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REVIEW

Immunological risks of minimization strategies

Renaud Snanoudj,[1,2] Claire Tinel[1,2] and Christophe Legendre[1,2]

1 Université Sorbonne Paris Cité, Paris, France
2 Service de Néphrologie – Transplantation, Hôpital Necker, Assistance Publique-Hôpitaux de Paris, Paris, France

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Correspondence
Dr. Renaud Snanoudj, Service de Néphrologie – Transplantation, Hôpital Necker, 149 rue de Sèvres, 75015 Paris, France.
Tel.: 33 1 44 49 54 32;
fax: 33 1 44 49 54 30;
e-mail: renaud.snanoudj@nck.aphp.fr

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Summary

During the past 10 years, minimization strategies have been legitimately initiated to decrease the many toxicities of calcineurin inhibitors, especially nephrotoxicity which was considered to be responsible for the majority of graft losses. Even though CNI-induced nephrotoxicity is undeniable, we have learned in the past 10 years that DSAs detected with solid-phase assays are excellent prognostic biomarkers in kidney transplantation (and in other organ transplantations as well) and that chronic antibody-mediated rejection has become the leading cause of graft loss. In this review, we will focus on the immunological risks linked to various strategies aiming at decreasing CNI doses either at time of transplantation or later in the course of follow-up. Some of these interventions are associated with an increase in acute cellular rejection rates but also with an improvement in renal function. The effects on antibody-mediated rejection and occurrence of *de novo* donor-specific antibodies are still under-reported. We are currently missing long-term data to appreciate the influence of these minimization strategies on graft and patient survival. This then leads to a cautious attitude regarding reducing immunosuppression.

Introduction

In his landmark paper in 1984 [1], Brian Myers stated 'We recommend that cyclosporine be used with restraint and caution until ways are found to mitigate its nephrotoxicity'. Since that time and during the next two decades, various strategies were designed to avoid or decrease the nephrotoxicity of calcineurin inhibitors (CNIs).

In this review article, we chose to focus on the immunological risks linked to these strategies: the incidence of acute rejection, the consequences regarding graft survival and the occurrence of *de novo* donor-specific antibodies (DSAs). However, it is important to stress that in most studies, the follow-up time was rather short, and *de novo* DSAs have not been examined until recently. Therefore, it is only possible to draw conclusions regarding these minimization strategies based on short-term or, at best, mid-term consequences.

Global results with primary avoidance or minimization of CNI

Sharif *et al.* [2] recently performed a meta-analysis of 56 randomized clinical trials, providing data for 11 337 renal transplant recipients. This study gave an overview of three early CNI-sparing strategies, that is CNI avoidance, CNI minimization and the delayed introduction of CNI (Table 1). The strength of such a large meta-analysis allowed it to assess hard endpoints and graft and patient survival rates, whereas the individual studies were underpowered to address these outcomes. They found no difference between standard and reduced CNI exposure regarding overall graft failure (OR: 1.05 [95% CI: 0.85–1.29], $P = 0.66$). However, these studies were short in length, ranging from 3 to 36 months at the longest. In this review, we will focus on the two strategies of minimization that were associated with a reduced risk of graft failure, that is the CNI avoidance based on newer immunosuppressants

Table 1. Strategies of CNI minimization.

1. Primary CNI avoidance:
   a. Steroids with azathioprine or MMF monotherapy
   b. Steroids with concomitant MMF and mTOR inhibitors
   c. Steroids with concomitant MMF and newer immunosuppressants (mainly Belatacept)
2. Primary CNI minimization (low-dose CNI-based immunosuppression):
   a. With MMF
   b. With mTOR inhibitors
   c. Induction with delayed introduction of CNI
3. Secondary CNI lowering or withdrawal:
   a. In patients with stable renal function
   b. In patients with deteriorating renal function
   c. In patients with a newly diagnosed cancer
4. Secondary CNI conversion:
   a. Early with mTOR inhibitors
   b. Late with mTOR inhibitors or belatacept
   c. In patients with cancer

– mainly belatacept – and the use of low-dose CNI in combination with either mycophenolate mofetil (MMF) or mTOR inhibitors.

