Immunosuppression minimization in kidney transplantation

Diego Cantarovich[1], Fabio Vistoli[1], Jean-Paul Soulillou[1]

[1]ITERT - Institut de Transplantation et de Recherche en Transplantation, Service de Nephrologie et d''Immunologie Clinique, Nantes University Hospital, Nantes, France

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1. ABSTRACT

Kidney transplantation is considered the best treatment for patients with end-stage renal failure, even in extreme age-groups. Immunosuppression for "life" is, however, mandatory. This chronic, somewhat unselected, inhibition of the host immune system may induce complications, such as cancer and infection, that could counterbalance the benefits achieved by the transplant. In addition, all currently used immunosuppressors have several side-effects, impeding their long-term use. Consequently, drug associations are frequently tested by different centres according to their own practices, resulting in different survival and tolerance profiles. Corticosteroids and calcineurin inhibitors are the cornerstones of current immunosuppressive regimens. However, they are also the main culprits of adverse-events and side-effects encountered after transplantation. Lowering the doses of each drug, or even eliminating them from the immunosuppressive menu, has been evaluated by many groups over the last two decades. This review summarises a huge number of studies dealing with corticosteroid and calcineurin inhibitor minimization, including withdrawal and avoidance trials. It is hard today to propose any practical guidelines on such a controversial topic. Good results are achieved by some groups and bad results by others. The lack of long-term follow-up in randomized studies contributes to this debate. Nevertheless, it seems possible and safe to avoid corticosteroids and/or calcineurin inhibitors in many patients. The application of protocol biopsies as well as new immunological tests to determine the degree of immunosuppression will certainly help transplant physicians to provide more personalized treatment strategies.

2. INTRODUCTION

The discovery and development of immunosuppressive drug therapies has led to the expansion and success of kidney transplantation over the last 50 years . The introduction of the calcineurin inhibitor (CNI) cyclosporine (CsA) into clinical practice in the early 1980s significantly improved one-year cadaver kidney allograft survival from approximately 60% to 85%. Moreover, CsA was the milestone responsible for the growing success of non-kidney organ transplantation. However, even under CsA, therapy the incidence of acute rejection remained high (approximately 50%) (1, 2) and long-term allograft survival improved only marginally (3). The introduction of mycophenolate mofetil (MMF), tacrolimus (TAC), CsA microemulsion, sirolimus (SRL), a new generation of monoclonal antibodies such as the anti–interleukin-2 receptor blockers (IL2r-Ab) daclizumab (DAC) and basiliximab (Bsx), the anti-CD52 monoclonal antibody Alemtuzumab (campath 1H), the polyclonal biologic anti-lymphocyte and anti-thymocyte sera, and the costimulatory blockade drug Belatacept, has provided transplant physicians a wide choice in selecting and minimizing the immunosuppressive protocols (4–10). On the other hand, attempts to obtain a state of "tolerance" using different strategies have been conducted using various immunosuppressive protocols (11-15). The aim of these protocols is not the minimization of immunosuppression but rather the establishment of a tolerant state that would ideally be maintained without immunosuppression. This type of approach will not be addressed in this review due to the radical difference in the approach and targets.The absence of reliable markers to monitor the degree and extent of immunosuppression is the main factor that

prevents progression within the field of minimization, the induction of a state of operational tolerance and the avoidance of malignancy. In addition, different infectious complications, such as BK-virus-induced nephropathy, are now emerging and are directly related to the amount and type of immunosuppression used (16). The threat of cancer is a permanent risk confronted by patients on long-term immunosuppression with around 40% of patients developing some kind of malignancy (primarily skin cancers) by 20 years after transplantation , the main cause of death of kidney recipients with functioning grafts (17, 18). The two most common causes of late graft loss (i.e., more than 12 months after transplantation) are chronic rejection or chronic allograft nephropathy (CAN), and death with a functioning graft (2, 3, 19, 20). Moreover, the majority of the current immunosuppressive drugs are associated with one or more risk factors predisposing to atherosclerotic cardiovascular disease. Corticosteroids and CNI are the most pro-atherogenic drugs (20). The cardiovascular risk of sirolimus is yet unclear (21). Because of their numerous and serious side-effects, the two main classes of drugs that are targeted for drug minimization are corticosteroids and CNIs. We shall therefore focus this review on these two classes of drugs.

3. CORTICOSTEROID MINIMIZATION PROTOCOLS

Nobody would deny that, without corticosteroids, kidney transplantation would not have seen the light of day. Because of their numerous mechanisms of action, effectiveness, low cost and no need for dose monitoring, corticosteroids are still used by the large majority of centers all over the world for induction, maintenance and ongoing rejection therapy. Fortunately, the doses used have been dramatically decreased over the years (mainly since the introduction of CNI) and, in parallel, corticosteroid-related side-effects and adverse events have also been diminished. The list of complications related to the use of corticosteroids is endless; the most frequent being osteoporosis, avascular necrosis, myopathy, cataracts, glaucoma, skin atrophy, weight gain, growth limitation in children, and changes in physical and psychological features. Corticosteroids also promote hypertension, hyperlipidemia, glucose intolerance and diabetes (22–25). Above all, long-term corticosteroid therapy can increase the risk of cardiovascular disease and infectious episodes, the current leading causes of post-transplant morbidity and mortality. Consequently, the question is whether a complete elimination of corticosteroids can be achieved with new immunosuppressive regimens, with the hope of improving patient quality of life as well as surveying and of course maintaining efficient graft function. Unfortunately, there is currently no answer to this question since studies on corticosteroid minimization and elimination focus mainly on the rejection rate rather than on graft and patient survival and quality of life. Since the introduction of CsA in our center in 1981, we systematically decided to withdraw corticosteroids as early as 3 months after transplantation in all kidney transplant patients receiving Thymoglobulin induction and a CsA-Azathioprine maintenance immunosuppression (26). This regimen,

which was ambitious at that time, gave us excellent results with no increased incidence of acute rejection or graft loss. In our experience, more than 80% of patients can be maintained without corticosteroids with a highly acceptable level of 20-year patient and graft survival. Several important issues, however, remain unresolved while planning corticosteroid minimization: timing of corticosteroid withdrawal, advantages and dangers associated with total avoidance, optimal concomitant immunosuppression, requirement for biologic induction therapy and need for immunological monitoring. Although the short-term results are very promising, it is more difficult to determine whether corticosteroid avoidance has a positive or negative impact on long-term outcome.

4. CORTICOSTEROID WITHDRAWAL

4.1. Late corticosteroid withdrawal (3 months post transplantation).

Traditionally, late withdrawal of corticosteroids is considered safer than early (first weeks) withdrawal, although results from recent studies no longer support this notion (27-33). Two well-designed double-blind, randomized trials of late corticosteroid withdrawal with concomitant maintenance therapy consisting of CsA and MMF demonstrated the potential risks and benefits of this minimization strategy (34, 35). The inclusion criteria for the US study were first transplant recipients on CsA, MMF (dose ≥ 2 g/day) and prednisone, without a history of previous rejection and with a serum creatinine of less than 2.4 mg/dl, in other words, the so called "low-risk" patients (34). Patients who satisfied these criteria at 3 months post-transplantation were randomized to continue on prednisone therapy or to withdraw prednisone over 2 months. Two hundred and sixty-six patients had been enrolled when the study was stopped because of an excess rejection rate in the prednisone withdrawal group. One-year after transplantation, the acute rejection rate was significantly higher in the withdrawal group compared with the corticosteroid maintenance group (31% vs. 10%, respectively). The high rejection rate in the corticosteroid withdrawal group was largely the result of a significantly higher rejection rate in African American patients. These patients are well known (due to genetic and social factors) to be a high-risk population for rejection. On the other hand, despite the increased risk of rejection, corticosteroid withdrawal was associated with significantly lower cholesterol levels and required less use of antihypertensive drugs. In the second multicenter trial (Europe, South Africa and Australia), 500 renal transplant recipients were randomized in a double-blind corticosteroid regimen for 6 months with an unblinded 6-month follow-up (35). The CsA and MMF maintenance protocol was similar in the two arms of the study, while corticosteroids were either maintained indefinitely or withdrawn at 3 months. The acute rejection rate was again statistically higher in the corticosteroid withdrawal group at 6 (23% vs. 14%) and 12 months (25% vs. 15%) following transplantation. However, blood pressure, total cholesterol and bone density at 1 year worsened in the corticosteroid maintenance group. The common conclusion of these two trials was that late withdrawal of corticosteroids with concomitant

immunosuppressive therapy consisting of CsA and MMF may result in a greater but acceptable risk of acute rejection (except in African American patients). Importantly, corticosteroid withdrawal was associated with a much better lipid and blood pressure profile. These secondary end-points may be much more relevant than the primary one in future trials. Much better results were reported with a TAC/MMF-based immunosuppression in a randomized, open-label, parallel-group trial (36). The incidence of acute rejection in 279 patients withdrawn from corticosteroids at 3 months was 6% compared to 1% in 277 patients who were maintained on corticosteroids during the first year following transplantation (36). This constant increase in rejection rate observed when corticosteroids were stopped was again confirmed in a meta-analysis performed by Pascual *et al.* including randomized and controlled trials under "modern" CNIs and MMF (34-39). Two large pharmaceutical-sponsored studies, the THOMAS study (40) and the COSTAMP study (41), investigated corticosteroid withdrawal after 3 months from a triple therapy together with TAC and MMF. No difference was found between the withdrawal group and the ongoing corticosteroid group as regards the rejection rate and recipient or graft survivals after 6 and 12 months. The THOMAS study confirmed the reduction of hyperlipidemia, hypertension and new onset diabetes mellitus and a long-term stable renal function in the absence of corticosteroids (40, 42). In addition, increase in bone mineral density, with a corresponding increase in serum osteocalcin, was noted 1 year following corticosteroid withdrawal (43). In a large European-based prospective study, Opelz *et al.* (44) analyzed deceased donor kidney and heart transplant recipients who were withdrawn from corticosteroids no earlier than 6 months following transplantation. Compared to other reports of corticosteroid withdrawal, the numbers of patients (1,015 kidney and 420 heart recipients) and the duration of follow-up (average 5 years) are impressive. In addition to the low rate of acute rejection observed (8.6 vs. 10.2%) the authors concluded that corticosteroid withdrawal significantly improves long-term patient survival, graft survival and death-censored graft survival. The clearest conclusion from this ambitious, multicenter study is that late withdrawal of corticosteroids is safe in the vast majority of Caucasian/European kidney and heart transplant recipients with 59% of the recipients remaining corticosteroid-free after a follow-up of 7 years.

