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CHAPTER 6

Summary and general discussion
Kidney transplantation is the preferred treatment for patients with end-stage renal disease (ESRD). However, the shortage of donor kidneys remains a major obstacle. Despite more deceased donor transplants with extended donor criteria and more (unrelated and older) living donors, waiting lists are still growing. Living donor transplants currently account for 50% of the total renal transplants in the Netherlands (RENINE data). Considering the lack of donor organs, extending graft survival as long as possible, and thereby preventing a return to the waiting list, has become a critical issue in renal transplantation.

Long-term graft survival is determined by death with a functioning graft and late graft loss. The major causes of mortality are cardiovascular disease, malignancy and infectious disease. Calcineurin inhibitors (CNIs) increase the cardiovascular risk via hypertension, hyperlipidaemia, diabetes mellitus and chronic renal failure by nephrotoxicity. Late allograft loss is mainly caused by chronic transplant dysfunction (CTD). CTD is a clinical syndrome of gradually deteriorating renal function, combined with hypertension and proteinuria, starting early after transplantation and ultimately ending in graft failure. Chronic allograft nephropathy (CAN), its histological counterpart, is currently defined by interstitial fibrosis and tubular atrophy (IF/TA). Immunological factors, such as chronic cellular- or antibody-mediated rejection, and non-immunological factors, including CNI nephrotoxicity, contribute to the evolution of CAN-IF/TA.

Intervention strategies to improve long-term graft survival include early CNI sparing to reduce cardiovascular adverse effects and nephrotoxicity. Although early CNI withdrawal with mycophenolate mofetil (MMF)-based therapy may improve renal allograft function, it is associated with an increased risk of acute rejection. Therefore, early minimisation of CNIs has become the trend, as low dose tacrolimus may ameliorate renal function and still protect against acute rejection. However, the 3-year follow-up data of the Symphony study did not demonstrate a significant difference in renal function in favour of the low dose tacrolimus group. Risk factors for acute rejection after CNI elimination are early withdrawal, subclinical rejection or underdosing of MMF. The optimal range of mycophenolic acid (MPA) exposure in patients who were withdrawn of cyclosporine (CsA) was found to be higher than the usual target of 30-60 µg·h/ml in combination with CNIs. Therapeutic drug monitoring (TDM) might prevent under-exposure to MPA.

This thesis studied the impact of late CNI withdrawal from a triple-drug regimen with corticosteroids, CNIs and MMF in renal transplant recipients, while providing adequate immunosuppression by using TDM of MPA, on renal function, the risk of acute rejection and surrogate markers for cardiovascular disease, including echocardiographic parameters, 24-hour ambulatory blood pressure and carotid intima media thickness (IMT).
In **chapter 2** the safety of either late concentration-controlled CNI or MMF withdrawal, in stable renal transplant recipients on a triple-drug regimen with corticosteroids, CNIs and MMF, was evaluated in a prospective randomised controlled study. In the safety phase, 58 patients were randomised (1:1:1) to CNI or MMF withdrawal or continuation of their immunosuppressive regimen. Subsequently, in the extension phase, another 119 patients were randomised (1:1) to either CNI or MMF withdrawal. The target exposures were 3250 ng·h/ml for CsA, 120 ng·h/ml for tacrolimus and 75 μg·h/ml for MPA. Only 1/79 patients (1.3%) in the MMF withdrawal group and 3/79 (3.8%) in the CNI withdrawal group experienced acute rejection during the first 6 months after withdrawal (p=0.62). The occurrence of acute rejection episodes was not significantly different between both groups at 3 years (MMF withdrawal: 2.5% vs. CNI withdrawal: 5.1%, p=0.68).