Regarding the overall risk of acute rejection, CNI-sparing strategies were associated with increased acute rejection rates compared with CNI-based regimens (OR: 1.24 [1.01–1.53], $P = 0.04$). In light of the observed heterogeneity, further subanalyses showed that azathioprine or MMF monotherapy was the sole strategy associated with increased acute rejection rates compared with CNI-based regimens (see below, OR: 2.34 [1.40–3.91], $P = 0.001$).

Regarding other outcome endpoints, there was no effect of reduced CNI exposure on mortality in the pooled analysis. The improvement of renal function, which is one of the goals of CNI-sparing strategies, was observed in the pooled analysis and with all of the individual strategies except for azathioprine/MMF monotherapy [2].

Results with the different strategies of immediate CNI avoidance

Azathioprine or MMF monotherapy

In the study by Sharif *et al.*, no difference in overall graft failure was apparent when azathioprine or MMF monotherapy was compared with the CNI-based regimens [2]. However, death-censored graft failure due to acute rejection was more common in the azathioprine or monotherapy arms. In all transplant centres, there are 'champions' of longevity, that is patients who were transplanted in the 70s or the 80s before cyclosporine A (CsA) introduction and who have maintained a functioning graft with good renal function after this treatment. Indeed, patients who do not experience acute rejection (AR) may have a long graft survival with good renal function. However, these protocols were specifically hampered by a high rate of AR ranging from 56% to 100%, even with the use of antilymphocyte induction therapy [3–7]. Many of these studies were conducted in the 80s. The comparator groups were often CsA monotherapy alone or in combination with steroids. These two factors may explain why the global allograft survival was similar in the meta-analysis by Sharif. Those high AR rates and the incapability to identify patients at a low risk of rejection may explain why these strategies of avoidance have been abandoned.

Combination of mTOR inhibitors and mycophenolate

The combination of mTORI and MMF was the sole strategy associated with increased overall graft failure (OR: 1.43 [1.08–1.90], $P < 0.01$) compared with CNI-based regimens [2]. This result was not explained by an increased rate of acute rejection, whereas many individual studies found an increased rate of AR.

Of the 16 studies analysed, only one study individually demonstrated a shorter graft survival: the Symphony study, in which the sirolimus/MMF group was compared with the tacrolimus group [8]. However, the objective of the trough levels for sirolimus was low and ranged from 4 to 8 ng/ml. Consequently, in this study, the rate of acute rejection was significantly higher (35% at 6 months). This target of sirolimus trough levels is not recommended in combination with MMF, which may explain the poor results observed.

One of the drawbacks of this *de novo* strategy is the necessity to reach rapidly sufficiently high trough levels of mTOR inhibitors in combination with mycophenolate; these levels must be much higher than in combination with CNI. Whether a loading dose is used or not, this results in an increased frequency of adverse events, including early post-transplantation period wound-healing complications. IMPDH inhibitors, such as mycophenolate, are also potent inhibitors of fibroblast proliferation. Several studies reported a significantly higher rate of incisional hernias, wound healing or lymphoceles with sirolimus/MMF compared with CsA-MMF [9–12]. This combination is also hampered by a twofold increased [2] risk of treatment withdrawals (40–50% in studies) because of the poor tolerance of this combination [9,13–15]. This results in frequent within-study crossover that may explain the difficulty in demonstrating a reno-protective effect.

In summary, these data do not support the use of this combination immediately following transplantation and lead to the conversion from CNI to mTORI 3 months post-transplantation.

Costimulation blockade with belatacept

In the study by Sharif *et al.* [2], the combination of MMF with newer immunosuppressants, that is belatacept or

tofacitinib, was associated with a benefit in terms of reduced overall graft failure. Because the development of tofacitinib was arrested in kidney transplantation, we will focus on the results obtained with belatacept.