4.2. Early corticosteroid withdrawal.

Very recently, strategies in corticosteroid minimization have favored immunosuppressive regimens in which corticosteroids are withdrawn very early after transplantation (usually in the first week) or completely avoided (27, 29-33, 45, 46). The potential advantages of these newer approaches in corticosteroid sparing are that acute rejection in patients with short-term exposure (or avoidance) to corticosteroids may occur early after transplantation, when renal allograft recipients are monitored closely and frequently. In contrast, late corticosteroid withdrawal is made at a time when visits to the clinic are less frequent and the follow-up course may not be under the direct supervision of the transplant center.

To reduce the risk of rejection and to provide a more effective immunosuppression in the early post-transplant period, trials with early corticosteroid withdrawal or avoidance have been designed to include the use of biologic induction therapy (27, 29-33,). From a theoretical standpoint, early corticosteroid withdrawal may be advantageous compared to chronic use and withdrawal: the host immune response is not modified by short courses of corticosteroids; there is no interference with the classical tolerogenic pathways of the allograft; there is a lack of corticosteroid dependency and the activation of the immune response following discontinuation could be prevented (46-48). Moreover, the side-effects of corticosteroids could be fully prevented (49, 50). One of the first, early corticosteroid withdrawal protocols was performed almost 20 years ago by Stratta *et al.* (51) In this CsA-based trial, corticosteroids were withdrawn two weeks after kidney transplantation. The authors reported no graft loss but a rejection rate of 50%. Interestingly, the authors concluded that early corticosteroid withdrawal was feasible, even though their study failed to find discriminating factors relative to the choice of the optimal recipient for this kind of strategy. More than a decade later, the same findings were described by Matas *et al.* (32, 33) following corticosteroid tapering within 6 days. Their findings were additionally confirmed by Ponticelli *et al.* (52). Irrespective of an increased rate of acute rejection, the long-term graft and patient survivals were not negatively affected by corticosteroid reduction. Fast corticosteroid withdrawal (within 7 days) on a TAC/MMF-based immunosuppression led to a rejection rate of 19% in the first year and an excellent graft and patient survival of 91% and 97%, respectively, after a median follow-up of 51 months (53). Another approach was the comparison of early corticosteroid withdrawal (3 days) with a slow corticosteroid tapering (over 16 weeks) using an induction therapy with DAC/TAC/MMF (54). Neither the number of acute rejections nor patient or graft survival differed. One can thus conclude that with the use of potent co-immunosuppressors like IL2r-mAb and anti-lymphocyte sera (55), corticosteroids can be withdrawn safely during the first days post surgery.

5. CORTICOSTEROID AVOIDANCE

Avoidance of corticosteroids was first practiced by Calne *et al.* in the early '80s (56). He demonstrated the utility of complete absence of corticosteroids in the transplant setting. Tarantino *et al.* (57) compared CsA monotherapy vs. CsA/azathioprine/corticosteroids. A higher number of severe rejections and graft losses were encountered in the CsA monotherapy group. Interestingly, 10 years later, similar outcomes were found in both groups with 50% of the recipients maintained under CsA monotherapy from the beginning (58). This percentage increased to 70% when induction and MMF were added (59). More recently, the ATLAS study compared two corticosteroid-free TAC-based protocols (TAC/MMF or Bsx/TAC) to a standard TAC/MMF/corticosteroid regimen (60). The corticosteroid-free groups showed significantly higher rejection rates than the controls (30 vs. 26 vs. 8%, respectively) but graft and patient survivals were not

different. In the CARMEN study, the same control group (TAC/MMF/corticosteroids) was compared with a DAC/TAC/MMF groups and one with no corticosteroids at all (61). The group without corticosteroids had a rejection rate identical to that of the controls (16.5%) with similar one-year patient and graft survivals . This study also demonstrated that recipients under TAC but without corticosteroids had a significantly lower incidence of new-onset insulin-dependent diabetes mellitus (0.4 vs. 5.4%). This study suggests that induction therapy can replace corticosteroids early after transplantation.Most of the beneficial effects of corticosteroids are related to inhibition of antigen presentation. Their avoidance may not need continuous replacement, but only compensation at the time of initial allograft recognition, as suggested by the CARMEN study. A similar approach was undertaken using the anti-CD52 monoclonal antibody Alemtuzumab (62). In a pilot study a rejection rate of 30% was reported. The rejecting recipients where switched to Alemtuzumab and SRL as the sole maintenance immunosuppression. Patient and graft survival rates were 100% and 97%, respectively, after a follow-up ranging from 3–29 months. In a well planned three-arm, randomized trial (63) (with 30 patients in each arm) induction with Thymoglobulin, alemtuzumab, and DAC was compared. All patients received maintenance immunosuppression with TAC/MMF/steroids, but the alemtuzumab group received half the dose of TAC and no steroids after the first week. In the interim report with a median follow-up of 15 months, there was no difference in patient or graft survival, acute rejection rate or renal function, nor was there any difference in infections or incidence of diabetes or hyperlipidemia. However, 80% of the alemtuzumab group remained steroid-free. Of particular interest in this study was the documentation of regulatory T cells that appeared in a higher proportion of the patients in the alemtuzumab arm. Gallon *et al.* (64) also tested SRL in combination with TAC compared to TAC/MMF, both involving induction with anti-CD25 monoclonal antibody. Acute rejection was 30% in the SRL/TAC group and 18% in the TAC/MMF group, with better graft function in the TAC/MMF group.An original and innovative study combined the elimination of corticosteroids with early discontinuation of CsA and later discontinuation of MMF (65). Ninety-six kidney transplant recipients were randomized into four subgroups of two pilot studies. All patients received induction with Thymoglobulin, SRL and the immunonutrients arginine and oil containing omega-3 fatty acids. MMF was started in standard doses and discontinued by 2 years. CsA was given in reduced doses for 4, 6 or 12 months. The cumulative 1-year acute rejection rate was 14%. At 3 –years, 90% of the recipients were corticosteroid-free, 87% were off CNI and SRL monotherapy was maintained in 57% (65). Attempts to minimize corticosteroid use have also been made in our center since 1986 in the context of simultaneous kidney/pancreas transplantation (66). This is a high-risk patient population in terms of rejection, infection, graft loss and death. To counter-balance the absence of corticosteroids, we systematically treated patients with rabbit anti-thymocyte globulin induction (67) and more recently with IL2r-mAb (68). These strategies were very successful, with an approximately 30% incidence of acute

rejection. With the introduction of MMF, the complete avoidance of corticosteroids was tested in a Thymoglobulin/CyA/MMF corticosteroid-free pilot protocol. A very low incidence of acute rejection (7%) was achieved and 75% of the included patients were able to remain without corticosteroids more than 7 years after transplantation (69). In the same setting, a corticosteroid-free and a late corticosteroid withdrawal (3 months) immunosuppressive regimen were randomly compared (70), again confirming the low acute rejection rate previously reported (4% in both groups). These two sparing strategies accounted for similar patient, kidney or pancreas survival rates at 1, 2 and 3 years (70). The first US experience of a successful prospective corticosteroid-free, maintenance protocol in simultaneous pancreas-kidney transplantation (SPKTx) reported the feasibility of using Thymoglobulin induction in combination with TAC-based maintenance therapy combined with either MMF or SRL (71). Other studies have validated the efficacy of steroid avoidance immunosuppression in SPKTx using other maintenance combinations (70, 72-75). Interestingly, all steroid avoidance immunosuppressive strategies in SPKTx recipients have relied on Thymoglobulin induction. No studies have reported on the use of alemtuzumab induction in conjunction with a steroid avoidance maintenance immunosuppression in SPKTx. Alemtuzumab is a reasonable induction agent to consider in steroid avoidance protocols for SPKTx because it is also a T-cell depleting agent and its efficacy has been demonstrated in tolerance achieving protocols in kidney transplantation (12, 13 ,62, 76-78). Alemtuzumab is a humanized monoclonal antibody that reacts against the CD52 cell surface antigen densely expressed on T- and B-cells, eosinophils and some populations of monocytes, macrophages and dendritic cells (79). Evidence of the utility of alemtuzumab (double-dose) to facilitate steroid-free immunosuppression in kidney transplantation has been described with CyA (77), TAC (12), or SRL monotherapy (13, 62), or based on a more conventional approach using combined TAC/MMF maintenance therapy (78). Recently in a retrospective single center sequential study in SPK transplantation were evaluated two main objectives: (i) to compare two different induction strategies, alemtuzumab and Thymoglobulin, in combination with TAC/SRL maintenance therapy and (ii) to report long-term outcomes in a corticosteroid-free immunosuppressive protocol (80). Overall 1- and 3-year patient and graft survival rates did not differ between patients treated with alemtuzumab and Thymoglobulin. Rejection rates were also nearly equivalent at 1 (6% vs. 2%) and 2 years (8% vs. 5%) for the alemtuzumab and Thymoglobulin group respectively. Interestingly, viral infectious complications were lower in the alemtuzumab group. Previous experience from the Pittsburgh group has shown that steroid withdrawal can be safely accomplished in pancreas transplant recipients maintained on tacrolimus-based immunosuppression without any induction. Steroid withdrawal was associated with excellent patient and graft survival with no increase in the cumulative risk of rejection (81, 82). The safety and benefits of very early withdrawal or complete avoidance of corticosteroids following kidney transplantation needs to be confirmed by long-term data and, most importantly, by designing large trials with end-

points other than acute rejection. Today, CAN would be the most suitable primary end-point. In the interim, transplant physicians should consider using corticosteroid minimization regimens selectively for patients who are at a high risk of complications from corticosteroid therapy: *1-* patients previously treated with corticosteroids; *2-* children with a low immunologic risk; *3-* patients at risk of developing skeletal disease; *4-* patients with atherosclerotic cardiovascular disease; *5-* patients with susceptibility to metabolic disorders; 6- obese patients, 7- diabetic patients, 8- elderly patients and 9- patients suffering from active hepatitis (83).