Late CNI withdrawal resulted in an immediate and significant improvement in renal function, which was maintained during the study follow-up of 3 years (59.5±2.1 ml/min/1.73 m² vs. 51.1±2.1 ml/min/1.73 m², p=0.006). Especially patients with an estimated glomerular filtration rate (eGFR)<50 ml/min/1.73 m² (but ≥30 ml/min/1.73 m²) benefitted from the late elimination of a CNI, with significant less decline in renal function compared to MMF withdrawal (slope: +0.008 ml/min/1.73 m²/month vs. -0.096 ml/min/1.73 m²/month, p=0.03). The defined higher MPA exposure was well tolerated. It was concluded, therefore, that either late concentration-controlled CNI or MMF withdrawal was safe, with a low probability of acute rejection in the large majority of stable renal transplant recipients. Area under the concentration-time curve (AUC) monitoring might prevent inadequate immunosuppression and CNI withdrawal had the advantage of improved renal function.

In the cardiovascular substudies, the impact of either concentration-controlled CNI or MMF withdrawal on surrogate markers of cardiovascular disease, including echocardiographic parameters, was investigated in **chapter 3** and the same for ambulatory blood pressure and common carotid IMT in **chapter 4**. Patients were treated for cardiovascular risk factors according to stringent predefined targets: goals for systolic blood pressure (SBP)<130 mm Hg and diastolic blood pressure (DBP)<85 mm Hg; in case of proteinuria SBP<125 mm Hg and DBP<75 mm Hg; for low-density lipoprotein (LDL)-cholesterol<2.6 mmol/L. Study visits were scheduled 3-monthly after trial entry during the first year, and subsequently after 2 and 3 years in a dedicated outpatient clinic, alternating with 3-monthly visits to their treating nephrologist in a regional outpatient clinic.

Echocardiographic measurements were performed in 108 patients at baseline and 2 years after withdrawal. The assessment of left ventricular (LV) diastolic function showed a significant prolongation of E-wave deceleration time after MMF withdrawal, whereas the E-wave deceleration time remained unchanged after CNI withdrawal at 2 years (p=0.013). Mitral annular e’ velocity improved in the CNI withdrawal group at 2 years,
but remained unchanged in the MMF withdrawal group (p=0.16). The left atrial volume index, an indicator of chronic LV diastolic dysfunction, was significantly increased in the MMF withdrawal group after 2 years (p<0.001). In addition, CNI withdrawal resulted in a lower SBP (135±18 vs. 141±14 mm Hg, p=0.06) and DBP (77±9 vs. 84±10 mm Hg, p=0.001) with the use of less antihypertensive drugs. A higher proportion of patients in the CNI withdrawal group achieved the target blood pressure (<130/85 mm Hg: 41.5% vs. 12.7%, p=0.001). Change in E-wave deceleration time was significantly associated with withdrawal group (p=0.025), but not with changes in SBP, DBP or renal function over time. Change in left atrial volume index was significantly associated with withdrawal group (p=0.03), changes in SBP (p=0.005) and DBP (p=0.005), and not with change in renal function over time. These findings indicated that CNI withdrawal prevented the progressive development of LV diastolic dysfunction and facilitated better treatment of hypertension.