The costimulation blockade with belatacept is associated with interesting immunological properties, including its effects on Tregs and on the antibody response. The B7/CD28-CTLA4 pathway has been shown to be critical for the activity of Tregs [16]. Although belatacept does not induce Treg expansion *in vivo,* an analysis of graft biopsies showed that the intragraft numbers of FoxP3+ Tregs among infiltrating CD3+ cells were significantly increased during acute rejection episodes in patients treated with belatacept compared with CNI-treated patients [17]. This has been suggested to favour recovery from rejection episodes [18]. The clinical effect of belatacept on Treg generation and function requires further investigation.

The effect of belatacept on the antibody response has also been assessed. Contacts between T follicular helper cells and B cells are critical for antibody production [19,20]. A number of surface signalling molecules, primarily CD40:CD40L but also CD28:B7, which is targeted by belatacept, play a critical role in this immunological synapse [21].

CTLA4-Ig was shown to block the T cell-dependent antibody response and chronic rejection in rodent [22] and non-human primate [23,24] models.

Results of clinical trials with belatacept

In the phase II trial, the rate of clinically suspected biopsy-proven AR (BPAR) after 6 and 12 months of maintenance immunosuppression with belatacept was comparable (7%) to that with cyclosporine, and the two agents resulted in similar patient/graft survival at 1 year [25].

Two large phase III studies with a similar design were then conducted: one, the BENEFIT study ($n = 666$), involved kidney transplants from standard donors [26], whereas the other, the BENEFIT-EXT study ($n = 543$), involved transplants from ECD donors [27].

At month 12 in the two studies, both belatacept regimens had similar patient/graft survival rates compared with cyclosporine (Table 2) and were associated with superior renal function until 3 years after transplantation [28,29].

Concerning the long-term follow-up of patients treated with belatacept, the benefit regarding renal function in the phase II trial was sustained at 5 years [30] and even at 10 years (in a little monocentric experience on 20 patients [31]), compared with cyclosporine. Similar results were observed at 5 years in patients who entered at 3 years the two BENEFIT Long-Term Extension studies: in the BENEFIT study, mean calculated GFR was 74 and 76 ml/min/1.73 m^2 in the two belatacept groups versus 53 in the cyclosporine one [32]; in the BENEFIT-EXT study, GFR was 56 and 59 ml/min/1.73 m^2 in the two belatacept groups versus 45 in the cyclosporine one [33]. Rates for infections and malignancies during extension studies were generally similar across belatacept and cyclosporine groups.

Table 2. Results of the two BENEFIT studies.

	BENEFIT			BENEFIT-EXT		
	MI	LI	CsA	MI	LI	CsA
12-Mo patient survival*,†	95	97	93	86	89	85
AR 12 moth	22	17	7	18	18	14
DSA 12 month whole group	3	1	7	n.a.	n.a.	n.a.
DSA after AR	5	0	7	n.a.	n.a.	n.a.
3-year patient survival†	92	92	89	80	82	80
AR at 3 years‡	27	22	14	22	24	23
DSA at 3 years‡	6	5	11	7	6	15
DSA at 3 years after AR	12	8	19	9	6	26

MI, belatacept with a more intensive regimen; LI, belatacept with a less intensive regimen; AR, acute rejection; DSAs, donor-specific antibodies.
*All values in the table are percentages.
†Patient survival with a functioning graft.
‡Cumulative rate of BPAR at 3 years.

The rates of acute cellular rejection – particularly grade II – were higher in the belatacept groups and did not meet the noninferiority criteria in the MI group compared with CsA (BENEFIT) (Table 2). This difference was observed at 3 years in the BENEFIT study but not in the BENEFIT-EXT study.

Furthermore, in the BENEFIT study, of the patients with acute rejection by month 12, 3 of 48 and 3 of 39 patients had lost their graft in the belatacept arms compared with 1 of 16 patients in the cyclosporine group.