6. CALCINEURIN INHIBITOR MINIMIZATION PROTOCOLS

The introduction of CsA, the first CNI widely available, into clinical practice in the early 1980s, dramatically improved graft survival at one year following transplantation. This drug was at the origin of the modern era of clinical kidney and non kidney organ transplantation. CsA-based immunosuppression was associated with new post-transplant morbidities, principally chronic nephrotoxicity, that has limited further improvement in long-term outcome (19, 20). While nephrotoxicity has been amply documented and is frequently cited as the Achilles' heel of the CNI-regimens, the proof of inexorable progression of CNI-induced nephrotoxicity remains controversial (84-87). Many factors have been found to play a role in the development of chronic allograft dysfunction, including input factors (donor disease or acute peri-transplant injuries), immunological risk factors (acute rejection episodes), non-immunological factors such as atherosclerotic risk factors (hypertension, dyslipidemia) and, probably, chronic CNI toxicity (1, 2, 88). Determining whether slow progression of graft failure is predominantly the result of immunological or nonimmunological events, and how this syndrome can be treated or prevented, remains challenging and one of the crucial unresolved issues in clinical practice today (89). Over the years, it has become clear that "under-immunosuppression" (whatever its cause) is associated with acute or progressive immunologic injury. In such cases, both cellular and humoral immune mechanisms play an important role in the pathogenesis of so-called CAN. Recently, donor-specific alloantibodies to human class I or II leukocyte antigen (HLA) have been shown to be associated with CAN, possibly reflecting an alloresponsive via the indirect pathway (90–94). It is not unusual to find that post-transplantation production of alloantibodies precedes the clinical manifestations of CAN, further implicating humoral immune mechanisms as a cause of CAN, rather than a consequence (95). In kidney biopsies, the presence of the complement split product C4d appears to be a good *in situ* marker of antibody-mediated rejection (92, 96-98). Although the role of CNI toxicity in the pathogenesis of CAN has been a matter of debate amongst transplant physicians and nephrologists, recent clinical-pathological studies have suggested that CNI nephrotoxicity also contributes to CAN, directly via drug toxicity or indirectly via hypertension and dyslipidemia (88, 99-102). Hence, patients who develop renal dysfunction from CNI

nephrotoxicity are at a greater risk of having a shortened graft half-life. CsA has also been shown to promote cancer progression by a direct cellular effect independent of its effect on host immune cells (103). The effect of CsA dosage on malignancy was initially explored in our center in a prospective, open label, randomized study (104). Two hundred and thirty-one patients were randomized 1 year after transplantation to either the continued use of the standard dose of cyclosporine or a reduced dose of CsA. With a 66-month follow-up period, significantly more patients in the standard dose group than in the low-dose group developed cancers, two thirds of which were skin cancers. Thus, renal toxicity and cancer are considered the two main limitations of chronic CsA use. TAC, the other available CNI approved for clinical use, has some differences as compared to CsA in terms of graft survival, acute rejection and adverse events. Although no difference in 5-year kidney allograft survival was found between these two drugs, kidney function was better in TAC-treated patients. (105). This finding suggests a lower nephrotoxic effect of TAC as compared to CsA. A switch study performed in CsA-treated patients with CAN supported this hypothesis (106). One possible explanation for the reduced nephrotoxicity observed with TAC may be that by inhibiting TGF beta, it induces less fibrosis (107-109). Clinical-histopathological studies using protocol biopsies may also provide key information concerning CsA and TAC-associated efficacy and nephrotoxicity. Preliminary data from recent studies suggest that, as compared to CsA, TAC-based immunosuppression may be associated with fewer inflammatory lesions and less transplant glomerulopathy and interstitial fibrosis. In the setting of biopsy-proven established CAN, the randomized conversion from CsA to TAC improves allograft function, lowers blood pressure, and reduces low-density lipoprotein (LDL) cholesterol compared to CsA continuation, suggesting that this superior profile may translate into improved long-term graft survival (106, 110). TAC also seems to be more effective than CsA in preventing acute rejection. In an initial study comparing TAC to oil-based CsA in 412 transplant recipients of deceased donor kidneys, the incidence of biopsy-confirmed acute rejection was significantly lower in the TAC group (31 vs. 46%) (111). In a multicenter European study including 560 kidney transplant recipients, the rate of biopsy-proven acute rejection was again significantly lower in the TAC group (9.4 vs. 21%) (112). Other studies have also indicated that TAC is associated with a lower incidence of hypertension and hyperlipidemia than CsA (84, 113-116). Conversion from CsA to TAC in stable kidney transplant recipients has been demonstrated to improve the cardiovascular risk profile (113-115). Serum levels of LDL cholesterol, triglycerides, apolipoprotein B, and fibrinogen decrease significantly after conversion from CsA to TAC (114). The Framingham risk score (although not initially designed for use in organ transplant recipients), is also reduced by conversion to TAC. A similar improvement in the cardiovascular risk profile was found in another study, as fibrinogen, total cholesterol, and LDL cholesterol decreased after conversion from CsA to TAC (115). Recently the results of the DIRECT study (sponsored by Novartis Pharma AG) were published. The aim of this

randomized, multicenter trial was to assess the incidence of new-onset diabetes 6 months after renal transplantation in patients receiving CsA microemulsion- (monitoring the 2-hour post-dose level) vs. TAC-based immunosuppression (117). All of the patients received similar co-medication (Bsx/MMF/corticosteroids). The intention to treat population consisted of 682 recipients, the vast majority were nondiabetic at baseline. The primary safety endpoint, post-transplant new-onset diabetes mellitus or impaired fasting glucose at 6 months, occurred more frequently in TAC (35%) than in CsA-microemulsion-treated patients (26%) (117). So far, although TAC seems superior to CsA as regards rejection and graft survival, side-effects such as diabetes can limit its use. It might, however, be worthwhile to keep in mind that the discovery and clinical use of CNI-based therapy was a revolution for all organ transplant recipients. Their use has been a key factor in achieving good allograft outcomes for most patients, and caution should be recommended before CNIs could be abandoned (118). It is important to continue to explore the possibility that lowering CNI dosages or withdrawal after the first months post-transplantation may help to prevent late allograft loss. The optimal dosage and type of CNI for long-term use should be further defined in order to optimize the cardiovascular risk profile, avoid nephrotoxicity and prevent tumors. Thus, serial prospective clinical and histological data (at 1, 3, and 5 years) of current clinical trials will be needed, with additional modern immunologic monitoring to ensure that what appears to be attractive in the short-term will also translate into improved medium-term and long-term outcomes. To be clinically useful, protocol biopsies must drive a beneficial change in therapy. Randomized trials have shown the benefit of protocol biopsies in patients with CNI toxicity . At present, they offer the potential for the diagnosis of intragraft pathological processes before they becoming symptomatic, at a time when they can be potentially treatable, and when the molecular and cellular mechanisms leading to late injury can be appreciated. Once validated in terms of sensitivity, protocol biopsies will be increasingly performed in routine clinical care and as a component of drug trials (119, 120).

7. CALCINEURIN INHIBITOR SPARING PROTOCOLS

CNI sparing is defined as the initial use after transplantation of a standard or low-dose of CNI with subsequent withdrawal. CNI avoidance protocols consist of immunosuppression regimens that completely avoid their use. Currently worldwide, regimens that are most widely used for induction and maintenance immunosuppressive therapy include CNIs. Therefore, the definition of optimal dosage and selection of CNI to be used in kidney transplantation still remains a major topic of discussion. In the "CsA-Corticosteroid-Azathioprine Era", reduction or withdrawal of CsA in stable kidney transplant recipients was associated with unacceptable rates of acute rejection and graft loss. As a consequence, there was a reluctance in most transplant programs to decrease long-term CsA dosages below an average of approximately 4 mg/kg/day, in an effort to avoid immunologic injury with resulting

chronic kidney allograft dysfunction (121). Indeed, it was well recognized that minimization of CNI dosages is a risk factor for poor outcomes (87). Since the introduction of MMF and SRL in 1997–1998 instead of azathioprine in CNI-based triple-drug regimens, there has been a renewed optimism that lowering or discontinuation of CNI might be feasible in stable kidney recipients.

7.1. Recipients with chronic kidney allograft dysfunction

The treatment of established CAN in kidney transplant recipients who are receiving CNI-based immunosuppression remains a challenge, and to date, no clear-cut therapeutic regimen or strategy has been shown to be consistently effective. Recent clinical studies, however, indicate that addition of MMF or SRL with a 30%–50% reduction of CNI dosages might be an effective means to stabilize or even improve kidney allograft function in this setting (122-126). In some reports, complete withdrawal of CNI also appeared to be beneficial and safe, even in the late post-transplant course in patients with established CAN (127). In general, the follow-up in these studies has been relatively short and no prospective serial immunologic monitoring has been reported, so definitive proof of long-term improvement is lacking. Moreover, MMF administration is frequently poorly tolerated (due to gastrointestinal and bone marrow associated side-effects), resulting from an accumulation of drug metabolites secondary to the kidney allograft dysfunction (in the absence of systematic MMF monitoring). Whether the subset of patients with true chronic rejection due mainly to immunological injury (with antidonor antibodies) should be treated preferentially with CNI or not remains to be determined in prospective studies (92, 94, 128). A recent systematic review of randomized trials of conversion from CNI protocols to SRL for chronic allograft dysfunction (129) identified 1,040 patients from 5 randomized trials and 977 patients from 25 non-randomized trials. In randomized trials, conversion to SRL improved short-term creatinine clearance by 6.4 ml/min. In non-randomized trials, renal function improved or stabilized in 66% of cases, creatinine clearance improved (by a mean of 5.7 ml/min) but cholesterol and triglycerides increased (by 20.8 mg/dl and 40.1 mg/dl, respectively). SRL was discontinued in 28-59% of the patients in randomized trials and 17% in non randomized trials because of side-effects. Better knowledge of SRL blood levels may improve the future clinical tolerance of this drug.

7.2. Recipients with stable kidney allograft function 3–12 months after transplantation

As already mentioned above, the optimal dosage and selection of CNI beyond the first 3–12 months after transplantation remains a matter of debate. The inclusion of MMF or SRL instead of azathioprine in triple drug, CNI-based regimens has clearly shown that both drugs are more potent immunosuppressors than azathioprine. As a consequence, complete CNI withdrawal has also been attempted, leaving patients with a regimen of either MMF-prednisone or SRL-prednisone alone. Important information has been recently gathered for the following two categories of stable patients.

