Therapeutic Monitoring of Calcineurin Inhibitors for the Nephrologist

Jeffrey Schiff,* Edward Cole,* and Marcelo Cantarovich†

Division of Nephrology, University Health Network, Toronto General Hospital, Toronto, Ontario, and †Multi-Organ Transplant Program, McGill University Health Centre, Royal Victoria Hospital, Montreal, Quebec, Canada

The calcineurin inhibitors (CNI) cyclosporine and tacrolimus remain the backbone of immunosuppression for most kidney transplant recipients. Despite many years of experience, protocols that optimize efficacy with minimal toxicity remain a subject of debate. Nevertheless, studies of the pharmacokinetic properties of the CNI, particularly cyclosporine, have led to improved dosing strategies. The purpose of this article is to review the current understanding of CNI pharmacokinetics and its relevance to proper dosing and monitoring of these medications. This article also reviews the trials that have helped to define the optimal dosages and discusses the effect of adjunctive immunosuppressive agents on CNI pharmacokinetics and dosing.

Clin J Am Soc Nephrol 2: 374–384, 2007. doi: 10.2215/CJN.03791106

Pharmacokinetics of Calcineurin Inhibitors

Both calcineurin inhibitors (CNI) cyclosporine and tacrolimus act through an interaction with a cytoplasmic protein, which subsequently binds to and inhibits calcineurin. In the case of cyclosporine, the target is cyclophilin, whereas tacrolimus binds to tacrolimus-binding protein. After a dose of CNI, there is an initial absorption phase, during which blood concentrations reach a peak level (C_{max}). Typically, C_{max} occurs during the first 2 to 3 h after the dose and corresponds to the time of maximal calcineurin inhibition (1,2). Drug levels then fall as a result of metabolism (also known as the elimination phase) until they are at the lowest, or trough, level (C_0) immediately before the next dose. Metabolism is performed chiefly by the cytochrome P450 3A enzyme system in the liver. Both CNI also are metabolized by the intestinal cytochrome P450 3A4 and by P-glycoprotein countertransport in the intestinal mucosa (3,4). The total drug exposure throughout the period from one dose until the next is the area under the concentration-time curve (AUC; Figure 1) (3). Determination of AUC can be made by formal pharmacokinetic testing, which requires blood samples to be drawn at multiple time points throughout the dosing interval. For both CNI, most of the inter- and intrapatient variability occurs in the absorption phase rather than in the elimination phase.

The original corn oil–based preparation of cyclosporine (Sandimmune, Novartis Pharma Canada Inc., Dorval, Canada) had widely varying inter- and intrapatient bioavailability, ranging between 1 and 89% (3,5). Absorption was affected by the need for solubilization of cyclosporine in bile, as well as the presence or absence of food, time of day, race, renal function, gastrointestinal transit time (*i.e.*, diarrhea), and gastrointestinal autonomic neuropathy, with some factors affecting AUC by up to 60% (3,6–9). As well, absorption increased in the early posttransplantation period, demonstrated as a decreasing dosage needed with time to achieve the same degree of total cyclosporine exposure during the first 2 wk after transplantation (10). Finally, cyclosporine metabolism is affected by liver disease and variations in CYP450 3A4 activity (11).

The microemulsion formulation of cyclosporine (Neoral, Novartis Pharma Canada Inc., Dorval, Canada) was developed to reduce this variability. Neoral was found to have increased and more consistent absorption of cyclosporine, leading to less intrapatient variability than Sandimmune (12), although there remains significant variability in absorption (Figure 2) (13). Randomized, controlled trials confirmed that Neoral was safe in stable (4) and *de novo* (14,15) renal transplant patients.

Tacrolimus behaves similarly to cyclosporine, with rapid absorption and peak levels being achieved within the first 3 h after a dose. It also shows marked intra- and interpatient variability in absorption (16). Its absorption is not bile dependent but does depend on gastrointestinal transit time and is affected by the presence or absence of food, as well as the lipid content of food (17). In addition, age, gender, race, body mass index, duration of time on tacrolimus, serum albumin, hematocrit, and presence of hepatitis B or C infection or other liver disease all have been shown to influence daily dosage requirements (18,19).

Recently, an extended-release, once-daily formulation of tacrolimus was developed. Modified-release tacrolimus was shown to have an equivalent pharmacokinetic profile in stable patients who were converted from standard tacrolimus in a 1:1 manner (20). Target trough levels for modified-release tacrolimus seem to be the same as for standard tacrolimus in both *de novo* and maintenance patients (21).

Cyclosporine Monitoring Strategies

Therapeutic drug monitoring is necessary for drugs with a narrow therapeutic index (*i.e.*, the exposure for efficacy is close

Published online ahead of print. Publication date available at www.cjasn.org.

Address correspondence to: Dr. Marcelo Cantarovich, Royal Victoria Hospital, 687 Pine Avenue West, Room R2.58, Montreal, Quebec, Canada, H3A 1A1. Phone: 514-934-1934, ext. 36223; Fax: 514-843-2815; E-mail: marcelo.cantarovich@muhc.mcgill.ca

ISSN: 1555-9041/202–0374

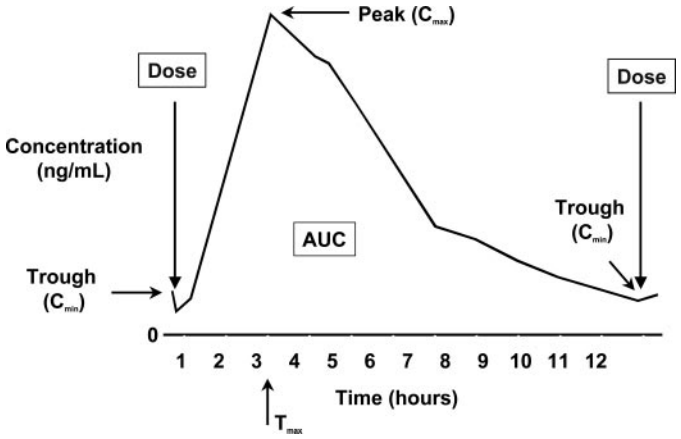

Figure 1. Drug levels during the course of a dosing interval. The drug concentration is lowest (C_{min}) just before the dose is taken, then rises to a peak level (C_{max}) at a certain time after the dose (T_{max}). The concentration then falls back to C_{min} before the next dose. The area under the concentration-time curve (AUC) describes total drug exposure during the entire dosing interval.

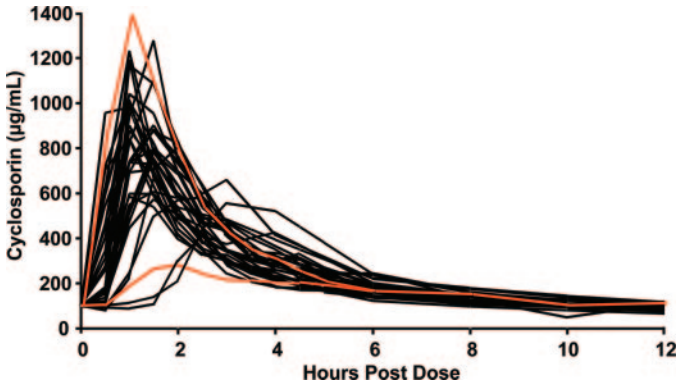

Figure 2. Intrapatient variability in cyclosporine blood levels in renal transplant patients. The *x* axis represents various time points after the cyclosporine dose when the cyclosporine level was measured. The *y* axis represents the whole-blood cyclosporine level. The lines in red highlight two patients with similar trough levels but very different peak concentrations. Early after transplantation, the patient with a low C_{max} would be at a higher risk for acute rejection, whereas the patient with a high C_{max} would be at risk for cyclosporine toxicity. In neither case would C_0 monitoring identify which patient was at risk. Adapted from Levy (56).

to that associated with toxicity) and when there is a high level of variability in the blood concentration of the drug between patients after a dose. In addition, it is most effective when there is a measurement that is a good surrogate for total drug exposure; when there is a clear relationship between drug exposure, efficacy, and toxicity; and when sampling is easy to perform. The CNI clearly require drug monitoring because of their narrow therapeutic index. The existence of a number of drug interactions that affect CNI levels is another motivating factor. Unfortunately, there is a less-than-ideal correlation between some drug levels and overall exposure and, therefore, clinical events.