In chapter 4 the results of ambulatory blood pressure monitoring (ABPM) and IMT of 119 patients were reported. Both measurements were performed at baseline and repeated every year. At 3 years, the CNI withdrawal group had significantly lower 24-hour ambulatory SBP (121.2±9.3 vs. 127.9±9.9 mm Hg, p=0.004) and DBP (72.2±6.9 vs. 78.5±5.3 mm Hg, p<0.001). During the 3-year follow-up, the CNI withdrawal group demonstrated a significant decline in ambulatory blood pressure (day-time: SBP: -1.6 mm Hg/yr, p=0.018; DBP: -1.3 mm Hg/yr, p=0.002; night-time SBP: -1.9 mm Hg/yr, p=0.008; DBP: -1.3 mm Hg/yr, p=0.014), whereas the MMF withdrawal group did not. There was a significant difference between the slopes of ambulatory blood pressure of both groups (day-time SBP: p=0.035; day-time DBP: p=0.001; night-time SBP: p=0.015; night-time DBP: p=0.003). The proportion of patients with >2 antihypertensive drugs was significantly higher after MMF withdrawal vs. CNI withdrawal (57% vs. 35%, p=0.03). A subgroup analysis of patients, who were treated with either CsA or tacrolimus at randomisation, demonstrated comparable results after CsA withdrawal (24-hour SBP: 123.1±7.9 vs. 128.3±9.5 mm Hg, p=0.05; 24-hour DBP: 72.9±6.1 vs. 78.0±4.2 mm Hg, p=0.001) and tacrolimus withdrawal (24-hour SBP: 115.9±11.3 vs. 127.2±11.0 mm Hg, p=0.058; 24-hour DBP: 70.1±8.8 vs. 79.5±6.8 mm Hg, p=0.058). The mean IMT did not change over time in both groups with no significant difference in IMT between the patients who had been withdrawn from either a CNI or MMF after 3 years (CNI withdrawal: 0.611±0.074 mm; MMF withdrawal: 0.591±0.070 mm, p=0.27). These results showed that late CNI withdrawal improved ambulatory blood pressure during both day-time and night-time. The significant reduction in ambulatory blood pressure after CNI withdrawal was not associated with a change in IMT after 3 years.
In chapter 5 a retrospective study was described investigating the safety and intermediate- to long-term impact on renal function of late CNI withdrawal and conversion to an immunosuppressive regimen with corticosteroids and MMF in renal transplant recipients, including those with deteriorating renal function. This study confirmed our clinical experience that late CNI withdrawal was not associated with an increased rate of acute rejection in the outpatient setting in the long-term. A total of 139 patients, who had been converted by their treating physician in our outpatient clinic, were included. Eighty-three (60%) patients had been withdrawn of the CNI because of reasons not related to renal function (group 1) and 56 (40%) because of declining renal function (group 2). The median follow-up time was 3.4 years (IQR 2.9-5.1 years). Conversion to MMF resulted in an immediate increase in renal function in both groups (group 1: 4.9±2.4 ml/min/1.73 m², p=0.04; group 2: 5.4±1.8 ml/min/1.73 m², p=0.004). Group 1 had a stable renal function before and after conversion. Group 2 demonstrated a progressive and gradual loss of eGFR towards conversion (−0.34±0.07 ml/min/1.73 m²/month), that stabilised after conversion (0.01±0.05 ml/min/1.73 m²/month) with a significant difference between the slopes (p<0.001). One patient in each group experienced an acute rejection episode. These results demonstrated that late CNI withdrawal was safe and led to improved renal function, with the stabilisation of renal function in the intermediate to long-term, in patients with stable function and in those with an already progressive loss of renal function.

LATE CNI WITHDRAWAL AND RENAL ALLOGRAFT OUTCOME

The introduction of CNIs and MMF has tremendously improved short-term graft survival due to the reduction of early acute rejection by better control of the alloimmune response in the immediate post-transplant period 16-20. However, long-term survival has not equivalently improved 21-23, which has partly been attributed to the adverse effects of CNIs, especially nephrotoxicity 24. Consequently, there has been an ongoing interest in interventions to eliminate or minimise CNI-based therapy. CNI withdrawal with MMF-based therapy has not become common practice, because of the documented increased risk of acute rejection 8.