Indisputably, belatacept has become a drug of choice in the immunosuppressive armamentarium. Its efficacy associated with the lack of nephrotoxicity is particularly useful for ECD kidneys, primarily or secondarily in case of severe dysfunction. However, the rate of high-grade acute cellular rejection reported in the above-mentioned studies as in our experience of switch from CNI for severe dysfunction is worrying. Associated drugs should be used at full dose, and a biopsy should be systematically performed before considering a conversion.

BENEFIT studies are poorly documented with respect to antibody-mediated rejection. The rate of DSA under belatacept seems reassuring and low compared with cyclosporine (Table 2) even if data are imprecise. Moreover, the diagnosis of ABMR or mixed rejection is never evoked, particularly those with a high grade associated with DSA [34]. This is probably because the study has been designed before edition of BANFF criteria. We personally observed cases of ABMR after early conversion from CNI to belatacept. In case of early conversion, doses used for late conversions [35] should probably be increased to fit with the *de novo* therapeutic schema. There is a need for studies with

modern tools (single antigen beads, histological diagnosis of ABMR) on the real risk of belatacept-based regimen regarding ABMR, particularly in comparison with tacrolimus. Indeed, patients at higher risk of ABMR may receive ECD kidneys and suffer from CNI nephrotoxicity.

Eventually, belatacept is the sole maintenance immunosuppressive drug used at fixed doses, with no currently available monitoring tool, either pharmacokinetic or pharmacodynamic. Even if the rate of infections and malignancy is similar in the long-term compared with cyclosporine, belatacept use is associated with a high rate of infections, particularly viral infections. Safety and efficacy of all immunosuppressive drugs have been improved by therapeutic monitoring. Given the very long half-life of belatacept, developing such tools should be very useful.

Results with the different strategies of CNI minimization

Delayed introduction of CNI

The goal of the delayed introduction of CNI after the first postoperative week is to avoid the additive deleterious effect of ischaemia–reperfusion injury and CNI toxicity.

Despite a reduction in delayed graft function, no effect of delayed CNI introduction on overall graft failure or death-censored graft failure was demonstrated in the meta-analysis by Sharif *et al.* [2].

Mycophenolate-based protocols of minimization

In most studies, reduced doses of CNI in combination with mycophenolate were not associated with an increased incidence of acute rejection or with a reduced short-term graft survival [2]. At variance, low-dose tacrolimus after alemtuzumab induction was associated with a reduced AR rate compared with tacrolimus standard exposure after basiliximab induction [36]. In many studies, renal function was similar with standard and reduced CNI exposure. This may be explained in some studies by the fact that exposure was not reduced by as much as the protocol indicated [8,37,38]. When a low dose of tacrolimus is compared with standard or reduced CsA, renal function may be better in the tacrolimus group [8,38], as shown in the Symphony study. Indeed, this protocol, which includes anti-IL2-R, steroids, MMF and low tacrolimus, has become the gold standard even if the tacrolimus levels observed (mean of approximately 7 ng/ml) are the upper limit of the target (3–7 ng/ml).

Everolimus-based protocols of minimization

mTOR inhibitors offer the opportunity to minimize CNI and have nonimmune properties that can be beneficial for reducing the risk of malignancies, viral infections and cardiovascular diseases. mTOR inhibitors have also interesting immunological properties that may warrant protection in the context of CNI sparing. Regarding the *in vitro* effect on antibody synthesis, CNI marginally inhibits the proliferation of purified B-cell and immunoglobulin production. In contrast, mycophenolate and mTOR inhibitors profoundly inhibit both B-cell proliferation, the differentiation of B cells into plasma cells and immunoglobulin production [39,40].

In vivo, this effect was not as evident. In stable renal transplant recipients, Struijk *et al.* [41] tested the effect of everolimus on immune responses after vaccination. Treatment with CsA partially inhibited the capacity to mount a primary humoral response and mycophenolate completely abolished this ability, whereas everolimus left this ability largely intact. Recall responses were inhibited only by mycophenolate.

