Long-Term Benefits and Risks of Early Conversion from Calcineurin Inhibitors to Mammalian Target of Rapamycin Inhibitors and Steroid Withdrawal

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Abstract

Mammalian target of rapamycin inhibitors are currently considered as an alternative immunosuppressive treatment to prevent nephrotoxicity, viral infections, and malignancies due to calcineurin inhibitor-based immunosuppressive regimens. Short-term results of the CONCEPT study have shown that early conversion to mammalian target of rapamycin in the first six months, in combination with mycophenolate mofetil, provided a renal benefit. This strategy is therefore appropriate for maintenance therapy in renal transplant recipients with a low immunological risk after careful screening at the time of conversion. The five-year results of the CONCEPT study, presented in this review, demonstrate that the renal benefit is maintained without an increased risk of acute and chronic rejection, allograft failure, and mortality.

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Key words

CNI conversion. mTOR inhibitor. CONCEPT. Renal function. Renal graft outcome. CNI nephrotoxicity.

Introduction

During the last two decades, short-term graft and patient survivals have been greatly improved. Among the many reasons which can explain this positive course, the use of calcineurin inhibitors (CNI) appears as a pivotal component[1]. However, long-term significant gains remain poor and disappointing[2].

Serious adverse events associated with the use of CNI can be involved in this setting. Indeed, CNI are considered as a risk factor for cardiovascular events[3] and malignancies, beyond the immunosuppressive effect[4], the two being leading causes of death in kidney transplant patients[5]. Most of all, CNI contribute to the development of chronic graft injuries[6]. In addition, corticosteroids, another component frequently associated with CNI, have been known for a long time to increase the cardiovascular risk of transplant patients[7]. The advent of new immunosuppressive agents, such as mammalian target of rapamycin (mTOR) inhibitors, has allowed to reduce or avoid the use of CNI regimens and consequently to test the hypothesis that CNI contribute to chronic allograft nephropathy[8,9].

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Sirolimus (SRL) binds to the mTOR complex and inhibits immune cell proliferation and differentiation. Pioneering trials of CNI withdrawal from SRL-based therapy has resulted in an improved four-year graft survival with a better renal function[10], showing that maintenance therapy with SRL and mycophenolate mofetil (MMF) was safe and effective, thus paving the way for conversion strategies. However, the CONVERT and ASCERTAIN[11-13] studies have shown that the renal benefit in late conversion strategies is restricted to patients with good renal function and weak proteinuria before CNI withdrawal, irrespective of the mTOR inhibitor used. These data lead to assessing the safety and efficacy of early conversion to SRL, before the appearance of renal injuries due to CNI.

Early conversion

Early conversion has been assessed in the CONCEPT study[14]. A total of 235 non-immunized patients transplanted with a kidney issued from a deceased donor received induction therapy with daclizumab and triple therapy with cyclosporine, MMF and steroids for three months. At three months, 192 patients with proteinuria < 1 g/day and glomerular filtration rate (GFR) > 40 ml/min were randomized to continue cyclosporin A (CsA group; n = 97) or be converted to sirolimus (SRL group; n = 95). At 12 months, daily dosages of CsA and SRL were 226 ± 49 (C2: 749 ± 233 ng/ml) and 3.2 ± 1.4 mg/day (C0: 9.6 ± 4.3 ng/ml), respectively. In the two groups, steroids were planned to be discontinued at eight months.

Both groups were similar with respect to demographic and medical characteristics such as donor and recipient age, time of dialysis before transplantation, human leukocyte antigen (HLA) and cytomegalovirus (CMV) matching, incidence of delayed graft function and GFR at one year. Patient and graft survival were excellent, with no deaths and only one graft loss, which occurred in the cyclosporine group.