In this study, late CNI withdrawal with TDM resulted in significantly better renal function in stable renal transplant patients with a low risk of acute rejection. The improvement in renal function directly after CNI withdrawal was sustained over time and most likely resulted from a reversal of renal vasoconstriction, since no change in the slopes of renal function could be demonstrated after 3 years. When comparing the change in eGFR from baseline to 3 years between the groups, a difference of 4.4±2.2 ml/min/1.73 m² (MMF withdrawal: -2.9±1.6 ml/min/1.73 m², CNI withdrawal: 1.5±1.6 ml/
min/1.73 m², p<0.05) in favour of the CNI withdrawal group was rather limited, but still clinically important and in accordance with a recent meta-analysis. Furthermore, the current study found a greater difference in the change in renal function from baseline of 8.7±2.3 ml/min/1.73 m² between the withdrawal groups in the subpopulation with an eGFR<50 ml/min/1.73 m² and ≥30 ml/min/1.73 m² after CNI withdrawal at 3 years (MMF withdrawal: -2.3±1.6 ml/min/1.73 m², CNI withdrawal: 6.4±1.7 ml/min/1.73 m², p<0.001), with a significant difference in the slopes of renal function over time. This finding suggested the elimination or reversal of chronic nephrotoxicity by the CNI in a subgroup with worse renal function and consequently more renal damage. These results are in line with 2 prospective trials investigating the effects of late CNI withdrawal with conversion to MMF (>5 years post-transplantation) in patients with deteriorating renal function.

The current results are in contrast with the CONVERT and ASCERTAIN studies, which have both evaluated late CNI withdrawal (3.2 and 5.4 years post-transplantation, respectively), but with conversion to a mammalian target of rapamycin (mTOR) inhibitor. Both studies failed to show better renal function in the intention-to-treat analysis. Only patients with an eGFR>40 ml/min in the CONVERT trial or a GFR>50 ml/min in the ASCERTAIN study and who carried on with mTOR inhibitor treatment for 2 years had a significant benefit in renal function, with a low risk of acute rejection. These data indicated that patients should be converted to an mTOR inhibitor before they develop chronic and irreversible structural (nephrotoxic) changes. Given the high rates of discontinuation of mTOR inhibitors in these studies, it is tempting to speculate that late CNI withdrawal with MMF may be more attractive than with an mTOR inhibitor, especially in patients with an eGFR between 30 and 50 ml/min/1.73 m² or deteriorating renal function. On the other hand, conversion to an mTOR inhibitor has the benefit of a lower incidence of malignancies. However, a randomised study comparing CNI withdrawal with adequate exposure of either MMF or an mTOR inhibitor would be needed to analyse this hypothesis. In general, it is desirable to stop or minimise CNI treatment before it is too late and irreversible damage has been done.

We hypothesise that concentration-controlled CNI withdrawal also might have been safe, if it had been performed prior to 3 years post-transplantation, but at least beyond the first year in view of the higher immunological risk after early elimination. The risk of acute rejection was low, but numerically higher after CNI withdrawal than MMF withdrawal at 3 years (5.1% vs. 2.5%, p=0.68). However, the current study was not designed to show a difference in this outcome. The rate of rejection was still lower than the incidence of between 11% and 22% reported in previous withdrawal studies. Also, numerically more patients developed a chronic transplant glomerulopathy after the CNI was withdrawn (2.5% vs. 0%, p=0.50). Most of the rejection episodes occurred during the first 6 months after either CNI or MMF withdrawal and could not have been predicted by the immunological risk profile of the patients.
TDM may have prevented inadequate exposure to MPA and consequently an excess rate of rejection after CNI withdrawal, in both the short- and long-term. The two patients in our study who experienced acute rejection after ≥1 year after either MMF or CNI withdrawal had underexposure to the remaining drug, due to therapeutic non-compliance and an intercurrent illness, respectively. Physicians (and patients) should be aware of the risk of rejection, in particular during the first 3-6 months after withdrawal and in case of an intercurrent illness, even when concentration-controlled dosing of the remaining drug is used. During these episodes, renal function and the AUC have to be monitored more frequently. TDM does not invariably detect non-compliance. In case there is serious doubt regarding the compliance, the patient should not be switched to dual therapy.

Although a high exposure to MPA was provided, the donor specific antibodies (DSA) or protocol biopsies were not included at the time of randomisation or during follow-up in order to identify patients with subclinical (chronic) rejection and CAN-IF/TA. According to the protocol, a renal biopsy was performed in case serum creatinine increased >15%. As serum creatinine is an insensitive marker, the rate of subclinical (chronic) rejection or CAN-IF/TA may have been underestimated. The follow-up time was 3 years, but it may still not be long enough to reveal the functional consequences of these subclinical histological changes.