Before the introduction of drug monitoring, cyclosporine usage was associated with less rejection but also dosage-related nephrotoxicity and acute renal failure after renal and cardiac transplantation (22,23). In the Sandimmune era, it was demonstrated that empiric cyclosporine dosage reduction was associated with rejection and that blood levels correlated with the degree of immune reactivity (1,24). Furthermore, patients with lower cyclosporine levels were at an increased risk for rejection and graft loss (25). Although patients who had an episode of acute rejection had a lower cyclosporine C_{max} and AUC, C_0 levels correlated poorly with the risk for rejection in individual patients (26). Despite this, C_0 monitoring of cyclosporine became the standard, because it was simpler than measuring AUC or determining C_{max} for each patient.

When Neoral was introduced into clinical use, a series of trials examined its pharmacokinetics in detail. Compared with Sandimmune, patients who received Neoral had similar C_0 levels but higher C_{max} and AUC (4,14,15,27). In addition, the rate of acute rejection was lower with Neoral in some studies (14). Although some studies showed more early nephrotoxicity with Neoral (28), long-term renal function was equivalent.

These studies also demonstrated that cyclosporine exposure during the first 4 h after a dose ($AUC_{0\ to\ 4}$) correlated well with exposure during the entire 12-h dosing interval ($AUC_{0\ to\ 12}$). This is consistent with the fact that most of the variability in cyclosporine exposure takes place during the absorption phase. In comparison with $AUC_{0\ to\ 4}$, C_0 levels correlated poorly ($r^2 = 0.53$) with $AUC_{0\ to\ 12}$. Although determining $AUC_{0\ to\ 4}$ required four or five blood samples to be drawn, it was also found that the combination of C_0 and the 2-h postdose cyclosporine level (C_2) provided excellent correlation ($r^2 = 0.945$) with $AUC_{0\ to\ 12}$ (4).

A retrospective study subsequently compared $AUC_{0\ to\ 4}$ with clinical events in *de novo* renal transplant recipients. In a group of patients who received cyclosporine, steroids, and a variety of adjunctive agents (azathioprine, mycophenolate mofetil [MMF], and sirolimus) but not antibody therapy, $AUC_{0\ to\ 4}$ was lower in patients who had an episode of acute rejection. In addition, patients with the highest $AUC_{0\ to\ 4}$ had the highest incidence of nephrotoxicity. Although the groups were small, there were no differences in the relationship among $AUC_{0\ to\ 4}$, acute rejection, and nephrotoxicity that was treated with different immunosuppressives. In this study, the optimal $AUC_{0\ to\ 4}$, defined by freedom from both acute rejection and nephrotoxicity, was 4400 to 5500 μg/h per L (29). This strategy was subsequently validated prospectively in *de novo* renal transplant patients (30). These studies also highlighted the importance of achieving adequate cyclosporine exposure early after transplantation. In the prospective study, only one of 11 rejection episodes occurred in a patient who achieved an $AUC_{0\ to\ 4}$ >4400 μg/h per L by day 5 after transplantation.

Another randomized, prospective study in patients who received cyclosporine, basiliximab, and prednisone compared a limited sampling strategy to C_0 monitoring during the first 3 mo after transplantation (31). In this study, two- or three-point algorithms were used to predict $AUC_{0\ to\ 12}$. This study confirmed that adequate early cyclosporine exposure was highly correlated with freedom from acute rejection. Despite this, oc-

currence of the primary end point of acute rejection, graft loss, or death was equal in both groups by the study's end, as was serum creatinine.

Although these limited sampling strategies were less cumbersome than performing a 12-h pharmacokinetic profile, they still required between two and five blood level measurements to be drawn, which was a deterrent to their implementation. Initial research in long-term heart and liver transplant patients determined that the C_2 level was the best single-point measurement that correlated with $AUC_{0\ to\ 4}$ (32,33). Further analysis in renal transplant patients confirmed that the C_2 level was the best correlate of $AUC_{0\ to\ 4}$ in predicting acute rejection (34). Other studies of renal transplant patients during the early posttransplantation period have confirmed that $AUC_{0\ to\ 4}$ is more predictive of rejection than $AUC_{0\ to\ 12}$ and that C_2 is the best single-point correlate of $AUC_{0\ to\ 4}$, with a correlation (r^2) of 0.83 to 0.85 (10,35).

On the basis of these studies, the CONCERT group published a consensus statement on Neoral monitoring in transplant recipients (36). It concluded that C_2 monitoring was the optimal method for monitoring Neoral, with the blood drawn within 15 min before or after the 2-h time point. The CONCERT group reiterated that C_0 monitoring poorly predicts clinical events. It also emphasized the importance of achieving adequate C_2 levels early after transplantation and that C_2 monitoring was not associated with impaired renal function, despite leading to the use of higher cyclosporine dosages in the early posttransplantation period. They also noted that some patients may be low absorbers (low C_{max}) or slow absorbers (delayed time to C_{max}), characteristics that may not be detected or distinguished with a single-point measurement but would be by a limited sampling strategy. In addition, some results from liver, heart, and lung transplant recipients suggested that C_2 monitoring may reduce nephrotoxicity (33,37,38). Finally, the authors noted that in pharmacoeconomic studies, C_2 monitoring is at least cost-neutral compared with C_0 monitoring and may result in cost savings, a finding that has since been confirmed (39,40). However, despite this suggestive evidence, there has never been a randomized, controlled trial of C_0 *versus* C_2 in renal transplantation demonstrating a clinical benefit of C_2 monitoring.

It is important to note that all of these studies were carried out using the Neoral formulation. Generic formulations of cyclosporine microemulsion are now available, but they may not have identical pharmacokinetics to Neoral or to each other. Although some studies have shown similar pharmacokinetics in transplant patients (41,42), others have not (43), whereas at least one trial showed an increased rate of acute rejection (44). If the cyclosporine formulation that a patient is using is changed, then more frequent monitoring after the switch is made is advisable (45). Furthermore, the optimal monitoring strategy could be different.

Several assays are available to measure cyclosporine. HPLC is less commonly used because of technical difficulties. Fluorescence polarization immunoassay, specific enzyme multiplied immunoassay technique, and cloned enzyme donor immunoassay all are suitable techniques, with whole-blood sampling recommended (3). Because the half-life of cyclosporine is approximately 8 h, the full effect of a dosage adjustment on the cyclosporine level will be seen only after approximately 2 d (4 to 5 half-lives).

Target Cyclosporine Levels in the First Year after Transplantation

Adequate cyclosporine exposure early after transplantation decreases the risk for rejection. In one study, a C_2 level >1700 ng/ml by day 3 after transplantation was associated with a 92% negative predictive value for acute rejection in the first 6 mo. Achieving this level required a mean cyclosporine dosage of 11.7 ± 2.0 mg/kg per d, with a range of 6.8 to 21.5 mg/kg per d. Achieving a C_2 level >1700 ng/ml by day 5 or 7 after transplantation did not have as strong a predictive value. This relationship did not hold for patients with delayed graft function. However, for patients with immediate graft function, rapid increases in cyclosporine dosage to reach this target level should be made (34).

Although the target C_2 level of >1700 ng/ml was derived from patients who received cyclosporine, an adjunctive agent, and steroids, this has not been seen in patients who received antibody therapy. In a retrospective analysis of patients who received basiliximab, cyclosporine, MMF, and steroids, a C_2 level of 1700 ng/ml on day 3 after transplantation did not discriminate between patients who went on to have acute rejection from those that did not (46). In a trial of patients who received basiliximab, cyclosporine, and prednisone without an adjunctive agent, a C_2 level of >1500 ng/ml by day 3 after transplantation was associated with the lowest risk for rejection (31). However, rather than a threshold value, the risk for rejection seems to be inversely correlated with C_2 levels during the first year after transplant for patients who receive induction therapy. In a retrospective analysis of a randomized, controlled trial that compared basiliximab with anti-thymocyte globulin followed by cyclosporine, MMF, and steroids, the risk for rejection was 40% at C_2 levels of 400 ng/ml but declined to 15% when the mean C_2 was >1500 ng/ml (47). Thymoglobulin allows C_2 levels to be targeted even lower. A randomized, controlled trial of Thymoglobulin induction, cyclosporine, MMF, and steroids compared C_2 monitoring with target levels of 1000 to 1200 ng/ml with C_0 monitoring with a target of 250 to 350 ng/ml during the first 3 mo after transplantation. Both regimens resulted in similar rates of acute rejection, graft loss, or death, but the C_2 group required lower cyclosporine dosages after the first month (40).