7.2.1 Patients receiving CsA/MMF/corticosteroids

In a prospective multicenter study, 212 stable primary transplant recipients receiving CsA, MMF, and prednisone were randomized at 6 months post-transplantation to either CsA withdrawal, prednisone withdrawal, or to continue their triple-drug therapy regimen. The objective of this study was to assess the safety of CsA withdrawal (50% tapering for 2 weeks before discontinuation) or prednisone at 6 months after transplantation compared with the continuation of triple therapy. Eighteen months after drug withdrawal, a biopsy-proven acute rejection occurred in 22%, 4%, and 1.4% of each group, respectively. Biopsy-proven chronic rejection was observed in 11%, 4% and 0%, respectively. Thus, CsA withdrawal was associated with a significantly increased incidence of not only acute rejection but also chronic rejection, as compared to the two other groups (37). Three months after withdrawal of either CsA or prednisone, a substantial decrease in total cholesterol was observed, but a lower total/HDL cholesterol ratio was only present in the CsA withdrawal group. Similarly, the mean arterial pressure was improved in both the CsA and prednisone withdrawal groups. Another similar European multicenter clinical trial enrolled 187 renal transplant recipients treated with triple therapy (CsA/MMF/prednisone) and randomized at 3 months to either CsA withdrawal or to continuation therapy. CsA withdrawal was gradual over 3 months. The primary end-point was creatinine clearance 6 months after complete withdrawal. Similar to the previous study, acute rejection episodes were significantly higher in the CsA withdrawal group (11% vs. 2%) with no graft loss. The lower rejection rate following CNI withdrawal reported may have been achieved because of the tapering preceding the withdrawal of CsA compared to the previous similar experience. At the end of the study, there was a significant improvement in creatinine clearance of 7.5 ml/min (*per* protocol population) in favor of the CsA withdrawal group. CsA withdrawal was associated with a significantly lower total and LDL cholesterol (130). During the study, the average daily dose of MMF was approximately 2 g/day in both treatment groups, whereas the mean daily dose of prednisone was 13 mg/day in the CsA withdrawal group and 7.5 mg/day in the CsA continuation group. The five-year results of the study recorded similar recipient and graft survival rates in the two groups. However, withdrawal of CsA resulted in an increased risk of acute rejection episodes and graft loss as a result of rejection throughout the 5-year study period. The improvement in renal function (in terms of creatinine clearance) observed at 1 year was maintained at 5 years. Blood pressure and cholesterol levels were well controlled in both groups. (131) Two other randomized CsA withdrawal studies in stable kidney transplant recipients receiving MMF therapy more than 3 months post-transplant were published with similar short-term results (132, 133). An interesting randomized, open-label trial was performed to compare the incidence of acute rejection after an early (3 months) withdrawal of CsA or MMF in renal transplantation (134). Non-sensitized, rejection-free patients who were under a triple drug regimen (CsA/MMF/prednisone) and had received a first kidney from a deceased donor were enrolled. Three months after transplantation, patients were gradually withdrawn from CsA (n = 54) or MMF (n = 54). A graft biopsy and a pharmacokinetic study of CsA and mycophenolic acid were systematically performed before the randomization. At 1 year, graft and patient survival rates were 100% in each group. Renal function was improved in the MMF group compared with the CsA group (Cockcroft-Gault calculated clearance + 8.2 ml/min). However, the probability of acute rejection was significantly higher in the MMF group (18% vs. 6%). The patients who developed acute rejection after CsA withdrawal had a significantly higher incidence of borderline changes on the randomization biopsy than the rejection-free patients and they displayed a lower area under the curve of mycophenolic acid. Multivariate analysis confirmed that borderline changes and area under the curve of mycophenolic acid were significant risk factors for acute rejection after CsA discontinuation. It was concluded that a systematic graft biopsy and a pharmacokinetic study of mycophenolic acid are needed to reduce the risk of acute rejection after CsA withdrawal (134).

A further trial of CNI withdrawal, the CAESAR trial, has enrolled 536 patients in three treatment groups. The purpose of this study was to evaluate whether a very low dose of CsA (with and without late withdrawal) in combination with the IL2r-mAb DAC and MMF is safe and provides effective immunosuppression. The key end points of this study were acute rejection, measured GFR, and histological analysis of protocol biopsy at 1 year. The mean GFR 12 months after transplantation (the primary end-point) was not statistically different in the CsA withdrawal and low-dose CsA groups (both 51 mL/min/1.73 m^2) vs. the standard-dose CsA group (48.6 mL/min/1.73 m^2). At 12 months, the incidence of biopsy-proven acute rejection was significantly higher in the CsA withdrawal group (38%) vs. the low or standard dose CsA groups (25% and 28%, respectively). In summary, a regimen of continuous low-dose CsA with MMF, corticosteroids and DAC induction seems to be clinically safe and effective (135). Overall, the available evidence suggests that complete discontinuation of CsA in stable kidney transplant recipients receiving CsA/MMF/prednisone is associated with a significantly increased risk of acute rejection. Such an approach may therefore be detrimental to a substantial proportion of patients. In addition, clinically evident acute rejection episodes may represent the tip of the iceberg, with unrecognized subclinical cellular rejection (or chronic humoral rejection) possibly occurring in some additional patients in the weeks or months after CsA withdrawal. Furthermore, it is well recognized that a history of acute rejection is a major risk-factor for CAN. Answers to these questions are lacking because study end-points of immunologic monitoring (serological or histopathological) were not systematically incorporated in these clinical trials and long-term follow-up was not reported. Because of these concerns, a safer strategy to prevent long-term toxicities associated with CNI use in stable patients might be the reduction (rather than discontinuation) of CsA dosages at 1 year post transplantation (136). To confirm this hypothesis, a prospective randomized trial was performed to determine whether CsA could be safely reduced by 50%. At 1 year post-transplantation, 64 stable kidney transplant recipients

were randomized to either continue their maintenance CsA dose (mean 3.5 mg/kg/day) or lower their CsA dose by approximately 50% over a 2-month period (mean CsA levels decreasing from 213 ng/ml to 86 ng/ml). During the study, the mean daily dose of MMF was approximately 2 g/day and the daily dose of prednisone was 9.5 mg/day in both treatment groups. Within 6 months of randomization, no episode of acute rejection or graft loss occurred in either group. Patients in the CsA reduction group had a significant increase in their glomerular filtration rate (from 57.7 ml/min to 64.6 ml/min) and a significant decrease in mean systolic blood pressure, triglycerides, and serum uric acid levels but no significant changes in homocysteine, C-reactive protein, or fibrinogen levels were observed (137, 138). Interestingly, the modest but significant improvement in kidney allograft function in the CsA reduction group was in the same order of magnitude as that observed in the CsA withdrawal studies mentioned above. Of note, no *de novo* anti-HLA antibody production after 50% CsA reduction was observed in the subgroup of patients that were tested (138).Newer methods of CsA monitoring (such as the 2-hour post-dose level of CsA) may help in selecting CsA-overexposed patients for a controlled dose-reduction of their CNI (139). However, it seems particularly important to emphasize that more long-term follow-up studies (up to 5 years) with sequential immunologic monitoring and protocol biopsies are needed, particularly for studies of complete CNI withdrawal (140, 141).

7.2.2. Patients receiving CsA/SRL/corticosteroids.

Two trials (142, 143) were designed to evaluate the efficacy of a maintenance regimen of SRL/prednisone following CsA withdrawal. These trials were conceived to minimize the enhanced nephrotoxicity that was observed when SRL was used in combination with full-dose CsA (5). These two studies (the first conducted in the US and in Europe, and the second conducted worldwide minus the US) have a slightly different design but the same underlying rationale: assessing the safety and the potential benefits of CsA withdrawal from a SRL/corticosteroid regimen. In the US-Europe trial, CsA was withdrawn at the end of month 2 after transplantation only in patients who had been rejection free (82% of patients were eligible). The incidence of acute rejection at 1 year was not statistically significant (19% vs. 22%, respectively) between patients who continued CsA therapy vs. patients who were withdrawn from CsA. Patients withdrawn from CsA experienced a significant increase in the calculated GFR (142). The Rapamune Maintenance Regimen Trial was conducted to examine whether concentration-controlled SRL dosing could be used to eliminate CsA from a CsA/SRL/prednisone maintenance regimen (143). In this study, 525 kidney transplant recipients of deceased (89%) or living (11%) donors received CsA/SRL/corticosteroids triple therapy for the first 3 months post-transplant. At 3 months, eligible patients (those with no recent episode of acute rejection) were randomized either to remain on the triple-drug therapy regimen or to have their CsA withdrawn. In the CsA withdrawal group, SRL was maintained at trough levels of 20-30 ng/ml in the first year, followed by 15-20 ng/ml thereafter. The results demonstrated that, at 12 months, overall graft and patient

survival were similar in both arms. The incidence of biopsy-proven acute rejection was 13% during the pre-randomization period. After randomization, acute rejection rates were significantly higher in the CsA withdrawal group (10% vs. 4%), but kidney function (63 ml/min vs. 57 ml/min) and blood pressure improved as compared to the control group. These 1-year results suggested that CsA elimination at 3 months from a CsA/SRL/prednisone regimen can result in subsequent improved kidney function and lower blood pressure, with a slight but significant increase in the acute rejection risk (143). Of note, during the pre-randomization period, 18% of the patients were discontinued from the study, and post-randomization there was an additional significantly different rate of discontinuation of 18% and 27%, in the CsA/SRL/prednisone group and SRL/prednisone group, respectively (143). The 36-month results of this multicenter trial showed that kidney function and blood pressure remained significantly better with SRL/prednisone. The incidence of biopsy-proven acute rejection between randomization and month 36 was 5% higher in the SRL/prednisone group, but this difference no longer achieved statistical significance. The discontinuation rate from the study at month 36 was significantly higher in the CsA/SRL/prednisone group (48% vs. 38%) (144). The major cause for discontinuation from the study was the occurrence of adverse events. Although the 3-year results of this trial are encouraging, the relatively large dropout rate observed needs to be considered when trying to apply such an immunosuppressive strategy to daily clinical practice. A common conclusion can be drawn from these trials: discontinuation of CsA from a SRL/corticosteroids regimen is associated with a modest increase in rejection but a significant and clinically relevant improvement in renal function. Whether this trade-off is deemed acceptable in clinical practice in stable patients remains to be determined. As previously mentioned, CNIs have been associated with an increased risk of diabetes, whereas SRL seems to be devoid of any effect on glucose metabolism. Recently, however, a small pivotal study was performed to investigate the effect of the withdrawal of CNI and the switch to SRL on peripheral insulin resistance and pancreatic beta-cell responses. This strategy failed to ameliorate the glycometabolic profile and was associated with a worsening of insulin resistance and an inappropriately low insulin response. These findings support the need for continual and specific monitoring of glucose metabolism in renal transplant recipients after CNI withdrawal and conversion to a new immunosuppressive protocol (145).