Renal function at one year

The primary endpoint, defined as the renal function estimated according to Cockcroft Gault at one year, was significantly better in the SRL group (68.9 vs. 64.4 ml/min; p = 0.017). Similar results were observed when GFR was calculated according to Modification of Diet in Renal Disease (MDRD) formula (61.2 vs. 53.9 ml/min; p = 0.002) or measured using iohexol (67.3 vs. 60.3 ml/min; p = 0.004). Despite this improved graft function in the SRL group, interstitial fibrosis quantified in protocol biopsies performed at year one was not reduced in the SRL group in comparison to the CsA group (26.3 ± 14.7 vs. 28.5 ± 16.2%, respectively)[15].

Acute rejection episodes during the first year after transplantation

Patients with clinically suspected acute rejection underwent a renal graft biopsy. After randomization, 43 biopsies (27 in the SRL group and 16 in the CsA group) were performed and centrally reassessed. The incidence of patients with biopsy-proven acute rejection (BPAR) was higher in the SRL group although not significantly different (17% vs. 8%; p = 0.071). The majority of BPAR in the SRL group occurred late after randomization while steroids were withdrawn, in contrast to those diagnosed in the CsA group where BPAR occurred mainly in the first month following randomization. At the time of rejection, mean serum creatinine was higher and creatinine clearance was lower in the CsA group than in the SRL group (174.1 ± 25.9 vs. 141.8 ± 54.5 µmol/l and 51.6 ± 7.8 vs. 62.4 ± 19.9 ml/min, respectively). All BPAR were mild in the SRL group, while five BPAR with grade ≥ 2 were observed in the CsA group. In addition, one patient

received antithymocyte globulins in the SRL group compared to three in the CsA group. After the rejection episodes, six patients in the SRL group and two in the CsA group were switched to tacrolimus. Interestingly, creatinine clearance at week 52 was similar in patients with or without previous episodes of acute rejection in the SRL group (67.2 ± 20.5 ml/min vs. 69.3 ± 17.5). Nevertheless, subclinical inflammation lesions, defined by borderline changes or subclinical acute rejection, were more frequently observed in protocol biopsies at one year in the 62 patients of the SRL group than in the 59 patients of the CsA group (45 vs. 15%; $p < 0.001$) as shown by Thierry, et al.[16]. Interestingly, subclinical inflammation lesions were observed in six of the nine patients of the SRL group who had presented a BPAR, even if BPAR between randomization and year one was not a risk factor for subclinical inflammation ($p = 0.126$). Protocol biopsy at one year could be therefore interesting to identify the subgroup of patients with potentially worse outcome.

Endothelial parameters at one year

Aortic stiffness and biomarkers of endothelial activation were studied in 44 of the patients enrolled in the CONCEPT study[17]. At one year after transplantation, the carotid-to-femoral pulse wave velocity was significantly lower in the SRL group. In parallel, plasma levels of endothelin-1 decreased in the SRL group during the study, suggesting a beneficial effect of SRL in preventing the development of cardiovascular complications after kidney transplantation.

Adverse events

The incidence of adverse events (stomatitis, acne, diarrhea, high triglyceride levels) was slightly increased in the SRL group (60 vs. 44%; $p = 0.025$) and more patients discontinued SRL (16 vs. 7%). Interestingly, hemoglobin, cholesterol, and proteinuria were similar in both groups. The number of patients with proteinuria > 0.5 g/day was also similar in both groups (12% in the SRL group and 9% in the CsA group). Some of the adverse events needed clinical adaptation of the daily dose of MMF (1.7 g/day in the SRL group vs. 1.9 g/day in the CsA group; $p < 0.001$). Therefore, conversion from CsA to SRL combined with MMF three months after transplantation was associated with an improvement in renal function with a good risk-to-benefit ratio.