These trials concentrated on the first 3 mo after transplantation. An international randomized, controlled trial compared two C_2 ranges in patients between 3 and 12 mo after transplantation. All patients received cyclosporine and steroids. Most patients received MMF, with the remainder (11%) receiving azathioprine. Target C_2 levels for all patients were 1700 ng/ml for the first month, 1500 ng/ml for month 2, and 1300 ng/ml for month 3. After 3 mo, patients were randomly assigned to a higher or lower C_2 group. Target C_2 levels were 1100 ng/ml for months 4 through 6 and 900 ng/ml for months 7 through 12 in the higher C_2 group, whereas patients in the lower C_2 group had target levels of 900 ng/ml for months 4 through 6 and 700

ng/ml for months 6 through 12. During the first 3 mo after transplantation, the rate of biopsy-proven acute rejection was 11.7% (48). There was no difference in acute rejection episodes between the two groups between months 3 and 12. At 12 mo, there was no significant difference in GFR, the study's primary end point. There also were no differences in BP, antihypertensive agent use, or serum total cholesterol, although more patients in the higher C_2 group were on lipid-lowering treatment. When patients were reclassified by their achieved C_2 levels, there was no difference in GFR at 12 mo, but there was a trend toward lower BP and serum cholesterol in patients with the lowest C_2 levels (49).

In summary, these trials have shown that C_2 monitoring is safe and effective during the first year after transplantation, and target levels have now been defined (Table 1). However, although C_2 is more accurate than C_0 monitoring, there is no evidence from randomized, controlled trials that C_2 monitoring leads to a reduction in acute rejection, graft loss, or death. For patients who receive antibody therapy, the need to achieve target C_2 levels rapidly after transplantation is diminished, although there continues to be a relationship between C_2 levels and the risk for rejection. Use of more potent adjunctive immunosuppressive agents, such as MMF, likely also reduces the need to achieve high C_2 levels early after transplantation, although the evidence here is not clear-cut (50).

Target Cyclosporine Levels after the First Year after Transplantation

Long-term graft function and survival often are compromised by chronic allograft nephropathy (CAN), which in some cases seems to be related to CNI toxicity. In addition, higher dosages of CNI increase the incidence of malignancy, hypertension, and hyperlipidemia (51,52). As in *de novo* renal transplant patients, C_0 monitoring correlates poorly with AUC. In a group of long-term patients who were maintained with C_0 levels of 206 ± 75 ng/ml, C_2 levels ranged from 140 to 2440 ng/ml. Patients with progressively rising serum creatinine values had lower C_2 levels (mean 492 ± 327 *versus* 1054 ± 579 ng/ml) and $AUC_{0\ to\ 12}$ (mean 3798 ± 1145 *versus* 6462 ± 1886 μg/h per L), and most had evidence of CAN on biopsy (53). This suggests that underexposure to cyclosporine in long-term transplant recipients is a risk factor for CAN and that C_2 monitoring might identify these patients.

C_2 monitoring can also identify patients who are receiving excessive cyclosporine dosing. One study showed that patients with a C_2 level between 700 and 800 ng/ml had lower serum creatinine values than patients with C_2 levels <450 or >950 ng/ml (54). However, this was a cross-sectional study and could not determine whether patients were being kept at lower or higher levels because of renal dysfunction or previous episodes of acute rejection.

In a prospective study (55), 175 patients were converted to C_2 monitoring, >90% of whom were >1 yr after transplantation. The target C_2 level was set at 800 ng/ml, on the basis of previously published recommendations (56). Approximately half of the patients had a C_2 level of >10% above the target C_2 after 1 yr after transplantation. C_2-guided dosing allowed the mean cyclosporine dosage to fall from 3.5 ± 1.4 to 2.8 ± 1 mg/kg. This reduction in cyclosporine dosage did not result in any episodes of acute rejection. There were improvements in BP and lipid profile, but these did not reach statistical significance. Among the group with a C_2 level >10% above the target level, serum creatinine decreased in half of the patients after cyclosporine dosage reduction, from 153 ± 55 to 132 ± 49 μmol/L.

In another study, patients who were maintained on cyclosporine and steroids were converted from C_0 to C_2 monitoring and followed for 3 yr. Target levels were 800 to 1000 ng/ml. C_2 monitoring showed that half of the patients were above the target range and allowed the mean daily dosage to be reduced by approximately 20%. At 3 yr, few (7.3%) patients had developed CAN. Serum creatinine remained stable through the study period and was accompanied by decreased use of antihypertensive agents and mean total cholesterol levels (57).

When histology has been used as an end point to compare cyclosporine and tacrolimus, some trials in *de novo* recipients have shown more fibrotic changes in patients who received cyclosporine (58–61), but these used C_0 monitoring. It is unknown whether C_2 monitoring from the time of transplantation will reduce the histologic changes of CAN.

Conversion of stable renal transplant recipients to C_2 monitoring is safe and does not lead to an increased risk for acute rejection. It is associated with improvements in BP and lipids and may also improve renal function in patients who are receiving excessive cyclosporine exposure. This improvement in metabolic parameters might decrease the risk for cardiovascular events in this high-risk population. Although reducing cyclosporine overexposure may prevent the development of CAN, no randomized, controlled trials have demonstrated that

Table 1. Suggested target ranges for renal transplant patients who receive cyclosporine[a]

Time	Without Induction Therapy	With IL-2 Receptor Antibody Therapy	Induction with Thymoglobulin	With mTOR Inhibitor
0 to 3 mo	C_2 >1700 ng/ml by day 5 (34); 1600 to 2000 ng/ml month 1, C_2 1400 to 1600 ng/ml month 2, C_2 1200 to 1400 ng/ml month 3 (48)	C_2 >1500 ng/ml for first 2 mo, C_2 1200 to 1400 ng/ml month 3 (46)	C_2 1000 to 1200 ng/ml (40)	C_0 75 to 125 ng/ml months 1 through 2, C_0 50 to 100 ng/ml months 3 through 6 (95,96); reduce C_2 target by 50 to 75%?
>3 to 12 mo	C_2 800 to 1000 ng/ml months 4 through 6, C_2 600 to 800 ng/ml months 7 through 12 (49)	C_2 600 to 1000 ng/ml (46)	C_2 600 to 1000 ng/ml (40)	C_0 50 to 100 ng/ml (95,96); reduce C_2 target by 50 to 75%?
>12 mo	C_2 approximately 800 ng/ml (54–56)	C_2 approximately 800 ng/ml (54–56)	C_2 approximately 800 ng/ml (54–56)	C_0 50 to 100 ng/ml (95,96); reduce C_2 target by 50 to 75%?

[a]C_0, trough level; C_2, 2-h postdose cyclosporine level; mTOR, mammalian target of rapamycin.

C_2 monitoring reduces CAN, graft loss, or death compared with C_0 monitoring.

Therapeutic Drug Monitoring of Tacrolimus

Trough-level monitoring of tacrolimus has been standard practice since its introduction. Similar to cyclosporine, achieving early adequate tacrolimus exposure significantly reduces the risk for acute rejection. In a retrospective analysis of a randomized, controlled trial, the tacrolimus AUC by day 2 after transplantation was found to be a strong predictor of the risk for acute rejection. Patients with a tacrolimus AUC >200 ng/h per ml had a markedly lower risk for acute rejection (17 *versus* 41%), regardless of whether they received MMF. Tacrolimus C_{max} did not correlate with the risk for rejection. The threshold value of 200 ng/h per ml correlated to a tacrolimus C_0 of 10 ng/ml (62).

Several small trials have assessed the ability of tacrolimus trough levels to predict the tacrolimus AUC. Two trials showed that the tacrolimus C_0 correlated poorly with AUC (r^2 = 0.11 and 0.362) and that C_4 was the best single-point correlate of AUC (r^2 = 0.79 and 0.81). Both studies suggested that a two- or three-point limited sampling strategy, both of which incorporated C_4, would predict AUC better than C_0 levels (63,64). Other studies identified C_2 or C_3 as the best correlates of AUC (65,66). However, some studies have shown excellent correlations (r^2) in the range of 0.79 to 0.86 between tacrolimus C_0 levels and AUC (65,67,68). No prospective trials have compared outcomes with an AUC, C_2-, C_3-, or C_4-guided dosing strategy with those with C_0 monitoring in patients who were treated with tacrolimus. Although C_4 may be the best single time point for monitoring tacrolimus, the correlation of C_2 with AUC for tacrolimus (r^2 = 0.87) is similar to that of cyclosporine (61). From a practical point of view, a comparison in the clinic setting of C_0 *versus* C_2 monitoring of tacrolimus might be interesting. Although such a strategy may not further reduce the already low rate of acute rejection that is seen in patients who are treated with the combination of tacrolimus and MMF, it may decrease the incidence of CNI toxicity.