Results of the clinical trials

We will focus on the use of *de novo* everolimus, which has recently been assessed in several studies of CNI minimization and has demonstrated a good efficacy/safety profile. The first pivotal studies showed that everolimus was safe and efficacious in the *de novo* setting in association with CNI standard exposure [42,43]. However, in the everolimus groups, creatinine clearance remained stable during the 3-year follow-up, but constantly lower compared with the control group (mycophenolate, CsA) [42]: this supports a recommendation for lower CsA exposure to avoid the potential of CNI nephrotoxicity.

Two similarly designed randomized phase III studies (A2306 and A2307, the latter including basiliximab induction) compared the efficacy and safety of everolimus initiated at 1.5 or 3 mg/day (and maintained at >3 ng/ml) in combination with a low CsA exposure [44]. BPAR occurred in 25.0% and 15.2% of patients in the 1.5 and 3 mg/day groups in the A2306 study and less frequently after induction in the A2307 study in 13.7% and 15.1% of patients, respectively. The incidence of BPAR was significantly higher in the patients with an everolimus trough <3 ng/ml. There were no significant between-group differences in the composite endpoint of BPAR, graft loss or death [44,45].

Another minimization study with two regimens of everolimus (standard with low-dose CsA and a higher everolimus exposure with a very low dose of CsA) found similar and low rates of BPAR (11.9% and 14.7%, respectively) [46].

These three studies lacked a control group with a standard-dose CNI. Recently, Cibrik *et al.* [47] published a 24-month phase IIIb trial of 833 *de novo* transplant recipients randomized to everolimus (with two trough concentrations of 3–8 or 6–12 ng/ml) plus reduced-CsA exposure or mycophenolate plus standard-CsA exposure. The composite efficacy failure rates (treated BPAR, graft loss,

death or loss to follow-up) were similar in the two everolimus groups (32.9% and 26.9%) and in the mycophenolate group (27.4%) at 24 months. Renal function was also similar in the three groups. The separate rates of treated BPAR were also similar: 19.9%, 15.1% and 19.1%, respectively. Grade II or III BPAR was observed in 5.0%, 4.6% and 7.9% of patients in the three groups, respectively. C4d staining was performed in approximately 30% of the patients and was positive in 4.7%, 0.7% and 3.6% of the patients, but no clear incidence of ABMR was available. Adverse events leading to discontinuation were more frequently reported in patients receiving 3–8 and 6–12 ng/ml everolimus (28.5% ($P = 0.03$ vs. mycophenolate) and 30.6% ($P = 0.007$ vs. mycophenolate), respectively) than in patients receiving mycophenolate (20.5%).

Two studies have been designed to explore the combination of everolimus with two regimens of tacrolimus. In the study by Chan *et al.*, 14% of the patients in each cohort experienced low-grade BPAR [48]. The small difference in tacrolimus exposure between the two arms was a major limitation of this study. In the ASSET study, the trough targets were 4–7 ng/ml during the first 3 months and 1.5–3 or 4–7 ng/ml thereafter [49]. The primary endpoint, which was to demonstrate a superior estimated glomerular filtration rate at month 12 in the low tacrolimus group, was not achieved (mean eGFR: 57.1 vs. 51.7 ml/min/1.73 m^2), most likely because of the overlapping of achieved tacrolimus exposure levels. Over the total treatment period of 12 months, the rates of efficacy failure (BPAR, graft loss, death or lost to follow-up) were higher in the tacrolimus 1.5–3 ng/ml group: 27.1 versus 12.0% ($P = 0.006$), but these differences were primarily observed prior to the month 4 randomization.

In summary, the safety and efficacy of low CNI/everolimus is now well supported. Each drug allows a reduced exposure of the other and therefore a better tolerability while ensuring a good efficacy. To date, the association between everolimus and CsA is the most studied relationship and has been compared to a control group and to everolimus/tacrolimus.