Long-term outcomes

Nevertheless, long-term clinical outcomes studies are necessary to confirm the short-term benefits of early CNI withdrawal[14]. Therefore, five-year data of 135 patients (SRL 65 vs. CsA 70) who have been enrolled in the post-CONCEPT study (48-month results available in 156 patients) have been recorded and are presented in this review, extending the previously reported four-year results[18]. Four deaths (SRL: 2, CsA: 2) and three graft losses (SRL: 2, CsA: 1) occurred during the first four years after randomization. During the fifth year, no graft loss but three deaths occurred (SRL: 1, CsA: 2). Thus, patient and graft survivals of randomized patients were 97.0 and 94.7% in the SRL group and 95.8 and 94.8% in the CsA group, respectively. The benefit on renal function in the SRL group, observed at one year, was maintained over the five years (Fig. 1). Renal function was significantly better in the SRL group in the intent-to-treat and on-treatment populations. Five-year mean GFR, estimated according to MDRD formula, was 59.1 vs. 49.3 ml/min ($p = 0.0012$). Interestingly, this difference was more pronounced in patients who remained at five years in their randomized arm, with a 14.9 ml/min difference. Moreover, a negative GFR slope with a progressive deterioration of renal function was observed in patients who received CsA, but

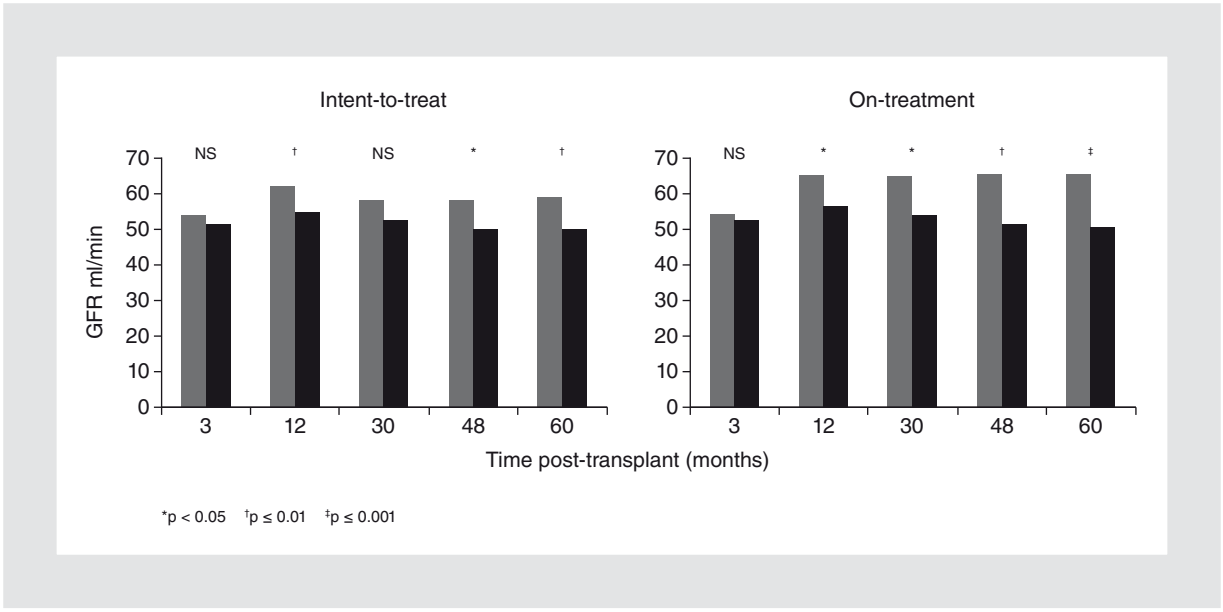

Figure 1. *Renal function at five years. Intent-to-treat (left panel) and on-treatment (right panel) estimated glomerular filtration rate according to Modification of Diet in Renal Disease in 135 patients from the CONCEPT study with available data at 60 months post-transplantation. *p < 0.05; †p ≤ 0.01; ‡p ≤ 0.001. NS: non significant; GFR: glomerular filtration rate.*

not in the SRL group, although the mean daily CsA dose was 170 ± 40 mg, with a mean C2 level of 527 ± 310 ng/ml. The occurrence of late BPAR after one year was low and similar in each group (SRL: 2, CsA: 6). Interestingly, no rejection was observed in both groups between 48 and 60 months, while the percentage of steroid-free patients was higher in the SRL group (73 vs. 61%). Among the 151 patients (SRL: 74, CsA: 77) screened for HLA antibodies by a Luminex method (One Lambda®) after transplantation, 33 (21%) had *de novo* HLA antibodies during the follow-up (SRL: 12, CsA: 21). No difference was observed between both groups (log rank: p = 0.103).