The present study has several other limitations. It is a single-centre study in a selected group of renal transplant recipients with stable renal function and a relatively low immunological risk profile. Therefore, the results of the present study may not be directly extrapolated to populations with a higher immunological risk. Outside the feasibility phase, there was no control group, but it is not likely that renal function would have improved in a control group with a CNI-based regimen with corticosteroids and MMF. Another shortcoming is that 75% of patients were treated with CsA in the MMF withdrawal group, whereas tacrolimus has become the most frequently prescribed CNI after renal transplantation.

LATE CNI WITHDRAWAL AND CARDIOVASCULAR RISK

Blood pressure

Hypertension is common in renal transplant recipients and an independent predictor of graft failure and cardiovascular mortality. CNIs cause hypertension in transplant recipients by renal vasoconstriction by the activation of vasoconstrictive factors, including the renin-angiotensin-aldosterone system, endothelin and thromboxane A2, and the reduction of vasodilator factors, such as nitric oxide and prostacyclin. Activation of the sympathetic nervous system may also play a role. Furthermore, sodium and
water retention can be increased by the activation of the renin-angiotensin-aldosterone system and inactivation of the natriuretic peptide 35. Late CNI withdrawal resulted in a significant decline in ambulatory blood pressure after 3 years, while less patients had to be treated with >2 antihypertensive drugs (35% vs. 57%, p=0.03). A subgroup analysis of patients, who were on either on CsA (68.1%) or tacrolimus (31.9%), showed comparable results with lower ambulatory blood pressures after CsA as well as tacrolimus withdrawal at 3 years. Although the study protocol did not include a subgroup analysis and the number of patients was small, these results suggest that both CsA and tacrolimus have adverse effects on arterial blood pressure, though CsA and tacrolimus inhibit calcineurin activity by binding to different immunophyllins 36.

Despite the treatment of hypertension according to a predefined target (blood pressure <130/85 mm Hg) and a treatment protocol, blood pressure was still insufficiently controlled in a relatively large proportion of patients. These findings are in accordance with a large cohort study, which reported that 46% of renal recipients had an SBP≥140 mm Hg at 1 year after transplantation 34. In the current cardiovascular substudy, only 12.7% of the patients in the MMF withdrawal group and 41.5% in the CNI withdrawal group had a blood pressure below the target of 130/85 mm Hg at 2 years (p=0.001).

The vigorous regulation of hypertension merits more attention, since it would confer a benefit in graft survival 33-34. After the first year, the majority of patients were followed by their treating physician 3-monthly, alternating with yearly visits to the dedicated outpatient clinic. Part of the disappointing results may be explained by non-adherence to the treatment protocol, although these were based on commonly accepted practice guidelines for renal transplant recipients. On the other hand, non-compliance and the tendency of many patients to bargain for as few antihypertensive drugs as possible, may have contributed to the poor control of blood pressure. A greater awareness of the importance and implications for long-term outcome among patients and their treating physicians, as well as the use of collective treatment protocols by physicians or their nurse practitioners/physician assistants, may help to achieve targets in a higher proportion of patients. Measures such as self-management may provide better patient understanding and promote compliance.

**Left ventricular function**

Patients with ESRD already have a high cardiovascular burden at the start of dialysis therapy with LV hypertrophy (LVH) as the most prevalent cardiac alteration 37. LVH and also higher left atrial volume are independent predictors of mortality and cardiovascular outcome in dialysis patients 37-39. Renal transplantation can reverse the structural cardiac changes associated with ESRD 40-42 and reduce cardiovascular mortality, but the cardiovascular risk remains high 43.
There have been conflicting reports concerning the effects of CNIs on the myocardium\textsuperscript{44-49}. An observational study reported worsening of LV diastolic function after renal transplantation, despite LVH regression and improvement in LV systolic function\textsuperscript{41}. The authors speculated that CsA treatment might cause the progression of LV diastolic dysfunction in renal transplant recipients\textsuperscript{41}. In cardiac and renal transplant recipients, replacing the CNI with sirolimus resulted in LV mass regression\textsuperscript{44-45} and improvement in LV diastolic function\textsuperscript{44}. However, in experimental animals, calcineurin activation appeared to be a key player in mediating the development of LVH and calcineurin inhibition could prevent LVH without affecting LV systolic function\textsuperscript{46-48}. Genetic inhibition of calcineurin reduced LVH development in a mouse model, but resulted in LV diastolic dysfunction\textsuperscript{49}.