Secondary CNI lowering or withdrawal

This third category of minimization corresponds to a secondary decrease in the CNI dosage or withdrawal after transplantation either in stable patients or in patients with chronic allograft dysfunction. The CNI dose decrease/withdrawal may be the only modification performed in the immunosuppressive regimen, or it may be combined with the introduction of another immunosuppressive drug (particularly MMF or mTOR inhibitors). The purpose of these designs was not to convert the CNI to another drug but to allow safer decreases/withdrawals of CNI.

In patients with stable renal function

The issue of elective CNI withdrawal (primarily CsA) was raised as early as the 1990s, as outlined in the meta-analysis by Kasiske *et al.* in 1993 [50]. The use of cyclosporine was associated with a reduced number of acute rejection episodes. However, the question of improved graft loss was difficult to ascertain because of a small number of controlled trials and the lack of mid- to long-term data. However, a few years later, the same authors concluded from additional data that in contrast to the results obtained from steroid withdrawal studies, CsA withdrawal in selected patients seemed to impart little risk of long-term graft failure [51]. This difference might have occurred because of a higher heterogeneity of the studies of cyclosporine withdrawal. Of note, it was not possible to discern between patients receiving azathioprine or MMF mainly because the number of patients on MMF was too small.

Dantal *et al.* [52] performed an elegant study in which stable patients at 1 year post-transplantation were randomized to regular exposures of cyclosporine or to reduced exposures of half the level of cyclosporine. Clearly, the patients in the reduced exposure group developed less cancer, but at the same time, they experienced a higher incidence of rejection episodes that did not lead to a higher graft loss incidence.

With the introduction and generalization of MMF as part of most immunosuppressive regimens, the question of cyclosporine withdrawal safety was raised again.

Abramowicz *et al.* [53] reported a remarkable study with both a short- and mid- to long (5 years)-term results analysis [54]. One hundred and eighty-seven patients (12–30 months post-transplantation) received a triple immunosuppression over 3 months (steroids, cyclosporine and MMF) and were then randomized to either continue receiving cyclosporine or not. The primary endpoint was creatinine clearance 6 months after complete withdrawal. A significant increase in the acute rejection rate was observed (10.6 versus 2.4%, $P = 0.03$) without a difference in graft loss. At 5 years, patient and graft survival were 93% and 88%, respectively, in the MMF group, whereas patient and graft survival were 95% and 92% in the cyclosporine–MMF group, respectively. Nine grafts were lost to chronic rejection in the MMF group compared with three in the control group. The main conclusion regarding safety was that cyclosporine withdrawal resulted in an increased risk for acute rejection episodes and graft loss as a result of rejection throughout the 5-year study period.

Hazzan *et al.* reported on a study in which patients on the same triple immunosuppression (steroids–MMF–cyclosporine) were randomized at 3 months post-transplantation to either withdrawal of MMF or cyclosporine [55,56]. The incidence of acute rejection was significantly increased

in the cyclosporine withdrawal group with a trend towards an inferior 2-year graft survival. Of note was the higher incidence of C4d deposits at 1 year in the cyclosporine withdrawal group, which most likely indicates a higher incidence of chronic ABMR.

Finally, Ekberg *et al.* [57] reported a study comparing three groups of patients: daclizumab–steroids–MMF–standard cyclosporine dosage, daclizumab–steroids–MMF–low-dose cyclosporine withdrawn at 6 months and daclizumab–steroids–MMF–low-dose cyclosporine without withdrawal. At 12 months, the incidence of biopsy-proven acute rejection was significantly higher in the CsA withdrawal group (38%) versus the low- or standard-dose CsA groups (25.4% and 27.5%, respectively; $P < 0.05$).

From these above-described studies in stable patients, secondary but early withdrawal of cyclosporine appears to be associated with an increased risk of acute and chronic rejection and is inconsistently associated with a decreased graft survival.