Like cyclosporine, tacrolimus monitoring should be done with whole-blood samples (16). Its half-life is 12 to 18 h, which suggests that a period of approximately 2.5 d should elapse to assess the effect of a dosage adjustment on the tacrolimus level. Both microparticle enzyme immunoassay and ELISA have excellent correlation with the reference methods of liquid chromatography and mass spectrometry (69).

Target Tacrolimus Levels

Target tacrolimus levels in renal transplant patients have been defined by clinical trials (Table 2). These trials usually compared tacrolimus with cyclosporine (both Sandimmune and Neoral) with trough-level monitoring. The trials also varied according to the type of adjunctive therapy, induction therapy, and follow-up.

Data from a phase II clinical trial in renal transplant patients were used to examine the relationship among tacrolimus level, acute rejection, and toxicity. This trial randomly assigned patients to three groups, with tacrolimus trough concentrations between 5 and 14, 15 and 25, and 26 and 40 ng/ml. There were no statistically significant differences among the three groups in terms of acute rejection, but there were more tacrolimus-related adverse events in the two higher dosage groups. In a logistic regression analysis, the risk for acute rejection decreased with increasing tacrolimus levels but at the expense of increased adverse events and nephrotoxicity (70).

The initial phase III clinical trials used tacrolimus C_0 levels as high as 10 to 20 ng/ml during the first 3 mo after transplantation, followed by levels of 5 to 10 ng/ml (71–75). However, significant toxicity was seen with C_0 levels of >15 ng/ml. Subsequent trials often used C_0 ranges between 10 and 15 ng/ml in the early posttransplantation period and 5 to 10 ng/ml after 3 mo, although there is significant variation around these ranges (58,76,77). Patients who receive IL-2 receptor blockade require tacrolimus levels of 10 to 15 ng/ml for only the first 6 wk after transplantation, followed by levels of 5 to 10 ng/ml thereafter (78). More recently, lower levels of tacrolimus (3 to 7 ng/ml) in the early posttransplantation period have been

Table 2. Suggested target ranges for renal transplant patients who receive tacrolimus

Time	Without Induction Therapy	With IL-2 Receptor Antibody Therapy	Induction with Thymoglobulin	With mTOR Inhibitor
0 to 3 mo	C_0 10 to 15 ng/ml (71–75)	C_0 10 to 15 ng/ml first 6 wk, C_0 5 to 10 ng/ml after week 6 (78); C_0 3 to 7 ng/ml throughout may be adequate (79)	C_0 5 to 10 ng/ml (76,77)	C_0 3 to 7 ng/ml (97)
>3 to 12 mo	C_0 5 to 15 ng/ml (71–75)	C_0 10 to 15 ng/ml first 6 wk, C_0 5 to 10 ng/ml after week 6 (78); C_0 3 to 7 ng/ml throughout may be adequate (79)	C_0 5 to 10 ng/ml (76,77)	C_0 3 to 7 ng/ml (97)
>12 mo	C_0 5 to 10 ng/ml (71–75)	C_0 10 to 15 ng/ml first 6 wk, C_0 5 to 10 ng/ml after week 6 (78); C_0 3 to 7 ng/ml throughout may be adequate (79)	C_0 5 to 10 ng/ml (76,77)	C_0 3 to 7 ng/ml (97)

assessed in a randomized, controlled trial against low- and standard-dosage cyclosporine (both monitored with C_0 levels) and sirolimus in a quadruple regimen that included daclizumab, MMF, and steroids. Tacrolimus was associated with the lowest risk for acute rejection as well as the highest GFR at 12 mo compared with the other groups (79). Thymoglobulin induction allows for reduction of tacrolimus C_0 levels to 5 to 10 ng/ml from the time of transplantation (80).

Comparison of Efficacy of Cyclosporine and Tacrolimus

Many trials have compared cyclosporine and tacrolimus in renal transplant recipients (75,81). A recent meta-analysis found fewer acute rejection episodes and graft losses with tacrolimus (82). However, the immunosuppressive protocols in these trials were highly heterogeneous and used a variety of target levels and therapeutic drug-monitoring strategies for cyclosporine and tacrolimus. These trials also used cyclosporine C_0 monitoring and therefore may have underdosed cyclosporine. A recent retrospective study showed a more rapid decline in GFR in patients who were treated with cyclosporine with C_2 monitoring compared with tacrolimus, although there was no difference in mean arterial pressure, total cholesterol, or new-onset diabetes (83). In a recent randomized, controlled trial that compared cyclosporine with C_2 monitoring and tacrolimus, there was no difference in the primary end point of acute rejection, graft loss, or death. However, GFR was slightly but significantly lower with cyclosporine. BP was similar in both groups, but patients who were treated with cyclosporine had higher LDL and HDL cholesterol, whereas there was a higher incidence of new-onset diabetes or impaired fasting glucose in the tacrolimus group (84). In comparison, a study in liver transplant recipients that compared cyclosporine C_2 with tacrolimus found no difference in renal function or acute rejection (85).

CNI in Combination with Mammalian Target of Rapamycin Inhibitors

The original trials that evaluated sirolimus used full-dosage cyclosporine monitored by C_0 levels (86,87). Although the acute rejection rate was reduced compared with patients who received cyclosporine and azathioprine, serum creatinine levels were higher. Similar findings were seen in trials that combined full-dosage cyclosporine with everolimus (88,89), and tacrolimus with sirolimus (90–92). There is a small increase in CNI exposure with the addition of mammalian target of rapamycin (mTOR) inhibitors. It therefore is believed that there also must be a substantial increase in tissue CNI exposure with the addition of an mTOR inhibitor, but the mechanism has not yet been elucidated. In addition, cyclosporine and sirolimus should be taken separately, because co-administration increases sirolimus AUC and nephrotoxicity. Whether there is a similar need to separate tacrolimus and sirolimus is unclear (93). Everolimus does not seem to be affected by co-administration with cyclosporine and has been given simultaneously (94).

Patients who are on a combination of CNI and mTOR inhibitor require reduction of the CNI to avoid nephrotoxicity. No randomized, controlled trials have established target C_2 levels for cyclosporine in combination with sirolimus or everolimus, but CNI dosage reductions of 50 to 75% (or even more) may be necessary to avoid nephrotoxicity (Tables 1 and 2) (95–97). Registry analysis has demonstrated that the combination of a CNI with sirolimus is associated with decreased graft survival compared with a CNI combined with MMF (98,99), but this may be due to nephrotoxicity from the combination of full-dosage CNI and sirolimus. Whether the combination of a low-dosage CNI with an mTOR inhibitor will give equivalent long-term results to a CNI combined with MMF is unknown.

CNI Levels in Steroid-Withdrawal Regimens

Interest has increased in protocols in which corticosteroids are stopped early after transplantation (100–102). Trials with cyclosporine have used trough-level monitoring, either following the same levels as per center practice (103) or choosing levels similar to usual practice (104,105). Trials with tacrolimus have used levels similar to protocols that contain steroids (Table 2), both with (81,106–108) and without (105) induction therapy. These regimens may lead to fewer metabolic complications after transplantation. However, reduction of CNI dosages to avoid nephrotoxicity may be more difficult in the absence of the immunosuppressive effects of steroids.

Limitations of Therapeutic Drug Monitoring

For therapeutic drug monitoring to be useful in clinical practice, it requires consistency in terms of drug administration and sampling. For example, meals may decrease the C_{max} and AUC of CNI (3,17). Although this may lead to higher dosage requirements for patients who take their medication with meals, as long as they are consistent, this should not affect drug levels. However, patients who take their medications with meals sometimes and fasting at other times may have more variability in measured levels, which could lead to under- or overdosing.

In addition, blood samples must be drawn at the correct time. For cyclosporine C_2 monitoring, blood should be drawn within 15 min of the 2-h postdose time point (36). For cyclosporine or tacrolimus trough-level monitoring, blood should be drawn 12 h after the last dose (*i.e.*, immediately before the next dose). Although C_0 monitoring probably does not require as narrow a therapeutic window as C_2 monitoring, levels that are drawn at other time points, such as 10 or 15 h after the last dose, may lead to unnecessary dosage adjustments, again leading to under- or overdosing.