The 15% increased incidence of discontinuations observed at one year in the SRL group was maintained at five years (40.0 and 44.6% vs. 24.2 and 21.6%, respectively), with an increased incidence of side-effects such as edema, stomatitis, pneumonia, and pyelonephritis. Among 16 patients who developed malignancy during the follow-up, 12 received CNI at the diagnosis (nine were randomized in the CsA group and three patients were converted to CNI before malignancy diagnosis in the SRL group). Risk of new-onset diabetes after transplantation, as suggested at four years, was significantly increased in the SRL group (p = 0.035). Lipid values (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides) and the percentage of patients receiving lipid-lowering agents were similar at five years in the two treatment groups.

There were no differences in hemoglobin values, either in the percentage of anemic patients (defined as hemoglobin < 11 g/dl), or in the percentage of patients receiving an erythropoietin-stimulating agent, between the groups. However, mean red blood cell counts were higher, whereas the mean corpuscular volumes were lower in the SRL group. Interestingly, mean proteinuria was similar in both groups at five years (0.38 vs. 0.41 g/24 hours). Moreover, the percentage of patients with proteinuria > 0.3 g/24 hours and the percentage of patients treated either with an angiotensin converting enzyme inhibitor and/or an angiotensin receptor blocker was similar in both groups.

Therefore, five-year results of CNI elimination with a SRL plus MMF regimen demonstrated that the renal benefit observed one year after transplantation was maintained and even increased, with a stability of GFR in patients remaining on assigned SRL therapy, contrary to patients remaining on assigned CsA therapy, in which GFR progressively declined. Moreover, fewer malignancies were observed. These benefits were observed despite more SRL discontinuations due to early adverse events.

Discussion

Protocols of CNI early withdrawal with conversion to mTOR inhibitors in the maintenance phase have been performed in order to:

– Achieve the one-year optimal renal function, as long-term graft and patient survivals have been associated with one-year renal function[19-21]. A 10 ml/min decrease in GFR at one year is associated with a 2.1 odds ratio of kidney allograft loss three years after transplantation[22].

– Reduce the incidence of viral infection, as previous studies have shown a low incidence of CMV infections in SRL-treated patients in comparison with CNI-treated patients[23]. A recent meta-analysis has shown that mTOR-inhibitor treatment, either alone or in combination with CNI, significantly reduced the CMV incidence after organ transplantation, suggesting that, with the use of mTOR inhibitors, CMV prophylaxis may be dispensable[24]. Furthermore, a significant increase in CMV-specific $CD8^+$ T-cell count has been observed in everolimus-treated renal recipients compared to cyclosporine-treated patients[25], and it has recently been reported that functional mTOR was essential for CMV replication, suggesting a direct antiviral effect of mTOR inhibitors[26]. A study has similarly suggested that mTOR inhibitors also reduce the incidence of BK virus infection after transplantation[27]. We could hope that mTOR inhibitors prevent indirect effects of CMV, including long-term graft dysfunction[28].

– Decrease the incidence of malignancies. This aim is supported by several studies, which have shown that mTOR inhibitor regimens could reduce the incidence of neoplasia[29]. Moreover, it has recently been shown that conversion from CNI to SRL in kidney transplant patients following a first skin cancer episode prevented its recurrence[30]. The mTOR inhibitors have mTOR anti-neoplasic properties[31,32] in contrast to CNI, which may induce cancer progression through mechanisms independent of host immunity[4].

The five-year results of our study demonstrated that most of these endpoints have been achieved in the CONCEPT study. The renal benefit observed at one year is maintained and even extended at year five after transplantation, in absence of renal function decline in the SRL group contrary to the CsA group. As conversion was performed at three months after transplantation, no difference between the two groups was observed for CMV and BK virus infection, in contrast with results observed when SRL was used just after transplantation[23].