In patients with deteriorating renal function

Similar studies were performed in patients with a chronic allograft dysfunction, formerly called chronic allograft nephropathy. A retrospective analysis of the data is difficult because the cause of renal dysfunction was not always precisely defined, particularly the occurrence of chronic ABMR.

In the so-called 'creeping creatinine' study, kidney transplant recipients with deteriorating renal function received MMF and concomitantly were randomized to cyclosporine withdrawal or not [58]. The primary endpoint was the stabilization/reduction of serum creatinine 6 months after the cyclosporine withdrawal. The mean follow-up time post-transplantation was 6.6 and 6.1 years in the two groups, respectively. No rejection was observed during the study period, and renal function improved significantly in the CsA withdrawal arm. However, the results were not obvious for establishing conclusions on the respective roles of MMF introduction and cyclosporine withdrawal.

Conversely, in the study by Suwelack *et al.* [59], patients with chronic allograft dysfunction (mean post-transplantation time of 7 years) who were on steroids and cyclosporine/tacrolimus first received MMF for 1 month and were then randomized to continue receiving CNI or not. This design was able to test the role of both MMF introduction and CNI interruption. These authors clearly demonstrated that CNI withdrawal was safe and that improved renal function was associated with CNI withdrawal and not MMF introduction without CNI withdrawal.

Other studies adopted a different design in patients with a deteriorating function, such as a 50% decrease in the cyclosporine dosage in the study reported by Frimat *et al.* [60]. After a mean post-transplantation time of 6.7 years, the patients were randomized to either maintain the same cyclosporine-based immunosuppression or to undergo a 50% decrease in the dosage of cyclosporine combined with the introduction of MMF. After a total of 96 weeks post-randomization, renal function improved in the MMF group without an increased incidence of rejection. However, after a 3-year follow-up, firm conclusions were not possible because of the small number of patients in the control group [61].

From these studies, in patients with a deteriorating renal function of an uncertain cause, CNI withdrawal was associated with improved renal function, and no increased incidence of acute or chronic rejection suggested that CNI nephrotoxicity was the true cause of renal dysfunction.

In patients with a newly diagnosed cancer

In patients with a diagnosis of cancer, it is or has been current practice to decrease immunosuppression. The consequences of such an attitude have not been carefully explored in the literature. For example, in patients with a PTLD, comparing our experience at Necker hospital (which involves CNI withdrawal without reintroduction) to the Lyon's group experience (which involves low-dose CNI maintenance), a multivariate analysis showed that stopping CNI was a deleterious prognostic factor with regard to long-term graft loss [62,63].

Secondary CNI conversion

This fourth category of minimization corresponds to a secondary conversion from a CNI-based immunosuppression to an immunosuppression based on a non-nephrotoxic drug. This conversion may occur early after transplantation (roughly during the first year) or later in the course of transplantation. The two main drugs used in conversion protocols are mTOR inhibitors and belatacept. An excellent systematic review was recently published regarding the conversion from CNI to mTOR inhibitors [64].

Early conversion

The most important studies regarding early conversion from a CNI to an mTOR inhibitor all showed a significantly increased risk of BPAR compared with the CNI group: 17 versus 8% in the Concept trial [65], 10 versus 3% in the Zeus trial [66] and 27.5 versus 11% in the Central trial [67]. Overall, these results were confirmed by a recently published systematic analysis [64]. It was not possible to draw conclusions regarding long-term graft survival because the majority of studies had a follow-up shorter than 2 years. The risk of drug discontinuation was also

greater in the mTOR inhibitor group. It is important to stress that some studies suggest that early conversion to mTOR inhibitors may be associated with an increased risk of developing donor-specific antibodies and acute and chronic ABMR [68,69]. The role of the drug itself is not clear, but underdosage linked to the search for a better tolerance of the drug may lead to overall underimmunosuppression. Conversely, it is important to note that the conversion was also associated with an improved GFR that may be responsible for better graft survival in a longer follow-up.