In the present cardiovascular substudy, deterioration of LV diastolic function, as determined by mitral deceleration time and mitral annular e’ velocity, was prevented by CNI withdrawal. Increase in E-wave deceleration time was significantly associated with MMF withdrawal. Additionally, left atrial volume index, an indicator of chronic LV diastolic dysfunction, significantly increased in the MMF withdrawal group, which was associated with MMF withdrawal and SBP and DBP. These findings indicated that CNI withdrawal may stabilise LV diastolic dysfunction, probably by influencing blood pressure control. However, this study was not designed to discriminate between the indirect effects of better blood pressure control and improved renal function or the direct effect of CNI elimination on the myocardium.

Furthermore, CNI withdrawal had no effect on LVH, despite better regulation of blood pressure. Another possible explanation for this finding may be that only a minority of the study population (22.2% of female patients and 11.1% of male patients) had LVH at baseline and, therefore, it is not surprising that no significant reduction in left ventricular mass was documented. In addition, the use of angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) was high, with the majority of patients (70.4%) using ≥2 antihypertensive drugs at baseline. As the renin-angiotensin-aldosterone system is activated in CNI-treated patients\textsuperscript{50}, treatment with an ACEi or ARB may have suppressed the renin-angiotensin-aldosterone system-mediated cell growth and the development of LVH.

**Intima media thickness**

Intima media thickness of the common carotid artery reflects atherosclerosis and is associated with an increased incidence of cardiovascular and cerebrovascular events in the general population\textsuperscript{51-53}. Consequently, it is increasingly used as a surrogate cardiovascular endpoint in interventional trials and for risk assessment in individual patients. In renal transplant recipients, IMT is increased compared to healthy controls\textsuperscript{54-57}, but lower than in haemodialysis patients\textsuperscript{57-58}. Increased IMT is a risk factor for mortality in renal transplant recipients\textsuperscript{59} and has been associated with inferior graft outcome\textsuperscript{60}.
Previous studies remain inconclusive regarding the progression of IMT long-term after renal transplantation and have reported either a reduction, no change or progression in limited numbers of patients.

Although CNI withdrawal resulted in a significant reduction in ambulatory blood pressure, sequential IMT measurements did not show a significant alteration in IMT during 3 years. Theoretically, the observed difference in blood pressures in this study may have been too small or a longer follow-up period may be required to detect the beneficial effect of blood pressure lowering on IMT progression. However, randomised trials investigating the effect of antihypertensive medication have shown a reduction of IMT progression with smaller blood pressure differences. Furthermore, the reduction in LDL-cholesterol by treatment with lipid lowering drugs (from 3.1 to 2.6 mmol/L, 16% reduction) in this study was not associated with a change in IMT. As intensive lipid lowering is known to reduce the progression of IMT, statin treatment may have contributed to the stable IMT. One small prospective randomised study has evaluated the impact of pravastatin on IMT in renal transplant recipients and found less progression after 1 year (LDL-cholesterol fell from 3.8 to 2.8 mmol/L, 26% reduction).

