to calculating CrCl using the Cockcroft–Gault formula, the simplified Modified Diet in Renal Disease (MDRD) 4 equation was also used to estimate glomerular filtration rate (GFR) and provide a secondary estimate of renal function [17], reflecting current practice as it evolved during the course of the study.

Analysis of between- and within-patient variability in whole blood tacrolimus trough levels was planned in the protocol as a secondary analysis. The Statistical Analysis Plan and Clinical Study Report did not include this analysis, but referred to a later report. These additional secondary analyses have now been carried out. The between- and within-patient variability in tacrolimus whole blood trough levels were analyzed during the steady-state phase for each of the tacrolimus BID and QD treatments. Within-patient variability was also compared between the tacrolimus BID phase and the first 6 weeks of tacrolimus QD treatment in a *post hoc* analysis. To evaluate between-patient variability, the trough levels were measured for each patient during the 6-week tacrolimus BID phase, and during Weeks 1–6 and 7–12 of the tacrolimus QD phase. The standard deviation for each patient was then calculated for each of the three 6-week periods. Comparisons were made using a similar model to the primary analysis, using PROC MIXED, allowing for repeated measures within-subject, using an unstructured correlation matrix and a fixed effect for dose and period. The protocol specified the PPS. The FAS has been reported generally and, where relevant, the PPS has also been included. The protocol specified the comparison with tacrolimus QD steady state (Weeks 7–12 above) as the comparison of interest.

A *post hoc* analysis examined the link between tacrolimus dose and trough levels. Although dose-adjusted trough levels are generally applicable, especially in early pharmacokinetic studies in healthy volunteers, they are not appropriate in this study. It is not appropriate to give all patients the same dose, and the corresponding trough levels would be outside the clinical dosing range. Instead, trough-adjusted dose levels were determined. This involved calculating the dose corresponding to a trough level of 7 ng/ml. For each subject, trough-adjusted values were calculated at each time point of trough level measurement, as well as the mean, SD, and coefficient of variation (CoV).

Results

Study population

Overall, 112 patients were enrolled into the study (Fig. 2), comprising the safety analysis set. Of these, 14/112

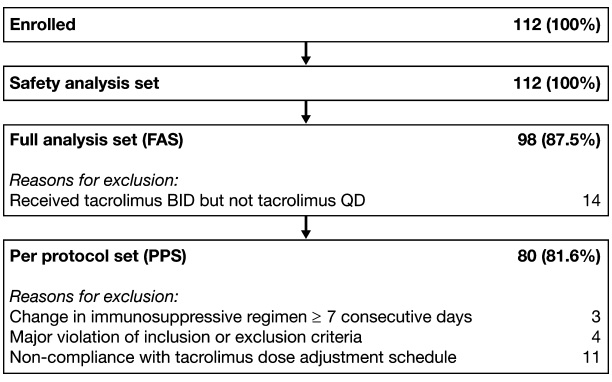

Figure 2 Patient disposition.

(12.5%) patients discontinued prematurely in the tacrolimus BID-treatment phase and therefore did not receive tacrolimus QD, thus excluding them from the FAS. A further 18/98 (18.4%) patients were excluded from the PPS attributable to major protocol violations (Fig. 2). In total, 16/112 (14.3%) patients in the safety analysis set discontinued before the end of the study.

Demographics for the PPS and FAS populations are presented in Table 1. The most common conditions leading to the need for transplant were cirrhosis (64.3%), of which alcoholic cirrhosis (38.8%) was the most frequently occurring type, and hepatocellular carcinoma (18.4%) (FAS). Prior to entering the study, 56 of 98 (57.1%) patients were hypertensive (FAS).