Finally, therapeutic drug monitoring is a method of monitoring a medication by its pharmacokinetics. However, the pharmacodynamic effects may not always correlate with pharmacokinetics. Previous studies have attempted to use calcineurin inhibition, IL-2 production, or cytokine mRNA production as a marker of the degree of calcineurin inhibition (1,106,107). A recent study measured expression of nuclear factor of activated T cells–regulated genes and found a close relationship between the degree of gene suppression and the incidence of infections and malignancies (108). However, no pharmacodynamic method has been validated yet in clinical practice.

Conclusion

Both cyclosporine and tacrolimus have a narrow therapeutic window, meaning that monitoring is required. Optimal monitoring can be achieved only with an understanding of the pharmacokinetics of these medications. Underdosing is associated with an increased risk for rejection, whereas overdosing is associated with toxicity and an increased risk for CAN. C_2 monitoring allows more accurate dosing of cyclosporine and better predicts which patients are at risk for acute rejection, and target C_2 levels early and late after transplantation have been defined. Even patients several years after transplantation may benefit from conversion to C_2 monitoring, because this may allow cyclosporine dosage reduction, possibly leading to improvements in renal function and adverse drug effects. Conversely, no randomized, controlled trials have proved conclusively that C_2 monitoring is associated with improved outcomes compared with C_0, and many centers have achieved excellent results using C_0 monitoring combined with the other available immunosuppressants. For this reason, adoption of C_2 monitoring has not been universal. Although tacrolimus C_0 levels correlate better with AUC than cyclosporine C_0 levels, there is new evidence that tacrolimus C_2 and C_4 levels are better surrogates of AUC than C_0. Further studies will be needed to determine whether these newly proposed time points will improve outcomes in patients who are treated with tacrolimus. The goal of such studies would be to reduce tacrolimus-related toxicity while maintaining the low rate of rejection that is seen with the current monitoring strategy.

Disclosures

M.C. has received honoraria from Astellas, Hoffman LaRoche, and Novartis. E.C. has received honoraria and been a member of the Speaker's Bureau for both Novartis and Astellas.

References

1. Halloran PF, Helms LM, Kung L, Noujaim J: The temporal profile of calcineurin inhibition by cyclosporine in vivo. *Transplantation* 68: 1356–1361, 1999
2. Sindhi R, LaVia MF, Paulling E, McMichael J, Burckart G, Shaw S, Sindhi LA, Livingston R, Sehgal S, Jaffe J: Stimulated response of peripheral lymphocytes may distinguish cyclosporine effect in renal transplant recipients receiving a cyclosporine+rapamycin regimen. *Transplantation* 69: 432–436, 2000
3. Dunn CJ, Wagstaff AJ, Perry CM, Plosker GL, Goa KL: Cyclosporin: An updated review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral)1 in organ transplantation. *Drugs* 61: 1957–2016, 2001
4. Keown P, Landsberg D, Halloran P, Shoker A, Rush D, Jeffery J, Russell D, Stiller C, Muirhead N, Cole E, Paul L, Zaltzman J, Loertscher R, Daloze P, Dandavino R, Boucher A, Handa P, Lawen J, Belitsky P, Parfrey P: A randomized, prospective multicenter pharmacoepidemiologic study of cyclosporine microemulsion in stable renal graft recipients. Report of the Canadian Neoral Renal Transplantation Study Group. *Transplantation* 62: 1744–1752, 1996
5. Kahan BD: Individualization of cyclosporine therapy using pharmacokinetic and pharmacodynamic parameters. *Transplantation* 40: 457–476, 1985
6. Kahan BD, Welsh M, Schoenberg L, Rutzky LP, Katz SM, Urbauer DL, Van Buren CT: Variable oral absorption of cyclosporine. A biopharmaceutical risk factor for chronic renal allograft rejection. *Transplantation* 62: 599–606, 1996
7. Tan KK, Trull AK, Uttridge JA, Metcalfe S, Heyes CS, Facey S, Evans DB: Effect of dietary fat on the pharmacokinetics and pharmacodynamics of cyclosporine in kidney transplant recipients. *Clin Pharmacol Ther* 57: 425–433, 1995
8. Kahan BD, Dunn J, Fitts C, Van Buren D, Wombolt D, Pollak R, Carson R, Alexander JW, Choc M, Wong R, *et al.*: Reduced inter- and intrasubject variability in cyclosporine pharmacokinetics in renal transplant recipients treated with a microemulsion formulation in conjunction with fasting, low-fat meals, or high-fat meals. *Transplantation* 59: 505–511, 1995
9. Ptachcinski RJ, Venkataramanan R, Rosenthal JT, Burckart GJ, Taylor RJ, Hakala TR: The effect of food on cyclosporine absorption. *Transplantation* 40: 174–176, 1985
10. Canadian Neoral Renal Transplantation Study Group: Absorption profiling of cyclosporine microemulsion (Neoral) during the first 2 weeks after renal transplantation. *Transplantation* 72:1024–1032, 2001
11. Christians U, Sewing KF: Alternative cyclosporine metabolic pathways and toxicity. *Clin Biochem* 28: 547–559, 1995
12. Kovarik JM, Mueller EA, van Bree JB, Fluckiger SS, Lange H, Schmidt B, Boesken WH, Lison AE, Kutz K: Cyclosporine pharmacokinetics and variability from a microemulsion formulation: A multicenter investigation in kidney transplant patients. *Transplantation* 58: 658–663, 1994
13. Johnston A, David OJ, Cooney GF: Pharmacokinetic validation of Neoral absorption profiling. *Transplant Proc* 32[Suppl]: 53S–56S, 2000
14. Keown P, Niese D: Cyclosporine microemulsion increases drug exposure and reduces acute rejection without incremental toxicity in de novo renal transplantation. International Sandimmune Neoral Study Group. *Kidney Int* 54: 938–944, 1998
15. Barone G, Bunke CM, Choc MG Jr, Hricik DE, Jin JH, Klein JB, Marsh CL, Min DI, Pescovitz MD, Pollak R, Pruett TL, Stinson JB, Thompson JS, Vasquez E, Waid T, Wombolt DG, Wong RL: The safety and tolerability of cyclosporine emulsion versus cyclosporine in a randomized, double-blind comparison in primary renal allograft recipients. The Neoral Study Group. *Transplantation* 61: 968–970, 1996
16. Scott LJ, McKeage K, Keam SJ, Plosker GL: Tacrolimus: A further update of its use in the management of organ transplantation. *Drugs* 63: 1247–1297, 2003
17. Christiaans M, van Duijnhoven E, Beysens T, Undre N, Schafer A, van Hooff J: Effect of breakfast on the oral bioavailability of tacrolimus and changes in pharmacokinetics at different times posttransplant in renal transplant recipients. *Transplant Proc* 30: 1271–1273, 1998
18. Hu RH, Lee PH, Tsai MK: Clinical influencing factors for daily dose, trough level, and relative clearance of tacrolimus in renal transplant recipients. *Transplant Proc* 32: 1689–1692, 2000
19. Fitzsimmons WE, Bekersky I, Dressler D, Raye K, Hodosh E, Mekki Q: Demographic considerations in tacrolimus pharmacokinetics. *Transplant Proc* 30: 1359–1364, 1998
20. Alloway R, Steinberg S, Khalil K, Gourishankar S, Miller J,