8. CALCINEURIN INHIBITOR AVOIDANCE IN IMMUNOSUPPRESSIVE PROTOCOLS

Initial trials combining SRL (instead of CsA) with corticosteroids and azathioprine or MMF resulted in an incidence of biopsy-proven acute rejection of 41% (with azathioprine) or 28% (with MMF), no different from that observed with CsA-based regimens (146, 147). The first European study utilized a CNI-free regimen combining SRL and MMF, in conjunction with corticosteroids but without antibody induction therapy (147). At 14 centers,

recipients of renal allografts from deceased donors were randomized to receive SRL (n= 40) or CsA (n= 38) in an open-label design. All patients received 2 g/d MMF and corticosteroids. The doses of SRL and CsA were concentration-controlled. At 1 year, patient and graft survival were similar between the two treatment groups. The incidence of biopsy-proven acute rejection was not statistically different (28% in the SRL arm vs. 18% in the CsA arm). The calculated GFR was consistently higher in the SRL-treated patients compared with CsA. A major concern with this trial was the very high doses of SRL that were required to achieve the target sirolimus blood levels (30 ng/ml for 2 months and 15 ng/ml thereafter). In addition, 43% of patients in the SRL treatment group were discontinued from the protocol for a number of reasons. Thus, while this trial demonstrated the potential efficacy of a regimen combining two antiproliferative drugs in the absence of a CNI, it fell short of being fully successful. The addition to this regimen of induction therapy with a biologic agent improved its tolerability, decreased the dose and target SRL levels required to provide efficacy, and reduced the acute rejection to below 20%. Such an approach consisting of DAC/MMF/corticosteroids was tested in two different clinical trials. In the absence of a CNI, these regimens showed relatively high acute rejection rates in the range of 40–50%, and consequently have generally been abandoned (148, 149). Interestingly, acute rejection episodes occurred despite complete blockade of IL2r, suggesting that blocking an important pathway of the immune response (the interleukin-2 pathway), together with oral administration of MMF and corticosteroids, does not confer complete protection against rejection (149). In the US-Europe multicenter trial, the rationale for such an approach to CNI avoidance was that anti–IL-2r-mAb blocking IL-2 binding to its receptor, could be substituted for CNI that inhibit cytokine transcription, particularly in the early post-transplant period when there is an increased risk of rejection (149). Ninety-eight patients were enrolled and followed for 1 year. Patients who experienced acute rejection were started on CNI. All patients were primary transplant recipients receiving kidneys from deceased or living donors. The biopsy-proven rejection rate at 1 year was 53%. Despite the high rejection rate, the overall 1-year outcome was excellent, with a patient survival of 97% and graft survival of 96%. On the basis of the findings that during acute rejection the IL-2 receptor on circulating and intragraft lymphocytes were fully saturated with DAC, it was hypothesized that rejection may have been mediated by redundant cytokines such as IL-15, which can induce T cell activation. In this setting, SRL blocks cytokine-mediated proliferative signals from the common gamma chain, a receptor that binds to several cytokines, including IL-15, and could provide greater efficacy to CNI-free regimens (150). Again recently, a prospective, randomized comparison of a similar immunosuppression (DAC/MMF/corticosteroids) to a CNI-based protocol (CsA/MMF/corticosteroids) in a population with low immunological risk (PRA negative, DR-matched deceased-donor kidney transplant recipients), was unsuccessful (151). The GFR at 1-year was significantly lower in the DAC/MMF/corticosteroids-group (- 17.9 ml/min). One-year patient and graft survival did not differ but the overall

acute rejection rate was higher in the CNI-free protocol (70.4%) as compared to the CsA-group (29.6%). The incidence of acute rejection was unacceptably high (even though anti-IL2r-mAb induction and initial higher MMF doses were applied) and renal function was significantly lower in the CNI-avoidance patients. (151). An initial pilot study evaluated a protocol consisting of DAC/MMF (2 g/day)/SRL (target blood levels= 10 to 20 ng/ml) (152). Nine primary renal transplant recipients were enrolled. At 3 months, only one of nine patients had an episode of mild acute rejection. While the immunosuppression regimen was well tolerated, anemia and hyperlipidemia were the most common side effects. Moreover, by adding Bsx induction to a SRL/MMF/corticosteroids regimen, it was reported that low acute rejection rates could be observed in 31 low-immunological risk kidney transplant recipients (153). At 2 years, SRL-treated patients had better renal function and a decreased prevalence of CAN as compared to CsA-treated patients, whose mean CsA trough levels were kept at 210–240 ng/ml during the first year (153, 154). In a single center US study 61 patients were randomized to either CsA or SRL in a Bsx/MMF/corticosteroids immunosuppression (153). At 1 year, patient and graft survival were not significantly different between the two treatment groups. The SRL-treated patients had a rejection rate of 6% compared with 17% in the CsA treatment group. At 6 and 12 months, the SRL-treated patients had significantly lower mean serum creatinine levels than the CsA-treated patients (1.3 mg/dl and 1.3 mg/dl vs. 1.7 mg/dl and 1.8 mg/dl, respectively). At 1 year, there were no significant differences in lipid levels between the two arms of the study. In the recently reported 5-year extended observation of this cohort, there were no significant differences in acute rejection rates (13% in the SRL group vs. 23% in the CsA group), total cholesterol (209.1 vs. 204.3 mg/dL, P=0.973) or urine protein/creatinine ratios (0.398 vs. 0.478 mg/dL, P=0.72) but the sirolimus-based CNI-free patients had a longer death-censored graft survival (96.4 vs. 76.7%, P=0.0265), a higher glomerular filtration rate by the abbreviated Modified Diet in Renal Disease (66.7 vs. 50.7 cc/min, P=0.0075), and fewer graft losses from CAN. The Banff chronic scores at two years were strong predictors of 5-year glomerular filtration rate (154, 155). On the other hand, the multinational, randomized study based on this pilot study (the ORION study) failed to replicate these good results and the SRL/MMF based arm of the study was terminated due to a higher than expected 18-month rate of acute rejection (30% in SRL/MMF vs. 11% in TAC/MMF vs. 17% in SRL/TAC). Despite more frequent rejection episodes, renal function was numerically better at 18 months in the SRL/MMF group, suggesting that in the setting of CNI-free immunosuppression, mild acute rejection is not associated with deterioration of renal allograft function as reflected by GFR, serum creatinine, and urinary protein excretion. (156). In another prospective, randomized controlled trial, 132 living donor renal allotransplant recipients were divided into two groups. All patients received corticosteroids and Bsx induction therapy. For maintenance immunosuppression, TAC/SRL or SRL/MMF was alternatively utilized (157). Patients were followed up for a minimum of 24 months. One-year patient and graft survival rates were not significantly different

between the groups. However, the incidence of biopsy-proven acute rejection was lower in the SRL/MMF group but the difference was not statistically significant (14% vs. 19%). Statistically significant better renal function was encountered among SRL/MMF patients at two years post-transplantation, as measured by serum creatinine and calculated GFR (+15.3 ml/min). One-year protocol biopsies showed insignificant differences relative to the chronic allograft damage index between the two groups. Assessment of the long-term impact of this observation on graft survival and function needs a longer follow-up (157). A registry analysis of outcomes with the SRL/MMF combination regimen in kidney transplant recipients transplanted between 2000 and 2005, taking into account data concerning 2,040 kidney transplant recipients on SRL/MMF concluded that SRL/MMF is associated with inferior renal transplant outcomes compared to other commonly used regimens (158). In this study, the 6-month acute rejection rate was higher with SRL/MMF (16% vs. 11% with other regimens). Overall graft survival was significantly lower on SRL/MMF and this approach was associated with twice the hazard for graft loss relative to TAC/MMF, also consistent in both living donor transplants and expanded criteria donor transplants. Among deceased donor transplants, the delayed graft function rate was higher in the SRL/MMF cohort (47% vs. 27%). However, adjusted graft survival was also significantly inferior with SRL/MMF in delayed graft function-free patients. In analyses restricted to patients who remained on the discharge regimen at 6 months post-transplantation, conditional graft survival in deceased-donor transplants was significantly lower with SRL/MMF compared to patients on TAC/MMF or CsA/MMF regimens at 5 years post-transplantation: 64%, 78% and 78%, respectively, and across all patient subgroups (158). More recently, powerful T-cell–depleting induction strategies using Thymoglobulin have been evaluated as a means to possibly induce hyporesponsiveness and thus enable minimization of maintenance immunosuppression (as proposed more than two decades ago by our group). In a pivotal study, high-dose Thymoglobulin administration (total dose of 20 mg/kg) for 8-10 days before transplantation followed by postoperative SRL monotherapy was tested in twelve nonsensitized primary kidney transplant recipients, but was associated with an acute rejection rate of 25% and frequent adverse events (159). In another prospective study in low immunologic risk recipients of suboptimal kidneys (n=30), it was found that Thymoglobulin induction followed by MMF (3 g/day)/corticosteroids was associated with a 24% acute rejection rate. However, side effects of MMF at the doses used were also common and overimmunosuppression was noted (160). In another prospective, randomized trial with Thymoglobulin induction comparing SRL/MMF/prednisone to TAC/MMF/prednisone, 81 patients in the SIR group and 84 patients in the TAC group were enrolled (161). At 1 year, patient and graft survival were similar between the two groups, the incidence of clinical acute rejection was 10% in the TAC group and 13% in the SIR group and there was no difference in mean GFR measured by iothalamate clearance at 1 or 2 years. At 1 year, according to the Banff score, there was no difference in interstitial, tubular or glomerular changes, but

fewer chronic vascular changes were detected in the SRL group. This study shows that a CNI-free regimen using SRL/MMF/prednisone produces similar acute rejection rates, graft survival and renal function 1 and 2 years after transplantation compared to TAC/MMF/prednisone (161). Other emerging CNI-free therapies are being developed for use in renal transplantation. These novel protocols avoid CNI because the mechanism of action of CNI (but not MMF or SRL) involves abrogation of lymphocyte response pathways to alloantigens that also blocks activation-induced apoptosis and the development of tolerance, relegating patients to a lifetime of immunosuppressive therapy with considerable toxicity. An important protocol, notably because it might represent a new and distinct way of preventing alloimmune injury in humans, is the incorporation of agents that inhibit T-cell costimulation signals in drug regimens with no CNIs. Belatacept, a recombinant fusion receptor protein consisting of the extracellular domain of CTLA4 (which binds with high affinity to CD80 and CD86 and blocks co-stimulation signals required for T cell activation) linked to the constant region of IgG1, can induce tolerance (in rodents) or indefinite graft survival (in non human primates) (162-164) when used instead of CsA, in maintenance triple drug regimen, with MMF and corticosteroids (165). A large multicenter prospective randomized study tested the efficacy and safety of a chronic intermittent intravenous therapy of an intensive or less-intensive Belatacept regimen or CsA regimen to demonstrate the non-inferiority of Belatacept over CsA in the incidence of acute rejection at six months (10). All patients received induction therapy with Bsx/MMF/corticosteroids. At six months, the incidence of acute rejection was similar among the groups: 7% for intensive Belatacept, 6% for less-intensive Belatacept, and 8% for CsA. At 12 months, the glomerular filtration rate was significantly higher with both intensive and less-intensive Belatacept than it was with CsA, and CAN was less common with both regimens of Belatacept than with CsA. Lipid levels and blood-pressure values were similar or slightly lower in the Belatacept groups, despite the greater use of lipid-lowering and antihypertensive medications in the CsA group (10). This trial represents a paradigm shift in immunosuppression therapy, replacing orally administered CNI and their requirements for therapeutic drug monitoring with intermittent parenteral therapy (administered at monthly or every other month intervals). This approach could not only be tested with molecules blocking costimulatory signals but also with a new generation of anti-CD25 monoclonal antibodies or even polyclonal antibodies.