Primary immunosuppressant administration and exposure

The mean total daily dose of tacrolimus BID remained unchanged at 3.7 mg (±1.8) during the entire 6-week period of the tacrolimus-treatment phase in the FAS. The mean total daily dose of tacrolimus QD was 3.7 mg (±1.8) during Week 1 and increased only slightly to 3.9 mg (±1.8) by Week 12. Mean total daily dose calculated in mg/kg showed the same pattern of dosing. During the 6-week tacrolimus BID-treatment phase, mean total daily dose remained stable at 0.05 mg/kg (±0.03) and doses of tacrolimus QD remained stable during the tacrolimus-treatment phase starting at 0.05 mg/kg (±0.03) at Day −1 and ending at 0.05 mg/kg (±0.03) at Week 12. Overall, there was no significant difference between the mean total daily doses for each treatment period ($P = 0.3201$). All patients in the FAS who entered the study on tacrolimus BID monotherapy remained on tacrolimus monotherapy during the QD phase.

Following conversion to tacrolimus QD, 73 patients (74.5%) did not require dose adjustment to remain within the target range for trough levels (FAS). Of those subjects who did require a dose adjustment (25.5%), one change was sufficient to achieve recommended trough levels in most cases (64.0%). A dose decrease was required in 15 patients and one or more dose increases was needed in 16 patients. Most dose adjustments occurred during Weeks 2–4, but were required up to 10 weeks after conversion in some patients (Fig. 3). No particular pattern could be determined for the minority of patients who required a dose adjustment.

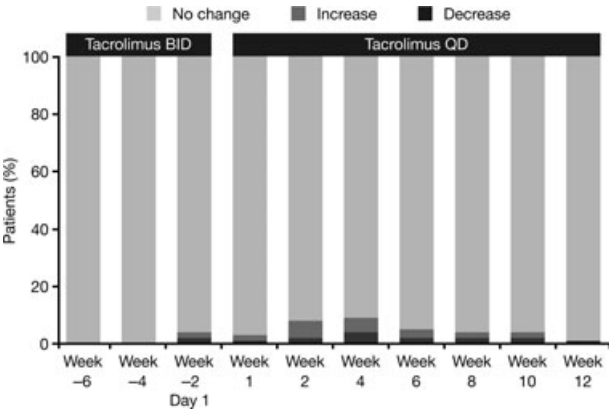

Figure 3 Patients requiring dose adjustments (FAS).

Table 1. Patient demographics and baseline characteristics (PPS and FAS).

Patient demographics and baseline characteristics	Per-protocol set (PPS) *n = 80*	Full analysis set (FAS) *n = 98*
Age (years), median (range)	54.5 (27–73)	55.0 (27–73)
Caucasian, *n* (%)	78 (97.5)	96 (98.0)
Male, *n* (%)	58 (72.5)	71 (72.4)
Height (cm), mean (SD)	171.2 (8.3)	171.1 (8.4)
Weight (kg), mean (SD)	82.2 (16.4)	81.8 (16.5)
Deceased organ donation, *n* (%)	79 (98.8)	96 (98.0)
Number of liver transplants, *n* (%)		
1	78 (97.5)	95 (96.9)
2	2 (2.5)	3 (3.1)
Time since last transplant (months), mean (SD)	42.9 (25.3)	41.2 (24.0)
Immunosuppression regimen, *n* (%)		
Tacrolimus BID monotherapy	41 (51.3)	52 (53.1)
Tacrolimus BID + mofetil	27 (33.8)	30 (30.6)
Tacrolimus BID + steroids	4 (5.0)	7 (7.1)
Tacrolimus BID + azathioprine	6 (7.5)	6 (6.1)
Tacrolimus BID + steroids + mycophenolate mofetil	2 (2.5)	3 (3.1)

SD, standard deviation.

Mean whole blood tacrolimus trough levels were within and toward the lower end of the recommended range during both treatment phases of the study (FAS; Table 2). After conversion to tacrolimus QD treatment, there was a reduction in the mean tacrolimus trough level from 7.36 ng/ml on Day −1 to 6.25 ng/ml at Week 1 (FAS), but levels were then stable throughout the remainder of the study period. The mean trough level during the tacrolimus QD phase was significantly lower compared with the tacrolimus BID period (7.5 ng/ml vs. 6.5 ng/ml; $P < 0.0001$). During the 6-week tacrolimus BID phase, median trough levels ranged between 7.4 and 7.6 ng/ml. Following conversion from tacrolimus BID to QD, the median whole blood trough level fell and then stabilized to 6.3–6.5 ng/ml for the remainder of the study (FAS; Fig. 4). Maximum trough values were generally lower during the tacrolimus QD phase (11.5–16.0 ng/ml) than during the tacrolimus BID phase (15.0–19.4 ng/ml). Minimum levels were <5 ng/ml with both the BID and QD tacrolimus formulations.