It may be necessary to include a larger number of patients with a longer follow-up time to draw definite conclusions on the influence of either CNI or MMF withdrawal on carotid atherosclerosis. The sample size calculation of the main study was based on the difference in renal function between the groups. Since data on the rate of IMT progression in patients long-term after transplantation are limited and indefinite and no studies have assessed the impact of CNI elimination on IMT change over time, a sample size calculation based on a change in IMT progression for our intervention would have a high level of uncertainty. A review by Bots provided sample size calculations based on a pooled common carotid IMT progression rate of 0.0147 mm/year with a median SD of 0.053, with a 2-sided α and a power of 90% for a period of 2 and 3 years. The number needed for each arm in a randomised controlled trial, varied from 339 (30% effect in 3 years) to 30 (100% effect in 3 years). Therefore, the current study may have been underpowered to find a smaller effect in 3 years.

**FUTURE PERSPECTIVES**

Late CNI withdrawal, while providing adequate exposure, is a potential approach to reduce CNI-related nephrotoxicity and cardiovascular side-effects, but earlier interventions within the first 6 months after renal transplantation are more preferable, since patients already show features of nephrotoxicity within the first year. There are several potential pre-emptive strategies to decrease CNI exposure:

1. De novo CNI minimisation with MMF.
The CAESAR and Symphony studies have evaluated de novo CNI minimisation under the umbrella of MMF \(^9,13\). The CAESAR study demonstrated that renal function and acute rejection rates were similar in either the low-dose or standard-dose CsA groups at 12 months after transplantation \(^13\). In the Symphony study, the low-dose tacrolimus regimen was superior to the low- and standard-dose CsA regimen with respect to the eGFR and the occurrence of acute rejection at 12 months post-transplantation \(^9\), although the observed benefit in renal function in favour of the low-dose tacrolimus treatment arm did not reach statistical significance at 3 years, suggesting less, but consistent nephrotoxicity, even at low doses of tacrolimus \(^10\). Further dose reduction to very low doses of tacrolimus with concentration-controlled dosing of MMF may be a future strategy, which has yet to be evaluated in randomised controlled trials.

2) De novo CNI minimisation or early CNI elimination with an mTOR inhibitor. This strategy includes the additional benefits on long-term outcome by the inhibition of angiogenesis/vascular remodeling and proliferation associated with the use of mTOR inhibitors (sirolimus and everolimus) \(^68\). They may have the capacity to prevent or delay the progression of CAN-IF/TA at an early stage, as previously demonstrated in rat models \(^69-70\). Additionally, the anti-tumour properties of mTOR inhibitors have been shown in clinical trials, reporting a decreased incidence of new malignancies among patients with sirolimus \(^29,71\). Furthermore, mTOR inhibitors protect against viral infections, which was demonstrated by a decrease in the incidence of cytomegalovirus (CMV) \(^72-74\) and BK virus infections \(^75\) in randomised studies comparing everolimus with MPA in de novo renal transplant recipients. CMV infection may play a role in the development of CAN-IF/TA \(^76\) and BK virus-associated nephropathy is an important cause of CTD \(^77\). There may be beneficial effects on cardiovascular outcome, as de novo everolimus reduced the incidence of cardiac allograft vasculopathy and its associated major cardiac adverse events in comparison to azathioprine in cardiac transplant recipients \(^78-79\). On the other hand, mTOR inhibitors may contribute to the elevated cardiovascular risk in transplant recipients by increasing the incidence of hypercholesterolaemia and new-onset diabetes \(^80\). Finally, mTOR inhibitors could potentially induce immunological tolerance, as indicated by in vitro experiments \(^81\).

Efficacy and safety has been demonstrated for de novo everolimus with CNI minimisation \(^75,82-86\), although renal function did not improve significantly compared to standard dose CNI in several randomised studies \(^75,84-86\), probably owing to relatively small differences in CNI blood levels between the groups \(^84-86\). De novo MPA-based therapy with a CNI followed by early conversion to an mTOR inhibitor with CNI elimination (between month 2 and 6) resulted in improved renal function in recently reported randomised trials \(^87-91\), but was associated with an increase in acute rejection rates \(^87,89-91\). Furthermore, the toxicity of mTOR inhibitors limits their usability. Considerable proportions of patients discontinued mTOR inhibitors in these trials (20-40%) due to various serious ad-
verse effects, including mucocutaneous and gastro-intestinal side-effects, proteinuria, delayed wound healing, bone marrow depression and pneumonitis.