9. SPARING OF STEROIDS AND CALCINEURIN INHIBITORS

Several new studies are ongoing with the use of Alemtuzumab (Campath-1H). This agent is a humanized rat monoclonal antibody (rat immunoglobulin IgG2b) directed against the CD52 antigen, which is expressed on all blood mononuclear cells and also on cells lining the male reproductive tract. It is a powerful cytolytic agent and has been used therapeutically in bone marrow transplantation,

several autoimmune diseases, and organ transplantation. It was first used in organ transplantation in 1998 to prevent rejection (77) and is being used with increasing frequency as induction therapy in many institutions with the goal of minimizing corticosteroids and CNI. In the absence of long-term results and randomized trials, no definitive conclusions can be made concerning this agent, although very promising short-term data is starting to be documented, as detailed in the recent review by Morris *et al* (166). Because of the long-lasting lymphopenia caused by alemtuzumab, especially of B and T lymphocytes, it had been hoped that its use might facilitate the development of steroid-free regimens and calcineurin-sparing or calcineurin-free regimens to avoid the long-term complications of these agents, particularly nephrotoxicity in the case of the latter. Most of the studies reported have used alemtuzumab induction with a steroid- and calcineurin inhibitor–free protocol (14, 62), a steroid-free and calcineurin-reduced protocol (12, 167, 168), or a steroid-free protocol (76, 169). As of yet, none of the retrospective studies of calcineurin inhibitor–free or -reduced immunosuppression and alemtuzumab induction in renal transplantation have been able to show any significant improvements in renal function compared with conventional therapies. This may be a reflection of the limited follow-up in these studies. The longest follow-up was reported by Watson *et al.* (167), who showed that, after 5 years, there was no significant difference in renal function in the alemtuzumab group that received reduced cyclosporine as maintenance immunosuppression, despite the fact that the patients in this group received significantly less cyclosporine than the control group for the first 2 years after transplantation. Shapiro *et al.* (12) showed that alemtuzumab induction allowed these patients to achieve spaced weaning of tacrolimus to every other day or less in 74% of patients, but at 1-year follow-up, there was no significant difference in renal function. Gruessner *et al.* (14), who employed a steroid- and calcineurin inhibitor–free protocol, did not observe a significant difference in renal function in their series of combined kidney/pancreas transplants, but follow-up was short. Conversely, improved renal function was observed in liver transplant recipients treated with alemtuzumab induction and low-dose tacrolimus monotherapy (170).

10. CONCLUSIONS

Today, the number of drugs (chemical, biological, etc) available to treat kidney allograft recipients is much greater than in the past. Because of multiple mechanisms of action, numerous immunosuppressive associations are possible, resulting in equal results from one center to another. The most relevant challenges are, however, to prevent acute rejection, to maintain good graft function indefinitely, to avoid cancer and to avoid death. None of the current drug regimens are capable of this. So far, and in order to be more effective and less toxic, physicians have tried to minimize their immunosuppressive protocols as much as possible. As reported here with corticosteroids and CNIs, the results are very confusing, making clear and secure guidelines impossible to be established. The lack of studies with long-term outcomes is an important issue that requires reflection. Despite these conflicting data, it appears possible and safe to eliminate, at least in a large majority of patients, the chronic use of corticosteroids and/or CNIs. Whether or not this approach is effective in the very long-term is still unknown.

11. ACKNOWLEDGMENTS

12. REFERENCES

1. P. F. Halloran, A. Melk and C. Barth: Rethinking chronic allograft nephropathy: the concept of accelerated senescence. *J Am Soc Nephrol*, 10(1), 167-81 (1999)

2. M. Pascual, T. Theruvath, T. Kawai, N. Tolkoff-Rubin and A. B. Cosimi: Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med*, 346(8), 580-90 (2002)

3. H. U. Meier-Kriesche, J. D. Schold, T. R. Srinivas and B. Kaplan: Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Am J Transplant*, 4(3), 378-83 (2004)

4. G. M. Danovitch: Immunosuppressive medications for renal transplantation: a multiple choice question. *Kidney Int*, 59(1), 388-402 (2001)

5. B. D. Kahan: Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Rapamune US Study Group. *Lancet*, 356(9225), 194-202 (2000)

6. F. Vincenti: The role of newer monoclonal antibodies in renal transplantation. *Transplant Proc*, 33(1-2), 1000-1 (2001)

7. F. Vincenti: What's in the pipeline? New immunosuppressive drugs in transplantation. *Am J Transpl*, 2, 898–903 (2002)

8. A. O. Gaber, M. R. First, R. J. Tesi, R. S. Gaston, R. Mendez, L. L. Mulloy, J. A. Light, L. W. Gaber, E. Squiers, R. J. Taylor, J. F. Neylan, R. W. Steiner, S. Knechtle, D. J. Norman, F. Shihab, G. Basadonna, D. C. Brennan, E. E. Hodge, B. D. Kahan, L. Kahan, S. Steinberg, E. S. Woodle, L. Chan, J. M. Ham, T. J. Schroeder and *et al.*: Results of the double-blind, randomized, multicenter, phase III clinical trial of Thymoglobulin versus Atgam in the treatment of acute graft rejection episodes after renal transplantation. *Transplantation*, 66(1), 29-37 (1998)

9. H. Tedesco-Silva, M. D. Pescovitz, D. Cibrik, M. A. Rees, S. Mulgaonkar, B. D. Kahan, K. K. Gugliuzza, P. R. Rajagopalan, M. Esmeraldo Rde, H. Lord, M. Salvadori and J. M. Slade: Randomized controlled trial of FTY720 versus MMF in de novo renal transplantation. *Transplantation*, 82(12), 1689-97 (2006)

10. F. Vincenti, C. Larsen, A. Durrbach, T. Wekerle, B. Nashan, G. Blancho, P. Lang, J. Grinyo, P. F. Halloran, K. Solez, D. Hagerty, E. Levy, W. Zhou, K. Natarajan and B. Charpentier: Costimulation blockade with belatacept in renal transplantation. *N Engl J Med*, 353(8), 770-81 (2005)

11. T. E. Starzl, N. Murase, K. Abu-Elmagd, E. A. Gray, R. Shapiro, B. Eghtesad, R. J. Corry, M. L. Jordan, P. Fontes, T. Gayowski, G. Bond, V. P. Scantlebury, S. Potdar, P. Randhawa, T. Wu, A. Zeevi, M. A. Nalesnik, J. Woodward, A. Marcos, M. Trucco, A. J. Demetris and J. J. Fung: Tolerogenic immunosuppression for organ transplantation. *Lancet*, 361(9368), 1502-10 (2003)

12. R. Shapiro, A. Basu, H. Tan, E. Gray, A. Kahn, P. Randhawa, N. Murase, A. Zeevi, A. Girnita, D. Metes, R. Ness, D. C. Bass, A. J. Demetris, J. J. Fung, A. Marcos and T. E. Starzl: Kidney transplantation under minimal immunosuppression after pretransplant lymphoid depletion with Thymoglobulin or Campath. *J Am Coll Surg*, 200(4), 505-15; quiz A59-61 (2005)

13. A. D. Kirk, D. A. Hale, R. B. Mannon, D. E. Kleiner, S. C. Hoffmann, R. L. Kampen, L. K. Cendales, D. K. Tadaki, D. M. Harlan and S. J. Swanson: Results from a human renal allograft tolerance trial evaluating the humanized CD52-specific monoclonal antibody alemtuzumab (CAMPATH-1H). *Transplantation*, 76(1), 120-9 (2003)

14. R. W. Gruessner, R. Kandaswamy, A. Humar, A. C. Gruessner and D. E. Sutherland: Calcineurin inhibitor- and steroid-free immunosuppression in pancreas-kidney and solitary pancreas transplantation. *Transplantation*, 79(9), 1184-9 (2005)

15. R. J. Corry, P. K. Chakrabarti, R. Shapiro, A. S. Rao, I. Dvorchik, M. L. Jordan, V. P. Scantlebury, C. A. Vivas, J. J. Fung and T. E. Starzl: Simultaneous administration of adjuvant donor bone marrow in pancreas transplant recipients. *Ann Surg*, 230(3), 372-9; discussion 379-81 (1999)

16. H. H. Hirsch, W. Knowles, M. Dickenmann, J. Passweg, T. Klimkait, M. J. Mihatsch and J. Steiger: Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. *N Engl J Med*, 347(7), 488-96 (2002)

17. N. J. London, S. M. Farmery, E. J. Will, A. M. Davison and J. P. Lodge: Risk of neoplasia in renal transplant patients. *Lancet*, 346(8972), 403-6 (1995)

18. C. M. Vajdic, S. P. McDonald, M. R. McCredie, M. T. van Leeuwen, J. H. Stewart, M. Law, J. R. Chapman, A. C. Webster, J. M. Kaldor and A. E. Grulich: Cancer incidence before and after kidney transplantation. *Jama*, 296(23), 2823-31 (2006)

19. A. J. Matas, A. Humar, K. J. Gillingham, W. D. Payne, R. W. Gruessner, R. Kandaswamy, D. L. Dunn, J. S. Najarian and D. E. Sutherland: Five preventable causes of kidney graft loss in the 1990s: a single-center analysis. *Kidney Int*, 62(2), 704-14 (2002)

20. L.W. Miller: Cardiovascular toxicities of immunosuppressive agents. *Am J Transplant,*; 2, 807–818 (2002)

21. T. S. Ikonen, J.F. Gummert, M. Hayase, Y. Honda, B. Hausen, U. Christians, G.J. Berry, P.G. Yock, R.E. Morris: Sirolimus (rapamycin) halts and reverses progression of allograft vascular disease in non-human primates. *Transplantation*, 70(6), 969–975 (2000)