There was an increase in mean trough-adjusted dose levels from tacrolimus BID (3.9 mg) to tacrolimus QD (4.5 mg; $P = 0.0003$). However, the highest trough-adjust dose level was 13.2 mg/day with tacrolimus BID vs. 11.8 mg/day with tacrolimus QD. There was also an observed decrease in mean within-subject variability of the adjusted dose levels between tacrolimus BID and tacrolimus QD, which was not statistically significant (SD 0.92 vs. 0.89, $P = 0.7474$; CoV 20.9 vs. 19.1, $P = 0.1263$). Within-subject variability ranged from 0.08 to 10.8 with tacrolimus BID and 0.04–3.9 with tacrolimus QD.

Following conversion, tacrolimus levels were more consistent showing both reduced between- and within-patient variability in trough levels. The reduction in between-patient variability is shown in Fig. 5, which demonstrates lower standard deviations of mean tacrolimus levels after conversion. Furthermore, the CoV for between-patient variability was lower with tacrolimus QD, ranging from 29.9% to 35.2% from Weeks 1 to 12, compared with 36.1% at Week −4, 39.0% at Week −2 and 37.3% on Day −1 with tacrolumus BID (Table 2). The within-patient CoV was also slightly lower with tacrolimus QD (19.5% and 20.6%) compared to tacrolimus BID (20.9%), but the differences were not statistically significant. The reduction in within-patient variability between the 6-week tacrolimus BID phase and the first 6 weeks of the tacrolimus QD phase was statistically significant ($P = 0.0028$), and there was also a reduction at steady state ($P = 0.0903$). The comparable results for the PPS are $P =$

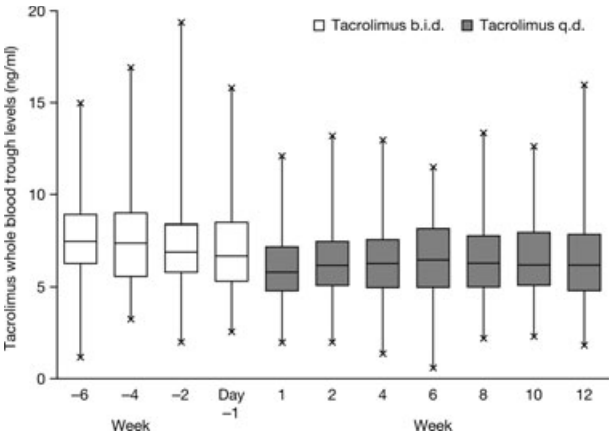

Figure 4 Median (minimum, maximum) tacrolimus whole blood trough levels (ng/ml) (FAS).

Table 2. Whole blood tacrolimus trough levels during the tacrolimus BID and tacrolimus QD treatment phases (FAS).

	n	Mean (SD) ng/ml	CoV (%)	Median (minimum–maximum) ng/ml
Tacrolimus BID phase				
Week −6	88	7.61 (2.35)	30.9	7.5 (1.2–15.0)
Week −4	98	7.62 (2.75)	36.1	7.4 (3.3–16.9)
Week −2	97	7.58 (3.00)	39.0	6.9 (2.0–19.4)
Day −1	93	7.36 (2.75)	37.3	6.7 (2.6–15.8)
Tacrolimus QD phase				
Week 1	95	6.25 (2.19)	35.0	5.8 (2.0–12.1)
Week 2	93	6.48 (1.94)	29.9	6.2 (2.0–13.2)
Week 4	96	6.52 (2.22)	34.0	6.3 (1.4–13.0)
Week 6	97	6.46 (2.21)	34.2	6.5 (0.6–11.5)
Week 8	96	6.45 (2.23)	34.5	6.3 (2.2–13.4)
Week 10	96	6.54 (2.30)	35.2	6.2 (2.3–12.6)
Week 12	94	6.42 (2.16)	33.6	6.2 (1.8–16.0)

SD, standard deviation; CoV, coefficient of variation.