Other studies have confirmed the CONCEPT study results in terms of renal function, irrespective of mTOR inhibitor used. So, a one-year renal benefit of early conversion from CNI to mTOR inhibitors has been observed with either SRL[33,34] or everolimus[35]. In the Spare-the-Nephron trial[34], 299 patients were randomized at one to six months after transplantation (mean 3.8 months) to continue CNI or to convert to SRL (cyclosporine, n = 31; tacrolimus, n = 120). After one year, the mean percentage change from baseline of measured GFR was significantly higher in the MMF/SRL

group compared with the MMF/CNI group (24.4 vs. 5.2%; p = 0.012). The GFR, calculated according to Nankivell formula, was higher in the SRL group, but the difference was not significant (74.6 vs. 71.5 ml/min). In the SMART study[33], 161 patients with a low-to-moderate immunological risk were randomized at 10 to 24 days after transplantation to be converted to SRL or to continue CsA. The primary endpoint, renal function estimated at one year according to Nankivell, was significantly better in the SRL group (64.5 vs. 53.4 ml/min; p = 0.0019). In ZEUS[35], 300 patients were randomized at 4.5 months to continue CsA or to be converted to everolimus. At one year, the everolimus regimen was associated with a better renal function evaluated according to Nankivell (71.8 vs. 61.9 ml/min; p < 0.0001). Finally, similar results were reported in the HERAKLES study at the last meeting of the American Congress of Transplantation. These studies, assessing substitution of an mTOR inhibitor for cyclosporine (SMART, ZEUS, HERAKLES), show the renal benefit at one year (about 8-10 ml/min) was similar to that observed in CONCEPT, whereas it was reduced with tacrolimus[34,36].

Nevertheless, concerns have been raised concerning the risk of acute and chronic rejection[37,38]. The percentage of BPAR at one year was low and similar in both groups in two randomized clinical trials previously described (11.3 vs. 9.5% in SPN, 17 vs. 16% in SMART). The CONCEPT study reported a non-significant increased risk of acute rejection in the SRL group (17 vs. 8%; p = 0.07). Most rejection occurred following steroid withdrawal at eight months and not just after randomization. An increased risk of acute rejection following steroid withdrawal has already been reported in CNI-based regimens[39,40]. However, the impact of steroid withdrawal is highly dependent on the training and patients' characteristics. In two randomized clinical trials which have evaluated late (six months after transplantation) and progressive steroid withdrawal in patients receiving MMF and cyclosporine[41,42], the incidence of acute rejection after steroid withdrawal was not increased. Moreover, a significantly increased incidence was reported in the randomized period in ZEUS in the everolimus group (10 vs. 3%; p = 0.04), while steroids were maintained in each group (mean dose: 7.9 and 9.3 mg/day in the CNI and SRL groups, respectively). Taken together, these results show that steroids may be withdrawn in mTOR inhibitor regimens.

A recent meta-analysis[38] has reported that the use of mTOR inhibitors with MMF increased the risk of graft failure (OR: 1.43 [1.08-1.90]; p = 0.01). Nevertheless, randomized clinical trials assessing conversion strategies have not been included in this study, which focused on avoidance strategies using mTOR/MMF combination. A US registry study has also reported worse patient and graft outcome in patients receiving mTOR inhibitors in their primary immunosuppressive regimen. This study is in accordance with the meta-analysis previously described and highlights that early conversion from CNI to mTOR inhibitors is safer and has more efficacy than *de novo* full CNI-avoidance strategies. In addition, risk of *de novo* immunization was similar in both groups in the CONCEPT study, in contrast to results reported by Liefeld, et al.[43], and the risk of anti-HLA antibodies appearance remains uncertain.

Conclusion

Early conversion to mTOR in combination with MMF could be an appropriate strategy for maintenance therapy in renal transplant recipients with a low immunological risk after careful screening at the time of conversion. Five-year results of the CONCEPT study are rather reassuring, without increased risk of anti-HLA antibodies appearance and graft loss. Nevertheless, whether the benefits observed in these trials could influence long-term graft and patient survivals remains to be determined.

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