Norman D, Hariharan S, Pirsch J, Matas A, Zaltzman J, Wisemandle K, Fitzsimmons W, First MR: Conversion of stable kidney transplant recipients from a twice daily Prograf-based regimen to a once daily modified release tacrolimus-based regimen. *Transplant Proc* 37: 867–870, 2005
21. Wente MN, Sauer P, Mehrabi A, Weitz J, Buchler MW, Schmidt J, Schemmer P: Review of the clinical experience with a modified release form of tacrolimus [FK506E (MR4)] in transplantation. *Clin Transplant* 20[Suppl 17]: 80–84, 2006
22. Calne RY, Rolles K, White DJ, Thiru S, Evans DB, McMaster P, Dunn DC, Craddock GN, Henderson RG, Aziz S, Lewis P: Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreases, and 2 livers. *Lancet* 2: 1033–1036, 1979
23. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M: Cyclosporine-associated chronic nephropathy. *N Engl J Med* 311: 699–705, 1984
24. Keown PA, Stiller CR, Ulan RA, Sinclair NR, Wall WJ, Carruthers G, Howson W: Immunological and pharmacological monitoring in the clinical use of cyclosporin A. *Lancet* 1: 686–689, 1981
25. Lindholm A, Kahan BD: Influence of cyclosporine pharmacokinetics, trough concentrations, and AUC monitoring on outcome after kidney transplantation. *Clin Pharmacol Ther* 54: 205–218, 1993
26. Kasiske BL, Heim-Duthoy K, Rao KV, Awni WM: The relationship between cyclosporine pharmacokinetic parameters and subsequent acute rejection in renal transplant recipients. *Transplantation* 46: 716–722, 1988
27. Barone G, Chang CT, Choc MG Jr, Klein JB, Marsh CL, Meligeni JA, Min DI, Pescovitz MD, Pollak R, Pruett TL, Stinson JB, Thompson JS, Vasquez E, Waid T, Wombolt DG, Wong RL: The pharmacokinetics of a microemulsion formulation of cyclosporine in primary renal allograft recipients. The Neoral Study Group. *Transplantation* 61: 875–880, 1996
28. Cole E, Keown P, Landsberg D, Halloran P, Shoker A, Rush D, Jeffrey J, Russell D, Stiller C, Muirhead N, Paul L, Zaltzman J, Loertscher R, Daloze P, Dandavino R, Boucher A, Handa P, Lawen J, Belitsky P, Parfrey P, Tan A, Hendricks L: Safety and tolerability of cyclosporine and cyclosporine microemulsion during 18 months of follow-up in stable renal transplant recipients: A report of the Canadian Neoral Renal Study Group. *Transplantation* 65: 505–510, 1998
29. Mahalati K, Belitsky P, Sketris I, West K, Panek R: Neoral monitoring by simplified sparse sampling area under the concentration-time curve: Its relationship to acute rejection and cyclosporine nephrotoxicity early after kidney transplantation. *Transplantation* 68: 55–62, 1999
30. Mahalati K, Belitsky P, West K, Kiberd B, Fraser A, Sketris I, Macdonald AS, McAlister V, Lawen J: Approaching the therapeutic window for cyclosporine in kidney transplantation: A prospective study. *J Am Soc Nephrol* 12: 828–833, 2001
31. International Neoral Renal Transplantation Study Group: Randomized, international study of cyclosporine microemulsion absorption profiling in renal transplantation with basiliximab immunoprophylaxis. *Am J Transplant* 2: 157–166, 2002
32. Cantarovich M, Besner JG, Barkun JS, Elstein E, Loertscher R: Two-hour cyclosporine level determination is the appropriate tool to monitor Neoral therapy. *Clin Transplant* 12: 243–249, 1998
33. Cantarovich M, Barkun JS, Tchervenkov JI, Besner JG, Aspeslet L, Metrakos P: Comparison of Neoral dose monitoring with cyclosporine through levels versus 2-hr postdose levels in stable liver transplant patients. *Transplantation* 66: 1621–1627, 1998
34. Clase CM, Mahalati K, Kiberd BA, Lawen JG, West KA, Fraser AD, Belitsky P: Adequate early cyclosporin exposure is critical to prevent renal allograft rejection: Patients monitored by absorption profiling. *Am J Transplant* 2: 789–795, 2002
35. International Neoral Renal Transplantation Study Group: Cyclosporine microemulsion (Neoral) absorption profiling and sparse-sample predictors during the first 3 months after renal transplantation. *Am J Transplant* 2: 148–156, 2002
36. Levy G, Thervet E, Lake J, Uchida K: Patient management by Neoral C(2) monitoring: An international consensus statement. *Transplantation* 73[Suppl]: S12–S18, 2002
37. Caforio AL, Tona F, Piaserico S, Gambino A, Feltrin G, Fortina AB, Angelini A, Alaibac M, Bontorin M, Calzolari D, Peserico A, Thiene G, Iliceto S, Gerosa G: C2 is superior to C0 as predictor of renal toxicity and rejection risk profile in stable heart transplant recipients. *Transpl Int* 18: 116–124, 2005
38. Glanville AR, Morton JM, Aboyoun CL, Plit ML, Malouf MA: Cyclosporine C2 monitoring improves renal dysfunction after lung transplantation. *J Heart Lung Transplant* 23: 1170–1174, 2004
39. Keown PA, Kiberd B, Balshaw R, Khorasheh S, Marra C, Belitsky P, Kalo Z: An economic model of 2-hour post-dose ciclosporin monitoring in renal transplantation. *Pharmacoeconomics* 22: 621–632, 2004
40. Hardinger KL, Schnitzler MA, Koch MJ, Enkvetchakul D, Desai N, Jendrisak M, Lowell JA, Miller B, Shenoy S, Brennan DC: Cyclosporine minimization and cost reduction in renal transplant recipients receiving a C2-monitored, cyclosporine-based quadruple immunosuppressive regimen. *Transplantation* 78: 1198–1203, 2004
41. Hibberd AD, Trevillian PR, Roger SD, Wlodarczyk JH, Stein AM, Bohringer EG, Milson-Hawke SM: Assessment of the bioequivalence of a generic cyclosporine A by a randomized controlled trial in stable renal recipients. *Transplantation* 81: 711–717, 2006
42. Sharma A, Shekhar C, Heer M, Minz M: Comparison of generic cyclosporine microemulsion versus Neoral in de novo renal transplant recipients managed by 2-hour postdose monitoring. *Transplant Proc* 38: 2051–2053, 2006
43. Qazi YA, Forrest A, Tornatore K, Venuto RC: The clinical impact of 1:1 conversion from Neoral to a generic cyclosporine (Gengraf) in renal transplant recipients with stable graft function. *Clin Transplant* 20: 313–317, 2006
44. Taber DJ, Baillie GM, Ashcraft EE, Rogers J, Lin A, Afzal F, Baliga P, Rajagopalan PR, Chavin KD: Does bioequivalence between modified cyclosporine formulations translate into equal outcomes? *Transplantation* 80: 1633–1635, 2005
45. Johnston A, Belitsky P, Frei U, Horvath J, Hoyer P, Helderman JH, Oellerich M, Pollard S, Riad H, Rigotti P, Keown P, Nashan B: Potential clinical implications of substitution of generic cyclosporine formulations for cyclo-