Late conversion

Late conversion from CNI to mTOR inhibitors (see refs in [64]) has occurred both in patients with stable renal function and in patients with former chronic allograft nephropathy or suboptimal renal function. Although conclusions may differ regarding the influence of the conversion on GFR, the risk of subsequent rejection was not significantly different. Long-term data on graft survival were unavailable.

Late conversion from CNI to belatacept [35] has also been studied in patients with a stable renal function and a mean follow-up of approximately 20 months post-transplantation. Six of the 84 patients who were converted to belatacept developed a mild-to-moderate centrally confirmed acute rejection compared with none in the control group during the 12-month follow-up without any influence on graft survival.

In patients with cancer

The majority of conversion studies from CNI to mTOR inhibitors have been performed in patients with nonmelanoma skin cancers [70–72]. Most of the studies concluded that mTOR inhibitors decreased the incidence of squamous cell skin cancers without an increased risk of acute or chronic rejection or a decreased graft survival.

With regard to other types of cancers, no data are yet available on both the risk and efficacy of conversion to mTOR inhibitors.

From the conversion studies, it is possible to conclude that the earlier the conversion is, the higher the risk of acute rejection; however, the influence of these rejections on graft survival is still unknown. Data must be generated to precisely determine the risk of DSA occurrence.

A more personal viewpoint as a summary

Minimization strategies have been legitimately initiated to decrease the many toxicities of calcineurin inhibitors, especially nephrotoxicity which was considered to be responsible for the majority of graft losses. Even though CNI-induced nephrotoxicity is undeniable, we have learned in the past 10 years that DSAs detected with solid-phase assays are excellent prognostic biomarkers in kidney transplantation (and in other organ transplantations as well) and that chronic antibody-mediated rejection has become the leading cause of graft loss. In some selected cases (namely early conversions), there is a clear link between CNI minimization and occurrence of *de novo* DSAs further followed by rejection. There is also a strong association between decreased overall immunosuppression, particularly due to noncompliance, and occurrence of *de novo* DSAs. As a consequence, as long as we do not have immunological monitoring allowing us to measure the burden of immunosuppression, it is probably cautious not to minimize immunosuppression without very good reasons such as overt and obvious toxicities or overimmunosuppression-induced infections and cancer. Reducing immunosuppression because of an imperfect safety profile should probably be avoided as long as we do not know what are efficient therapeutical options in case of *de novo* DSA occurrence. DSA monitoring is therefore useful but prevention of its occurrence is clearly a better option! In this review we focused deliberately on the immunological risks of minimization strategies. It would not be fair to forget the many side effects due to other than CNI immunosuppressants because they are responsible for frequent discontinuation of these drugs and increased noncompliance. CNIs have been and still are, in the vast majority of patients, the backbone of our immunosuppressive regimens.

Conclusions

For 30 years, reducing the toxicity, mainly the nephrotoxicity, of CNI has been attempted in many ways, which are called ‘minimization’ strategies. The risks of these strategies have been outlined in this review paper. The primary strategies currently used appear to be safe in terms of rejection risk and may afford a gain of graft survival, even with a short-term design. Secondary modifications of immunosuppression are associated with an increased risk of graft rejection and *de novo* DSA occurrence for conversion to mTOR inhibitors. One must note that these studies included only low-risk patients and should not be extrapolated to high immunological risk patients. Another limitation of the studies presented here is that even the most recent studies did not take into account the definition of ABMR and very rarely examined the occurrence of DSAs. However, the landscape of chronic graft dysfunction has changed significantly over the past 10 years since chronic ABMR has become the primary cause of graft loss. Consequently, it is of utmost importance to balance the risk of underimmunosuppression linked to CNI minimization with the risk of overimmunosuppression and nephrotoxicity.

All of these studies are short- or mid-term studies. The benefits in terms of renal function for many strategies may be associated with a gain of graft survival. However, we are missing long-term data to appreciate the influence of these minimization strategies on graft and patient survival.

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