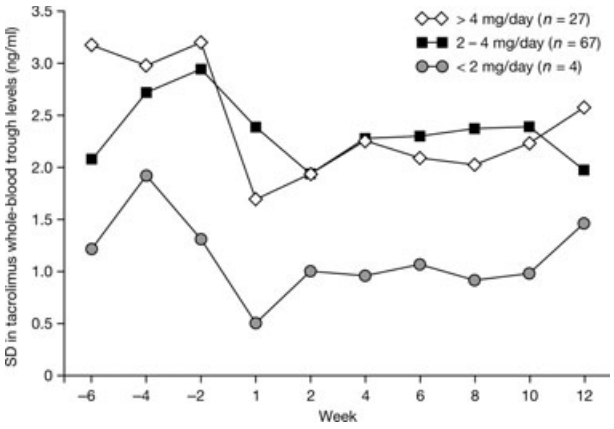

Figure 5 Standard deviations in tacrolimus whole blood trough levels (ng/ml) (FAS). Box represents 25% quartile, median value and 75% quartile of range. Highest and lowest values designated by diamond shape.

0.0051 and $P = 0.0473$, respectively, and thus statistically significant according to the prespecified comparison.

Adjunctive immunosuppressant use

No patient required a change in concomitant immunosuppressive treatment at any point during the study (FAS). For the 10 patients (10.2%) receiving corticosteroids, the mean (SD) total daily dose was 5.8 (2.4) mg during both phases of the study. For the 33 patients (33.7%) who were taking mycophenolate mofetil (MMF) as adjunctive treatment, daily doses of MMF remained constant during both treatment phases. The maximum daily dose of MMF was 2.0 g. Six patients in the FAS (6.1%) were taking azathioprine as adjuvant treatment. No additional adjunct immunosuppressive medications were administered to patients during either treatment phase of the study. There were few changes in nonimmunosuppressant medications from baseline to Week 12.

Renal function

In the analysis of the primary endpoint (PPS), noninferiority of tacrolimus QD against tacrolimus BID was demonstrated with a relative difference in mean (SD) calculated CrCl (Cockcroft–Gault) during the steady-state phase for each treatment of −0.1% (±6.3%; Table 3a). The 95% CI for the relative difference (−1.4, 1.1) was well above the prespecified noninferiority margin of −10% of the tacrolimus BID mean. Results of the primary endpoint were similar for the FAS. Similar results for estimated GFR were found in the additional analysis using the abbreviated MDRD 4 formula in both the FAS and the PPS (Table 3a). Mean (SD) CrCl and mean (SD) serum creatinine levels remained stable throughout the two treatment phases (FAS; Fig. 6 and Table 3b).

Efficacy

There were no cases of clinically diagnosed or BPAR, deaths or graft losses during either treatment phase of the

Table 3. Renal function (a) during the steady-state phase for each treatment (primary endpoint; FAS and PPS), and (b) over time (FAS).

(a)

	Tacrolimus BID Week −6 to Day −1	Tacrolimus QD Day 1 to Week 12	Relative difference Tacrolimus BID–Tacrolimus QD % ± SD (95% CI)
FAS, *n*	98	98	98
Mean (SD) CrCl (Cockcroft–Gault), ml/min	85.7 (24.4)	85.6 (24.1)	−0.1 ± 6.3 (−1.4, 1.1)
Mean (SD) GFR (MDRD 4 formula), ml/min/1.73 m^2	69.9 (16.5)	69.3 (15.9)	−0.4 ± 6.9 (−1.8, 1.0)
PPS, *n*	80	80	80
Mean (SD) CrCl (Cockcroft–Gault), ml/min	85.7 (24.2)	85.5 (23.7)	−0.0 ± 6.2 (−1.4, 1.3)
Mean (SD) GFR (MDRD 4 formula), ml/min/1.73 m^2	69.2 (15.8)	68.6 (14.7)	−0.4 ± 6.9 (−1.9, 1.2)

CrCl, creatinine clearance; GFR, glomerular filtration rate; FAS, full analysis set; MDRD 4, Modified Diet in Renal Disease 4; PPS, per-protocol set; SD, standard deviation.