22. J. P. Fryer, E. Benedetti, K. Gillingham, J. S. Najarian and A. J. Matas: Steroid-related complications in pediatric kidney transplant recipients in the cyclosporine era. *Transplant Proc*, 26(1), 91-2 (1994)

23. H. Schacke, W.D. Docke, K. Asadullah: Mechanisms involved in the side effects of glucocosteroids. *Pharmacol The*r; 96: 23 (2002)

24. T. Rhen, J.A. Cidlowski: Antiflammatory action of glucocosteroids – new mechanism for old drugs. *N Engl J Med*, 353: 1711-1723 (2005)

25. M. N. Hill, R. A. Grossman, H. I. Feldman, S. Hurwitz and D. C. Dafoe: Changes in causes of death after renal transplantation, 1966 to 1987. *Am J Kidney Dis*, 17(5), 512-8 (1991)

26. D. Cantarovich: Prevention of acute rejection with antithymocyte globulin (Thymoglobuline): its potential to reduce corticosteroids. *J Nephrol*, 17 Suppl 8, S40-6 (2004)

27. M. M. Sarwal, J. R. Vidhun, S. R. Alexander, T. Satterwhite, M. Millan and O. Salvatierra, Jr.: Continued superior outcomes with modification and lengthened follow-up of a steroid-avoidance pilot with extended daclizumab induction in pediatric renal transplantation. *Transplantation*, 76(9), 1331-9 (2003)

28. D. E. Hricik, C. C. Whalen, J. Lautman, M. R. Bartucci, E. J. Moir, J. T. Mayes and J. A. Schulak: Withdrawal of steroids after renal transplantation--clinical predictors of outcome. *Transplantation*, 53(1), 41-5 (1992)

29. E. Cole, D. Landsberg, D. Russell, J. Zaltzman, B. Kiberd, C. Caravaggio, A. R. Vasquez and P. Halloran: A pilot study of steroid-free immunosuppression in the prevention of acute rejection in renal allograft recipients. *Transplantation*, 72(5), 845-50 (2001)

30. F. Vincenti, A. Monaco, J. Grinyo, M. Kinkhabwala and A. Roza: Multicenter randomized prospective trial of steroid withdrawal in renal transplant recipients receiving basiliximab, cyclosporine microemulsion and mycophenolate mofetil. *Am J Transplant*, 3(3), 306-11 (2003)

31. E. S. Woodle, F. Vincenti, M. I. Lorber, H. A. Gritsch, D. Hricik, K. Washburn, A. J. Matas, M. Gallichio and J.

Neylan: A multicenter pilot study of early (4-day) steroid cessation in renal transplant recipients under simulect, tacrolimus and sirolimus. *Am J Transplant*, 5(1), 157-66 (2005)

32. A. J. Matas, T. Ramcharan, S. Paraskevas, K. J. Gillingham, D. L. Dunn, R. W. Gruessner, A. Humar, R. Kandaswamy, J. S. Najarian, W. D. Payne and D. E. Sutherland: Rapid discontinuation of steroids in living donor kidney transplantation: a pilot study. *Am J Transplant*, 1(3), 278-83 (2001)

33. A. J. Matas, R. Kandaswamy, K. J. Gillingham, L. McHugh, H. Ibrahim, B. Kasiske and A. Humar: Prednisone-free maintenance immunosuppression-a 5-year experience. *Am J Transplant*, 5(10), 2473-8 (2005)

34. N. Ahsan, D. Hricik, A. Matas, S. Rose, S. Tomlanovich, A. Wilkinson, M. Ewell, M. McIntosh, D. Stablein and E. Hodge: Prednisone withdrawal in kidney transplant recipients on cyclosporine and mycophenolate mofetil--a prospective randomized study. Steroid Withdrawal Study Group. *Transplantation*, 68(12), 1865-74 (1999)

35. Y. Vanrenterghem, Y. Lebranchu, R. Hene, F. Oppenheimer and H. Ekberg: Double-blind comparison of two corticosteroid regimens plus mycophenolate mofetil and cyclosporine for prevention of acute renal allograft rejection. *Transplantation*, 70(9), 1352-9 (2000)

36. J. P. Squifflet, Y. Vanrenterghem, J. P. van Hooff, K. Salmela and P. Rigotti: Safe withdrawal of corticosteroids or mycophenolate mofetil: results of a large, prospective, multicenter, randomized study. *Transplant Proc*, 34(5), 1584-6 (2002)

37. P. J. Smak Gregoor, R. G. de Sevaux, G. Ligtenberg, A. J. Hoitsma, R. J. Hene, W. Weimar, L. B. Hilbrands and T. van Gelder: Withdrawal of cyclosporine or prednisone six months after kidney transplantation in patients on triple drug therapy: a randomized, prospective, multicenter study. *J Am Soc Nephrol*, 13(5), 1365-73 (2002)

38. J. N. Boletis, I. Konstadinidou, H. Chelioti, H. Theodoropoulou, K. Avdikou, A. Kostakis and C. P. Stathakis: Successful withdrawal of steroid after renal transplantation. *Transplant Proc*, 33(1-2), 1231-3 (2001)

39. E. Sola, M. J. Alferez, M. Cabello, D. Burgos and M. Gonzalez Molina: Low-dose and rapid steroid withdrawal in renal transplant patients treated with tacrolimus and mycophenolate mofetil. *Transplant Proc*, 34(5), 1689-90 (2002)

40. Y. Vanrenterghem, J. P. van Hooff, J. P. Squifflet, K. Salmela, P. Rigotti, R. M. Jindal, J. Pascual, H. Ekberg, L. S. Sicilia, J. N. Boletis, J. M. Grinyo and M. A. Rodriguez: Minimization of immunosuppressive therapy after renal transplantation: results of a randomized controlled trial. *Am J Transplant*, 5(1), 87-95 (2005)

41. Z. Wlodarczyk, J. Walaszewski, F. Perner, S. Vitko, M. Ostrowski, P. Bachleda, F. Kokot, M. Klinger, P. Szenohradszky, P. Studenik, P. Navratil, L. Asztalos, B. Rutkowski, K. N. Kalmar and D. Hickey: Steroid withdrawal at 3 months after kidney transplantation: a comparison of two tacrolimus-based regimens. *Transpl Int*, 18(2), 157-62 (2005)

42. J. Pascual, J. P. van Hooff, K. Salmela, P. Lang, P. Rigotti and K. Budde: Three-year observational follow-up of a multicenter, randomized trial on tacrolimus-based therapy with withdrawal of steroids or mycophenolate mofetil after renal transplant. *Transplantation*, 82(1), 55-61 (2006)

43. C. K. Farmer, G. Hampson, I. C. Abbs, R. M. Hilton, C. G. Koffman, I. Fogelman and S. H. Sacks: Late low-dose steroid withdrawal in renal transplant recipients increases bone formation and bone mineral density. *Am J Transplant*, 6(12), 2929-36 (2006)

44. G. Opelz, B. Dohler and G. Laux: Long-term prospective study of steroid withdrawal in kidney and heart transplant recipients. *Am J Transplant*, 5(4 Pt 1), 720-8 (2005)

45. S. A. Birkeland: Steroid-free immunosuppression in renal transplantation: a long-term follow-up of 100 consecutive patients. *Transplantation*, 71(8), 1089-90 (2001)

46. D. E. Hricik, W. Y. Almawi and T. B. Strom: Trends in the use of glucocorticoids in renal transplantation. *Transplantation*, 57(7), 979-89 (1994)

47. R. W. Strickland, L.M. Wahl, D.S. Finbloom: Corticosteroids enhance the binding of recombinant interferon-γ to cultured human monocytes. *J Immunol,* 137(5), 1577–1580 (1986)

48. M. Y. Almawi, D.A. Hess, J. W. Assi , D. M. Chudzik, M. J. Rieder: Pretreatment with glucocorticoids enhances T-cell effector function: Possible implication for immune rebound accompanying glucocorticoid withdrawal. *Cell Transplant,* 8(6), 637–647 (1999)

49. J. Cremer, M. Struber, I. Wagenbreth, J. Nischelsky, S. Demertzis, T. Graeter, C. Abraham, A. Haverich: Progression of steroid-associated osteoporosis after heart transplantation. *Ann Thorac Surg,* 67(1), 130–133 (1999)

50. E. Shane, M. Rivas, D. J. McMahon, R. B. Staron, S. Silverberg, M.J. Seibel, D. Mancini, R. E. Michler, K. Aaronson, V. Addesso, S. H. Lo: Bone loss and turnover after cardiac transplantation. *J Clin Endocrinology Metabolism,* 82(5), 1497–1506 (1997)

51. R. J. Stratta, M. J. Armbrust, C. S. Oh, J. D. Pirsch, M. Kalayoglu, H. W. Sollinger and F. O. Belzer: Withdrawal of steroid immunosuppression in renal transplant recipients. *Transplantation*, 45(2), 323-8 (1988)

52. C. Ponticelli, A. Tarantino and G. Montagnino: Steroid withdrawal in renal transplant recipients. *Transplant Proc*, 33(1-2), 987-8 (2001)

53. R. Borrows, K. Chan, M. Loucaidou, C. Lawrence, J. Van Tromp, T. Cairns, M. Griffith, N. Hakim, A. McLean, A. Palmer, V. Papalois and D. Taube: Five years of steroid sparing in renal transplantation with tacrolimus and mycophenolate mofetil. *Transplantation*, 81(1), 125-8 (2006)

54. C. G. ter Meulen, I. van Riemsdijk, R. J. Hene, M. H. Christiaans, G. F. Borm, T. van Gelder, L. B. Hilbrands, W. Weimar and A. J. Hoitsma: Steroid-withdrawal at 3 days after renal transplantation with anti-IL-2 receptor alpha therapy: a prospective, randomized, multicenter study. *Am J Transplant*, 4(5), 803-10 (2004)

55. R. Kandaswamy, J. K. Melancon, T. Dunn, M. Tan, V. Casingal, A. Humar, W. D. Payne, R. W. Gruessner, D. L. Dunn, J. S. Najarian, D. E. Sutherland, K. J. Gillingham and A. J. Matas: A prospective randomized trial of steroid-free maintenance regimens in kidney transplant recipients--an interim analysis. *Am J Transplant*, 5(6), 1529-36 (2005)

56. R. Y. Calne: Cyclosporin in cadaveric renal transplantation: 5-year follow-up of a multicentre trial. *Lancet*, 2(8557), 506-7 (1987)

57. A. Tarantino, A. Aroldi, L. Stucchi, G. Montagnino, L. Mascaretti, A. Vegeto and C. Ponticelli: A randomized prospective trial comparing cyclosporine monotherapy with triple-drug therapy in renal transplantation. *Transplantation*, 52(1), 53-7 (1991)