sporine microemulsion (Neoral) in transplant recipients. *Eur J Clin Pharmacol* 60: 389–395, 2004
46. Balbontin F, Kiberd B, Fraser A, Kiberd M, Lawen J: Basiliximab lowers the cyclosporine therapeutic threshold in the early post-kidney transplant period. *Clin Transplant* 19: 225–229, 2005
47. Pescovitz MD, Barbeito R: Two-hour post-dose cyclosporine level is a better predictor than trough level of acute rejection of renal allografts. *Clin Transplant* 16: 378–382, 2002
48. Thervet E, Pfeffer P, Scolari MP, Toselli L, Pallardo LM, Chadban S, Pilmore H, Connolly J, Buchler M, Schena FP, Carreno CA, Dandavino R, Cole E: Clinical outcomes during the first three months posttransplant in renal allograft recipients managed by C2 monitoring of cyclosporine microemulsion. *Transplantation* 76: 903–908, 2003
49. Stefoni S, Midtved K, Cole E, Thervet E, Cockfield S, Buchler M, Toselli L, Pallardo LM, Schena FP, Pilmore H, Kessler M, Chadban S, Carreno CA: Efficacy and safety outcomes among de novo renal transplant recipients managed by C2 monitoring of cyclosporine a microemulsion: Results of a 12-month, randomized, multicenter study. *Transplantation* 79: 577–583, 2005
50. Remuzzi G, Lesti M, Gotti E, Ganeva M, Dimitrov BD, Ene-Iordache B, Gherardi G, Donati D, Salvadori M, Sandrini S, Valente U, Segoloni G, Mourad G, Federico S, Rigotti P, Sparacino V, Bosmans JL, Perico N, Ruggenenti P: Mycophenolate mofetil versus azathioprine for prevention of acute rejection in renal transplantation (MYSS): A randomised trial. *Lancet* 364: 503–512, 2004
51. Dantal J, Hourmant M, Cantarovich D, Giral M, Blancho G, Dreno B, Soulillou JP: Effect of long-term immunosuppression in kidney-graft recipients on cancer incidence: Randomised comparison of two cyclosporin regimens. *Lancet* 351: 623–628, 1998
52. Pascual M, Curtis J, Delmonico FL, Farrell ML, Williams WW Jr, Kalil R, Jones P, Cosimi AB, Tolkoff-Rubin N: A prospective, randomized clinical trial of cyclosporine reduction in stable patients greater than 12 months after renal transplantation. *Transplantation* 75: 1501–1505, 2003
53. Citterio F, Scata MC, Borzi MT, Pozzetto U, Castagneto M: C2 single-point sampling to evaluate cyclosporine exposure in long-term renal transplant recipients. *Transplant Proc* 33: 3133–3136, 2001
54. Midtvedt K, Fauchald P, Bergan S, Hoieggen A, Hallan S, Svarstad E, Bergrem H, Eriksen BO, Pfeffer PF, Dalen I, Leivestad T: C2 monitoring in maintenance renal transplant recipients: Is it worthwhile? *Transplantation* 76: 1236–1238, 2003
55. Cole E, Maham N, Cardella C, Cattran D, Fenton S, Hamel J, O'Grady C, Smith R: Clinical benefits of Neoral C2 monitoring in the long-term management of renal transplant recipients. *Transplantation* 75: 2086–2090, 2003
56. Levy GA: C2 monitoring strategy for optimising cyclosporin immunosuppression from the Neoral formulation. *BioDrugs* 15: 279–290, 2001
57. Citterio F, Scata MC, Romagnoli J, Nanni G, Castagneto M: Results of a three-year prospective study of C2 monitoring in long-term renal transplant recipients receiving cyclosporine microemulsion. *Transplantation* 79: 802–806, 2005
58. Murphy GJ, Waller JR, Sandford RS, Furness PN, Nicholson ML: Randomized clinical trial of the effect of microemulsion cyclosporin and tacrolimus on renal allograft fibrosis. *Br J Surg* 90: 680–686, 2003
59. Bicknell GR, Williams ST, Shaw JA, Pringle JH, Furness PN, Nicholson ML: Differential effects of cyclosporin and tacrolimus on the expression of fibrosis-associated genes in isolated glomeruli from renal transplants. *Br J Surg* 87: 1569–1575, 2000
60. Rowshani AT, Scholten EM, Bemelman F, Eikmans M, Idu M, Roos-van Groningen MC, Surachno JS, Mallat MJ, Paul LC, de Fijter JW, Bajema IM, ten Berge I, Florquin S: No difference in degree of interstitial Sirius red–stained area in serial biopsies from area under concentration-over-time curves–guided cyclosporine versus tacrolimus-treated renal transplant recipients at one year. *J Am Soc Nephrol* 17: 305–312, 2006
61. Roos-van Groningen MC, Scholten EM, Lelieveld PM, Rowshani AT, Baelde HJ, Bajema IM, Florquin S, Bemelman FJ, de Heer E, de Fijter JW, Bruijn JA, Eikmans M: Molecular comparison of calcineurin inhibitor–induced fibrogenic responses in protocol renal transplant biopsies. *J Am Soc Nephrol* 17: 881–888, 2006
62. Undre NA, van Hooff J, Christiaans M, Vanrenterghem Y, Donck J, Heeman U, Kohnle M, Zanker B, Land W, Morales JM, Andres A, Schafer A, Stevenson P: Low systemic exposure to tacrolimus correlates with acute rejection. *Transplant Proc* 31: 296–298, 1999
63. Wong KM, Shek CC, Chau KF, Li CS: Abbreviated tacrolimus area-under-the-curve monitoring for renal transplant recipients. *Am J Kidney Dis* 35: 660–666, 2000
64. Armendariz Y, Pou L, Cantarell C, Lopez R, Perello M, Capdevila L: Evaluation of a limited sampling strategy to estimate area under the curve of tacrolimus in adult renal transplant patients. *Ther Drug Monit* 27: 431–434, 2005
65. Balbontin FG, Kiberd B, Squires J, Singh D, Fraser A, Belitsky P, Lawen J: Tacrolimus monitoring by simplified sparse sampling under the concentration time curve. *Transplant Proc* 35: 2445–2448, 2003
66. Mardigyan V, Giannetti N, Cecere R, Besner JG, Cantarovich M: Best single time points to predict the area-under-the-curve in long-term heart transplant patients taking mycophenolate mofetil in combination with cyclosporine or tacrolimus. *J Heart Lung Transplant* 24: 1614–1618, 2005
67. Scholten EM, Cremers SC, Schoemaker RC, Rowshani AT, van Kan EJ, den Hartigh J, Paul LC, de Fijter JW: AUC-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients. *Kidney Int* 67: 2440–2447, 2005
68. Undre NA: Pharmacokinetics of tacrolimus-based combination therapies. *Nephrol Dial Transplant* 18[Suppl 1]: i12–i15, 2003
69. Oellerich M, Armstrong VW, Schutz E, Shaw LM: Therapeutic drug monitoring of cyclosporine and tacrolimus. Update on Lake Louise Consensus Conference on cyclosporin and tacrolimus. *Clin Biochem* 31: 309–316, 1998
70. Kershner RP, Fitzsimmons WE: Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation* 62: 920–926, 1996
71. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS: A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation.

FK506 Kidney Transplant Study Group. *Transplantation* 63: 977–983, 1997
72. Mayer AD, Dmitrewski J, Squifflet JP, Besse T, Grabensee B, Klein B, Eigler FW, Heemann U, Pichlmayr R, Behrend M, Vanrenterghem Y, Donck J, van Hooff J, Christiaans M, Morales JM, Andres A, Johnson RW, Short C, Buchholz B, Rehmert N, Land W, Schleibner S, Forsythe JL, Talbot D, Pohanka E, *et al.*: Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: A report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation* 64: 436–443, 1997
73. Morris-Stiff G, Ostrowski K, Balaji V, Moore R, Darby C, Lord R, Jurewicz WA: Prospective randomised study comparing tacrolimus (Prograf) and cyclosporin (Neoral) as primary immunosuppression in cadaveric renal transplants at a single institution: Interim report of the first 80 cases. *Transpl Int* 11[Suppl 1]: S334–S336, 1998
74. Busque S, Shoker A, Landsberg D, McAlister V, Halloran P, Shapiro J, Peets J, Schulz M: Canadian multicentre trial of tacrolimus/azathioprine/steroids versus tacrolimus/mycophenolate mofetil/steroids versus neoral/mycophenolate mofetil/steroids in renal transplantation. *Transplant Proc* 33: 1266–1267, 2001
75. Margreiter R: Efficacy and safety of tacrolimus compared with ciclosporin microemulsion in renal transplantation: A randomised multicentre study. *Lancet* 359: 741–746, 2002
76. Charpentier B, Rostaing L, Berthoux F, Lang P, Civati G, Touraine JL, Squifflet JP, Vialtel P, Abramowicz D, Mourad G, Wolf P, Cassuto E, Moulin B, Rifle G, Pruna A, Merville P, Mignon F, Legendre C, Le Pogamp P, Lebranchu Y, Toupance O, Hurault De Ligny B, Touchard G, Olmer M, Purgus R, Pouteil-Noble C, Glotz D, Bourbigot B, Leski M, Wauters JP, Kessler M: A three-arm study comparing immediate tacrolimus therapy with antithymocyte globulin induction therapy followed by tacrolimus or cyclosporine A in adult renal transplant recipients. *Transplantation* 75: 844–851, 2003
77. Hardinger KL, Bohl DL, Schnitzler MA, Lockwood M, Storch GA, Brennan DC: A randomized, prospective, pharmacoeconomic trial of tacrolimus versus cyclosporine in combination with Thymoglobulin in renal transplant recipients. *Transplantation* 80: 41–46, 2005
78. Rostaing L, Cantarovich D, Mourad G, Budde K, Rigotti P, Mariat C, Margreiter R, Capdevilla L, Lang P, Vialtel P, Ortuno-Mirete J, Charpentier B, Legendre C, Sanchez-Plumed J, Oppenheimer F, Kessler M: Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. *Transplantation* 79: 807–814, 2005
79. Ekberg H, Tededsco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A, Margreiter R, Hugo C, Grinyo J, Frei U, Vanrenterghem Y, Daloze P, Halloran P: Symphony: Comparing standard immunosuppression to low-dose cyclosporine, tacrolimus or sirolimus in combination with MMF, daclizumab and corticosteroids in renal transplantation. *Transplantation* 82: 83, 2006
80. Brennan DC, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, Lockwood M, Torrence S, Schuessler R, Roby T, Gaudreault-Keener M, Storch GA: Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 5: 582–594, 2005
81. Kramer BK, Montagnino G, Del Castillo D, Margreiter R, Sperschneider H, Olbricht CJ, Kruger B, Ortuno J, Kohler H, Kunzendorf U, Stummvoll HK, Tabernero JM, Muhlbacher F, Rivero M, Arias M: Efficacy and safety of tacrolimus compared with cyclosporin A microemulsion in renal transplantation: 2 year follow-up results. *Nephrol Dial Transplant* 20: 968–973, 2005
82. Webster AC, Woodroffe RC, Taylor RS, Chapman JR, Craig JC: Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: Meta-analysis and meta-regression of randomised trial data. *BMJ* 331: 810, 2005
83. Kim SJ, Prasad GV, Huang M, Nash MM, Famure O, Park J, Thenganatt MA, Chowdhury N, Cole EH, Fenton SS, Cattran DC, Zaltzman JS, Cardella CJ: A comparison of the effects of C2-cyclosporine and C0-tacrolimus on renal function and cardiovascular risk factors in kidney transplant recipients. *Transplantation* 82: 924–930, 2006
84. Vincenti F, Tuncer M, Castagneto M, Klinger M, Friman S, Scheuermann EH, Wiecek A, Russ GR, Martinek A, Nonnast-Daniel B: Prospective, multicenter, randomized trial to compare incidence of new-onset diabetes mellitus and glucose metabolism in patients receiving cyclosporine microemulsion versus tacrolimus after de novo kidney transplantation. *Transplant Proc* 37: 1001–1004, 2005
85. Levy G, Villamil F, Samuel D, Sanjuan F, Grazi GL, Wu Y, Marotta P, Boillot O, Muehlbacher F, Klintmalm G: Results of lis2t, a multicenter, randomized study comparing cyclosporine microemulsion with C2 monitoring and tacrolimus with C0 monitoring in de novo liver transplantation. *Transplantation* 77: 1632–1638, 2004
86. Kahan BD: Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: A randomised multicentre study. The Rapamune US Study Group. *Lancet* 356: 194–202, 2000
87. MacDonald AS: A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. *Transplantation* 71: 271–280, 2001
88. Lorber MI, Mulgaonkar S, Butt KM, Elkhammas E, Mendez R, Rajagopalan PR, Kahan B, Sollinger H, Li Y, Cretin N, Tedesco H: Everolimus versus mycophenolate mofetil in the prevention of rejection in de novo renal transplant recipients: A 3-year randomized, multicenter, phase III study. *Transplantation* 80: 244–252, 2005
89. Vitko S, Margreiter R, Weimar W, Dantal J, Kuypers D, Winkler M, Oyen O, Viljoen HG, Filiptsev P, Sadek S, Li Y, Cretin N, Budde K: Three-year efficacy and safety results from a study of everolimus versus mycophenolate mofetil in de novo renal transplant patients. *Am J Transplant* 5: 2521–2530, 2005
90. Ciancio G, Burke GW, Gaynor JJ, Ruiz P, Roth D, Kupin W, Rosen A, Miller J: A randomized long-term trial of tacrolimus/sirolimus versus tacrolimus/mycophenolate versus cyclosporine/sirolimus in renal transplantation: Three-year analysis. *Transplantation* 81: 845–852, 2006
91. Augustine JJ, Chang PC, Knauss TC, Aeder MI, Bodziak KA, Schulak JA, Hricik DE: Improved renal function after conversion from tacrolimus/sirolimus to tacrolimus/mycophenolate mofetil in kidney transplant recipients. *Transplantation* 81: 1004–1009, 2006