(b)

	Tacrolimus BID *n* = 98		Tacrolimus QD *n* = 98		
	Week −6	Day −1	Week 1	Week 6	Week 12
Mean (SD) serum creatinine, μmol/l	101 (19)	101 (18)	100 (18)	100 (18)	102 (19)
Mean (SD) CrCl (Cockcroft–Gault formula), ml/min	85.7 (25.7)	85.6 (24.5)	85.6 (24.4)	86.7 (25.1)	85.0 (23.3)
Mean (SD) estimated GFR (MDRD 4 formula), ml/min/1.73 m^2	69.7 (17.1)	69.5 (17.0)	69.9 (17.4)	70.1 (16.5)	68.7 (16.2)

CrCl, creatinine clearance; GFR, glomerular filtration rate; MDRD 4, Modified Diet in Renal Disease 4; SD, standard deviation.

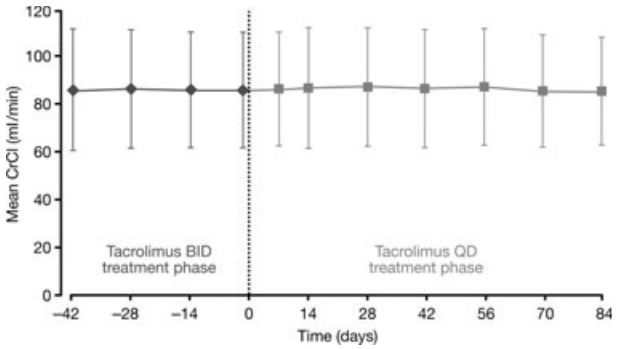

Figure 6 Mean (standard deviation) creatinine clearance (CrCl) over time (Cockcroft-Gault; FAS).

study. Furthermore, there were no deaths in patients prematurely withdrawn from study.

Safety

The incidence of treatment-related AEs was low during both treatment phases. The incidence of treatment-related AEs was higher during the 12-week tacrolimus QD phase than in the shorter 6-week tacrolimus BID phase (Table 4). The majority of AEs reported during the study were nonserious. The only serious AEs reported in at least 3% of patients in either treatment phase were hepatobiliary disorders, reported in 3.1% of patients during the tacrolimus QD phase. Specifically, these were cholangitis (two patients) and alcoholic hepatitis (one patient). None were considered to be causally related to the study drug.

During the tacrolimus BID phase, two patients withdrew ascribable to an AE (respiratory tract infection, possibly related to the study drug, and suicidal intention, not related to the study drug). No patients withdrew attributable to an AE during the tacrolimus QD phase.

One patient required modification of their tacrolimus BID dose because of a skin/subcutaneous tissue disorder (generalized pruritus, possibly related to study drug). In the tacrolimus QD treatment phase, each of the following AEs led to a modification of dose of study drug in one patient: ear/labyrinth disorders (tinnitus, probable relationship to study drug) with nervous system disorder (headache, probable relationship), hepatobiliary disorder (alcoholic hepatitis, possible relationship), and a vascular disorder (hypertension, not related to study drug).

Between Day −1 (tacrolimus BID phase) and Week 12 (tacrolimus QD phase), ambulatory 24-h mean arterial blood pressure decreased by 2.0 mmHg (from 101.9 to 100.0 mmHg; FAS; Table 5). This was statistically significant ($P = 0.0084$; one sample *t*-test). Systolic and diastolic blood pressure also decreased slightly, but the decrease was not statistically significant. There were no significant changes in HbA_{1c}, total bilirubin, SGPT/ALT, and SGOT/AST after conversion to tacrolimus QD (Table 5).