3) CNI avoidance with belatacept.

Belatacept is a CTLA-4 antibody that blocks T-cell co-stimulation by binding to CD80/CD86 on antigen presenting cells. Two phase III multicentre trials, BENEFIT and BENEFIT-EXT, in recipients with extended criteria donor kidneys, evaluated CNI-free more and less intensive belatacept-based immunosuppressive regimens with a standard CsA-based regimen. Although more acute rejection episodes occurred in the BENEFIT study during the first year (more intensive belatacept: 22%; less intensive belatacept: 17%; CsA: 7%) in the BENEFIT-EXT trial, the incidence of acute rejection was comparable in the belatacept-treated and the CsA-treated groups after 1 year. At 3 years, the eGFR was 11 ml/min/1.73 m² higher in the belatacept-treated groups. However, the risk of post-transplant lymphoproliferative disorder, especially with central nervous system involvement, was increased in both studies. Associated risk factors were primary Epstein Barr virus (EBV)-infection, T-cell depleting therapy and CMV disease. Belatacept is therefore only recommended in EBV-seropositive patients. The cardiovascular risk profile improved in the belatacept-treated groups at 1 year, and showed similar trends at 2 and 3 years. One of the limitations of these studies was the high target trough level range of CsA (100-250 ng/ml), which may have worsened renal function in the CsA group.

In recent years, several studies have questioned the dominant role of CNI-related nephrotoxicity in late renal graft failure. The application of the immunological markers C4d and DSA has revealed that chronic humoral alloreactivity is common in transplant recipients with new-onset late transplant dysfunction. In the DeKAF study, patients with for cause biopsies with C4d+ staining and DSA had an increased risk of losing their graft, whereas patients with only CNI toxicity had a lower risk of experiencing graft loss. A retrospective study from the Mayo clinic demonstrated cellular and antibody-mediated alloimmune and autoimmune injury to be important factors in graft loss, whereas CNI-related toxicity only rarely attributed. Two prospective studies following patients after for cause biopsies, indicated antibody-mediated rejection and glomerular disease as the major causes of late allograft failure, while CNIs did not play a role. Nevertheless, chronic CNI nephrotoxicity remains a serious problem, as shown in previous studies and in patients with non-renal transplantations and autoimmune diseases, but questions have risen regarding the consequence of reducing CNIs on chronic (humoral) immunity and what the best immunosuppressive protocol to prevent it would be. Therefore, improving the monitoring of the immunological status has become even more important for the individualisation of immunosuppression.
has been an extensive search for biomarkers that are able to predict rejection or organ tolerance in transplant recipients and could reduce the risk during CNI minimisation or withdrawal. Potential biomarker assays include analysis of gene expression profiles of either rejection or tolerance, assessment of regulatory T-cells, detection of urinary biomarkers of acute rejection (at the protein, peptide or mRNA level) and functional assays to monitor humoral or effector and memory T-cell alloimmune responses. Currently, none of these biomarkers or assays have been standardised and validated in prospective trials.

CNI-induced nephrotoxicity might be reduced by strategies for advanced TDM, including pharmacodynamic analysis and pharmacogenetic testing. Pharmacodynamic monitoring assessing calcineurin enzyme activity and determination of the genotype of drug metabolising genes as CYP3A5 and ABCB1 (encoding the efflux pump transporter P-glycoprotein) could further individualise and optimise CNI dosing. However, at present, the only parameter that may be clinically relevant is CYP3A5 genotyping and solely in relation to the initial dosing of tacrolimus.

In summary, CNI withdrawal using TDM of MPA may result in improved outcome. In this context, the timing of the intervention, early vs. late, and the immunological risk profile appear the most relevant parameters. It is not known whether comparable results can be achieved in patients with a higher immunological risk profile and who will develop DSA.
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Summary and general discussion