92. Vitko S, Wlodarczyk Z, Kyllonen L, Czajkowski Z, Margreiter R, Backman L, Perner F, Rigotti P, Jaques B, Abramowicz D, Kessler M, Sanchez-Plumed J, Rostaing L, Rodger RS, Donati D, Vanrenterghem Y: Tacrolimus combined with two different dosages of sirolimus in kidney transplantation: Results of a multicenter study. *Am J Transplant* 6: 531–538, 2006
93. Chueh SC, Kahan BD: Clinical application of sirolimus in renal transplantation: An update. *Transpl Int* 18: 261–277, 2005
94. Vitko S, Tedesco H, Eris J, Pascual J, Whelchel J, Magee JC, Campbell S, Civati G, Bourbigot B, Alves Filho G, Leone J, Garcia VD, Rigotti P, Esmeraldo R, Cambi V, Haas T, Jappe A, Bernhardt P, Geissler J, Cretin N: Everolimus with optimized cyclosporine dosing in renal transplant recipients: 6-month safety and efficacy results of two randomized studies. *Am J Transplant* 4: 626–635, 2004
95. Ferreira AN, Machado PG, Felipe CR, Motegi SA, Hosaka BH, Tanaka MK, Kamura LA, Park SI, Garcia R, Franco M, Alfieri F, Casarini DE, Tedesco-Silva H Jr, Medina-Pestana JO: Concentration-controlled use of sirolimus associated with reduced exposure of cyclosporine in black recipients of primarily living renal allograft donors: 12-month results. *Clin Transplant* 19: 607–615, 2005
96. Nashan B, Curtis J, Ponticelli C, Mourad G, Jaffe J, Haas T: Everolimus and reduced-exposure cyclosporine in de novo renal-transplant recipients: A three-year phase II, randomized, multicenter, open-label study. *Transplantation* 78: 1332–1340, 2004
97. Russ GR, Campbell S, Chadban S, Eris J, O'Connell P, Pussell B, Walker R: Reduced and standard target concentration tacrolimus with sirolimus in renal allograft recipients. *Transplant Proc* 35[Suppl]: 115S–117S, 2003
98. Meier-Kriesche HU, Steffen BJ, Chu AH, Loveland JJ, Gordon RD, Morris JA, Kaplan B: Sirolimus with Neoral versus mycophenolate mofetil with Neoral is associated with decreased renal allograft survival. *Am J Transplant* 4: 2058–2066, 2004
99. Meier-Kriesche HU, Schold JD, Srinivas TR, Howard RJ, Fujita S, Kaplan B: Sirolimus in combination with tacrolimus is associated with worse renal allograft survival compared to mycophenolate mofetil combined with tacrolimus. *Am J Transplant* 5: 2273–2280, 2005
100. Vanrenterghem Y, Lebranchu Y, Hene R, Oppenheimer F, Ekberg H: Double-blind comparison of two corticosteroid regimens plus mycophenolate mofetil and cyclosporine for prevention of acute renal allograft rejection. *Transplantation* 70: 1352–1359, 2000
101. Vincenti F, Monaco A, Grinyo J, Kinkhabwala M, Roza A: Multicenter randomized prospective trial of steroid withdrawal in renal transplant recipients receiving basiliximab, cyclosporine microemulsion and mycophenolate mofetil. *Am J Transplant* 3: 306–311, 2003
102. Park JB, Kim SJ, Oh HY, Han YS, Kim DJ, Park JW, Kwon CH, Joh JW, Lee SK: Steroid withdrawal in living donor renal transplant recipients using tacrolimus and cyclosporine: A randomized prospective study. *Transpl Int* 19: 478–484, 2006
103. Abramowicz D, Vanrenterghem Y, Squifflet JP, Kuypers D, Mourad M, Meurisse M, Wissing M: Efficacy and cardiovascular safety of daclizumab, mycophenolate mofetil, tacrolimus, and early steroid withdrawal in renal transplant recipients: A multicenter, prospective, pilot trial. *Clin Transplant* 19: 475–482, 2005
104. Kuypers DR, Evenepoel P, Maes B, Coosemans W, Pirenne J, Vanrenterghem Y: The use of an anti-CD25 monoclonal antibody and mycophenolate mofetil enables the use of a low-dose tacrolimus and early withdrawal of steroids in renal transplant recipients. *Clin Transplant* 17: 234–241, 2003
105. ter Meulen CG, van Riemsdijk I, Hene RJ, Christiaans MH, Borm GF, van Gelder T, Hilbrands LB, Weimar W, Hoitsma AJ: Steroid-withdrawal at 3 days after renal transplantation with anti-IL-2 receptor alpha therapy: A prospective, randomized, multicenter study. *Am J Transplant* 4: 803–810, 2004
106. Stein CM, Murray JJ, Wood AJ: Inhibition of stimulated interleukin-2 production in whole blood: A practical measure of cyclosporine effect. *Clin Chem* 45: 1477–1484, 1999
107. Hartel C, Fricke L, Schumacher N, Kirchner H, Muller-Steinhardt M: Delayed cytokine mRNA expression kinetics after T-lymphocyte costimulation: A quantitative measure of the efficacy of cyclosporin A-based immunosuppression. *Clin Chem* 48: 2225–2231, 2002
108. Sommerer C, Konstandin M, Dengler T, Schmidt J, Meuer S, Zeier M, Giese T: Pharmacodynamic monitoring of cyclosporine a in renal allograft recipients shows a quantitative relationship between immunosuppression and the occurrence of recurrent infections and malignancies. *Transplantation* 82: 1280–1285, 2006