There were no clinically meaningful changes in any hematology or biochemistry laboratory value during the study period with the exception of alkaline phosphatase,

Table 4. Overall incidence of reported AEs and most commonly reported causally related AE (safety analysis set).

	Tacrolimus BID Week −6 to Day −1 *n* = 112		Tacrolimus QD Day 1 to Week 12 *n* = 98	
	n (%)	Events	*n* (%)	Events
AEs	41 (36.6)	85	56 (57.1)	105
Causally related AEs	18 (16.1)	27	24 (24.5)	38
Serious AEs	3 (2.7)	6	6 (6.1)	6
Causally related serious AEs	1 (0.9)	1	1 (1.0)	1
Most commonly reported causally related AEs				
MedDRA Primary SOC				
MedDRA Preferred Term				
Metabolism/nutrition disorders	4 (3.6)	5	7 (7.1)	8
Hypertriglyceridemia	1 (0.9)	1	3 (3.1)	4
Infections	4 (3.6)	5	7 (7.1)	7
Nasopharyngitis	1 (0.9)	1	3 (3.1)	3
Nervous system disorders	3 (2.7)	4	4 (4.1)	9
Headache	1 (0.9)	1	4 (4.1)	9
Vascular disorders	1 (0.9)	1	4 (4.1)	4
Hypertension	1 (0.9)	1	4 (4.1)	4

AEs, adverse events; MedDRA, Medical Dictionary for Regulatory Activities; SOC, system organ class.
Reported in at least 3% of subjects during either treatment phase.

Table 5. Mean (SD) differences in blood pressure and laboratory measures from Day −1 to Week 12 (FAS).

	Arterial blood pressure (mmHg)	HbA_{1c} (%)	Total bilirubin (μmol/l)	SGPT/ALT (U/L)	SGOT/AST (U/L)
n	84	63*	98	98	98
Day −1	101.9 (9.0)	5.4 (0.6)	12.9 (6.2)	27.9 (18.7)	24.4 (10.5)
Week 12	100.0 (8.7)	5.5 (0.6)	13.2 (6.5)	29.4 (24.7)	25.1 (11.4)
Difference	−2.0 (6.7)†	0.1 (0.3)	0.3 (3.5)	1.5 (14.4)	0.7 (9.2)

SD, standard deviation.
*Nondiabetic subjects.
†$P = 0.0084$, one sample *t*-test.

which showed a mean increase of approximately 30% that could be attributed to one participant who entered the study with a high value that continued to increase through the study. Proteinuria (an indicator of renal damage) remained low with a mean (SD) value of 0.08 (0.03) g/d at Week 12.Vital signs (heart rate, blood pressure and body weight) were measured at each visit and showed no clinically significant changes during the study period.

Discussion

Tacrolimus QD demonstrated noninferiority to tacrolimus BID for the primary endpoint of renal function measured by the relative difference in steady-state CrCl (Cockcrof–Gault) in the PPS. This was confirmed by analysis in the FAS and by estimation of GFR using the MDRD 4 formula.

The whole blood trough levels of tacrolimus in both treatment phases were at the low end of the recommended therapeutic range (5–15 ng/ml), but both the BID and QD tacrolimus formulations provided effective immunosuppression throughout the study, with no cases of AR during either treatment phase. After conversion to tacrolimus QD, there was a small reduction in tacrolimus exposure, evidenced by a reduction in the median whole blood trough level at Week 1. However, this then stabilized (with a minority of patients requiring dose modifications) and mean values remained consistently within the range 6.2–6.5 ng/ml from Week 2 until the end of the study at Week 12.

Between- and within-patient variability in tacrolimus trough levels decreased after conversion to the prolonged-release formulation. Conversion to tacrolimus QD, therefore, provided more consistent and predictable tacrolimus exposure that generally remained within the recommended target trough level range, in line with previous findings [13]. Interestingly, with both the BID and QD formulations there were patients who were adequately immunosuppressed despite low exposure to tacrolimus, with no cases of AR even though individuals' minimum trough levels were below 2 ng/ml on isolated occasions.

Modification of the tacrolimus QD dose was required in 25% of patients, usually 2–4 weeks after conversion, but occurring up to 10 weeks postconversion in some patients. This need for dose adjustment indicates that close surveillance is initially necessary following conversion from tacrolimus BID to tacrolimus QD to ensure that appropriate exposure is achieved. However, one change of dose tended to be sufficient for maintaining recommended tacrolimus trough levels during the tacrolimus QD phase, and similar numbers of dose increases and decreases were required. No clear pattern of patients requiring dose changes emerged.

Approximately 50% of patients received tacrolimus monotherapy in both the BID and QD phases. Most patients did not require dose changes, which was consistent with a previous study [13]. These results suggest that conversion from tacrolimus BID to tacrolimus QD is straightforward for most stable liver transplant patients and can be undertaken safely on a 1:1 (mg:mg) basis, although close initial monitoring is required to identify patients who do require dose adjustment. In addition, the same therapeutic drug monitoring targets that transplant physicians are familiar with can be used with both tacrolimus BID and tacrolimus QD.

Renal function was stable throughout the two treatment phases, providing evidence that tacrolimus QD is noninferior to tacrolimus BID. The good renal function seen in this study is consistent with a previous study in stable liver recipients converted from tacrolimus BID to QD [13]. Well-maintained renal function is an important consideration in nonrenal transplant recipients since renal failure is a significant cause of morbidity and mortality [14–16]. Moreover, previous studies have shown that tacrolimus is superior in terms of renal function when compared with cyclosporine, as it has been shown to have little impact on blood flow to the kidneys [14,16,18–20].

Conversion from tacrolimus BID to tacrolimus QD in stable liver transplant recipients did not affect the

incidence (relative to treatment duration) or nature of AEs, and efficacy was well maintained without any cases of AR. There were no graft losses or deaths during or after discontinuation from the study. Patients had a small but statistically significant improvement in 24-h arterial blood pressure of −2 mmHg. However, this is likely to have limited clinical or physiological impact and there were no clinically meaningful changes in blood pressure during routine measurements at each study visit.

This large, phase III study reflects clinical practice in the conversion of stable liver transplant recipients from tacrolimus BID to tacrolimus QD, in which patients served as their own control group. Hence, the results presented herein are applicable to a significant proportion of liver transplant recipients who are maintained on tacrolimus BID and who may potentially benefit from switching to a once-daily immunosuppressive regimen. However, the inclusion and withdrawal criteria were fairly strict, which may limit the applicability of the results to the whole of the liver transplant recipient population. The study is also limited by the short duration of exposure for capturing AEs or changes in renal function or graft rejection; studies of longer duration are therefore warranted. Although the sample size calculation had indicated that 100 patients were required for the FAS to assess noninferiority, this was based on an assumed worst case of 5% decrease in CrCl with tacrolimus QD. The actual reduction in CrCl during tacrolimus QD treatment was markedly smaller, and so the reduced sample size achieved did not translate into a relevant loss of power.

Conclusion

Conversion of stable liver transplant recipients from tacrolimus BID to tacrolimus QD on a 1:1 (mg:mg) total-daily dose basis is straightforward and well tolerated and may lead to tacrolimus exposure remaining more consistently within the target range, reflecting the findings that maximum trough levels exceeded 15 ng/ml less frequently with tacrolimus QD than with tacrolimus BID. Dose adjustments were necessary in a quarter of patients to maintain tacrolimus trough levels within the target range. Renal function remained stable following conversion to tacrolimus QD, and there was a small but significant improvement in mean arterial blood pressure. Stable patients can be successfully switched from tacrolimus BID to tacrolimus QD without risk of AR in the short term. The reduced frequency of dosing with tacrolimus QD may help to optimize adherence and this, together with the consistency of exposure, has the potential to improve long-term outcomes in liver transplant recipients. Additional studies of longer duration and in larger populations are warranted to provide further clarification of the effects of conversion from tacrolimus BID to tacrolimus QD in stable liver transplant recipients.

Authorship

All authors performed research/study, collected and analyzed data, and contributed to the writing of the article.

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Study group

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