58. G. Montagnino, A. Tarantino, G. P. Segoloni, V. Cambi, G. Rizzo, P. Altieri, M. Castagneto, M. Salvadori, M. Cossu, F. Pisani, M. Carmellini, F. Mastrangelo, R. Ferrara and C. Ponticelli: Long-term results of a randomized study comparing three immunosuppressive schedules with cyclosporine in cadaveric kidney transplantation. *J Am Soc Nephrol*, 12(10), 2163-9 (2001)

59. M. El-Faramawi, N. Rohr and B. Jespersen: Steroid-free immunosuppression after renal transplantation-long-term experience from a single centre. *Nephrol Dial Transplant*, 21(7), 1966-73 (2006)

60. S. Vitko, M. Klinger, K. Salmela, Z. Wlodarczyk, G. Tyden, G. Senatorski, M. Ostrowski, P. Fauchald, F. Kokot, S. Stefoni, F. Perner, K. Claesson, M. Castagneto, U. Heemann, M. Carmellini, J. P. Squifflet, M. Weber, G. Segoloni, L. Backman, H. Sperschneider and B. K. Kramer: Two corticosteroid-free regimens-tacrolimus monotherapy after basiliximab administration and tacrolimus/mycophenolate mofetil-in comparison with a standard triple regimen in renal transplantation: results of the Atlas study. *Transplantation*, 80(12), 1734-41 (2005)

61. L. Rostaing, D. Cantarovich, G. Mourad, K. Budde, P. Rigotti, C. Mariat, R. Margreiter, L. Capdevilla, P. Lang, P. Vialtel, J. Ortuno-Mirete, B. Charpentier, C. Legendre, J. Sanchez-Plumed, F. Oppenheimer and M. Kessler: Corticosteroid-free immunosuppression with tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. *Transplantation*, 79(7), 807-14 (2005)

62. S. J. Knechtle, J. D. Pirsch, J. H. Fechner J, B. N. Becker, A. Friedl, R. B. Colvin, L. K. Lebeck, L. T. Chin, Y. T. Becker, J. S. Odorico, A. M. D'Alessandro, M. Kalayoglu, M. M. Hamawy, H. Hu, D. D. Bloom and H. W. Sollinger: Campath-1H induction plus rapamycin monotherapy for renal transplantation: results of a pilot study. *Am J Transplant*, 3(6), 722-30 (2003)

63. G. Ciancio, G. W. Burke, J. J. Gaynor, M. R. Carreno, R. E. Cirocco, J. M. Mathew, A. Mattiazzi, T. Cordovilla, D. Roth, W. Kupin, A. Rosen, V. Esquenazi, A. G. Tzakis and J. Miller: A randomized trial of three renal transplant induction antibodies: early comparison of tacrolimus, mycophenolate mofetil, and steroid dosing, and newer immune-monitoring. *Transplantation*, 80(4), 457-65 (2005)

64. L. Gallon, N. Perico, B. D. Dimitrov, J. Winoto, G. Remuzzi, J. Leventhal, F. Gaspari and D. Kaufman: Long-term renal allograft function on a tacrolimus-based, pred-free maintenance immunosuppression comparing sirolimus vs. MMF. *Am J Transplant*, 6(7), 1617-23 (2006)

65. J. W. Alexander, H. R. Goodman, M. Cardi, J. Austin, S. Goel, S. Safdar, S. Huang, R. Munda, J. P. Fidler, J. F. Buell, M. Hanaway, B. Susskind, P. Roy-Chaudhury, J. Trofe, R. Alloway and E. S. Woodle: Simultaneous corticosteroid avoidance and calcineurin inhibitor minimization in renal transplantation. *Transpl Int*, 19(4), 295-302 (2006)

66. D. Cantarovich, J. Palneau, J. P. Couderc, A. Murat, M. Hourmant, R. Boatard, J. Dantal, G. Karam, O. Bouchot and J. P. Soulillou: Maintenance immunosuppression without corticosteroids following combined pancreas and kidney transplantation. *Transplant Proc*, 23(4), 2224-5 (1991)

67. D. Cantarovich, G. Karam, M. Giral-Classe, M. Hourmant, J. Dantal, G. Blancho, L. Le Normand and J. P. Soulillou: Randomized comparison of triple therapy and antithymocyte globulin induction treatment after simultaneous pancreas-kidney transplantation. *Kidney Int*, 54(4), 1351-6 (1998)

68. D. Cantarovich, B. Le Mauff, M. Hourmant, J. Dantal, R. Baatard, M. Denis, Y. Jacques, G. Karam, J. Paineau and J. P. Soulillou: Prevention of acute rejection episodes with an anti-interleukin 2 receptor monoclonal antibody. I. Results after combined pancreas and kidney transplantation. *Transplantation*, 57(2), 198-203 (1994)

69. D. Cantarovich, M. Giral-Classe, M. Hourmant, J. Dantal, G. Blancho, G. Karam and J. P. Soulillou: Low incidence of kidney rejection after simultaneous kidney-pancreas transplantation after antithymocyte globulin induction and in the absence of corticosteroids: results of a

prospective pilot study in 28 consecutive cases. *Transplantation*, 69(7), 1505-8 (2000)

70. D. Cantarovich, G. Karam, M. Hourmant, J. Dantal, G. Blancho, M. Giral and J. P. Soulillou: Steroid avoidance versus steroid withdrawal after simultaneous pancreas-kidney transplantation. *Am J Transplant*, 5(6), 1332-8 (2005)

71. D. B. Kaufman, J. R. Leventhal, A. J. Koffron, L. G. Gallon, M. A. Parker, J. P. Fryer, M. M. Abecassis and F. P. Stuart: A prospective study of rapid corticosteroid elimination in simultaneous pancreas-kidney transplantation: comparison of two maintenance immunosuppression protocols: tacrolimus/mycophenolate mofetil versus tacrolimus/sirolimus. *Transplantation*, 73(2), 169-77 (2002)

72. C. E. Freise, S. M. Kang, S. Feng, R. Hirose and P. Stock: Excellent short-term results with steroid-free maintenance immunosuppression in low-risk simultaneous pancreas-kidney transplantation. *Arch Surg*, 138(10), 1121-5; discussion 1125-6 (2003)

73. A. Kahl, W. O. Bechstein, F. Lorenz, J. Steinberg, C. Pohle, D. Kampf, A. Muller, U. Settmacher, P. Neuhaus and U. Frei: Long-term prednisolone withdrawal after pancreas and kidney transplantation in patients treated with ATG, tacrolimus, and mycophenolate mofetil. *Transplant Proc*, 33(1-2), 1694-5 (2001)

74. M. Rossetti, G. B. Piccoli, M. Burdese, C. Guarena, R. Giraudi, E. Mezza, V. Consiglio, G. Soragna, M. Messina and G. P. Segoloni: Tailored immunosuppression and steroid withdrawal in pancreas-kidney transplantation. *Rev Diabet Stud*, 1(3), 129-36 (2004)

75. R. J. Knight, R. H. Kerman, E. McKissick, A. Lawless, H. Podder, S. Katz, C. T. Van Buren and B. D. Kahan: A pilot study of immunosuppression minimization after pancreas-kidney transplantation utilizing thymoglobulin induction and sirolimus maintenance therapy. *Transplant Proc*, 37(8), 3538-41 (2005)

76. D. B. Kaufman, J. R. Leventhal, D. Axelrod, L. G. Gallon, M. A. Parker and F. P. Stuart: Alemtuzumab induction and prednisone-free maintenance immunotherapy in kidney transplantation: comparison with basiliximab induction--long-term results. *Am J Transplant*, 5(10), 2539-48 (2005)

77. R. Calne, P. Friend, S. Moffatt, A. Bradley, G. Hale, J. Firth, J. Bradley, K. Smith and H. Waldmann: Prope tolerance, perioperative campath 1H, and low-dose cyclosporin monotherapy in renal allograft recipients. *Lancet*, 351(9117), 1701-2 (1998)

78. G. Ciancio, G. W. Burke, J. J. Gaynor, A. Mattiazzi, R. Roohipour, M. R. Carreno, D. Roth, P. Ruiz, W. Kupin, A. Rosen, V. Esquenazi, A. G. Tzakis and J. Miller: The use of Campath-1H as induction therapy in renal

transplantation: preliminary results. *Transplantation*, 78(3), 426-33 (2004)

79. M Q. Xia, G. Hale, H. Waldmann . Efficient complement-mediated lysis of cells containing the ALEMTUZUMAB-1 (CDw52) antigen. *Mol Immunol*, 30(12), 1089–1096 (1993)

80. D. B. Kaufman, J. R. Leventhal, L. G. Gallon and M. A. Parker: Alemtuzumab induction and prednisone-free maintenance immunotherapy in simultaneous pancreas-kidney transplantation comparison with rabbit antithymocyte globulin induction - long-term results. *Am J Transplant*, 6(2), 331-9 (2006)

81. M. L. Jordan, P. Chakrabarti, P. Luke, R. Shapiro, C. A. Vivas, V. P. Scantlebury, J. J. Fung, T. E. Starzl and R. J. Corry: Results of pancreas transplantation after steroid withdrawal under tacrolimus immunosuppression. *Transplantation*, 69(2), 265-71 (2000)

82. M. L. Jordan, R. Shapiro, H. A. Gritsch, F. Egidi, A. Khanna, C. A. Vivas, V. P. Scantlebury, J. J. Fung, T. E. Starzl and R. J. Corry: Long-term results of pancreas transplantation under tacrolius immunosuppression. *Transplantation*, 67(2), 266-72 (1999)

83. F. Vincenti: Immunosuppression minimization: current and future trends in transplant immunosuppression. *J Am Soc Nephrol*, 14(7), 1940-8 (2003)

84. F. Vincenti, S. C. Jensik, R. S. Filo, J. Miller and J. Pirsch: A long-term comparison of tacrolimus (FK506) and cyclosporine in kidney transplantation: evidence for improved allograft survival at five years. *Transplantation*, 73(5), 775-82 (2002)

85. W. M. Bennett, A. DeMattos, M. M. Meyer, T. Andoh and J. M. Barry: Chronic cyclosporine nephropathy: the Achilles' heel of immunosuppressive therapy. *Kidney Int*, 50(4), 1089-100 (1996)

86. A. O. Ojo, P. J. Held, F. K. Port, R. A. Wolfe, A. B. Leichtman, E. W. Young, J. Arndorfer, L. Christensen and R. M. Merion: Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med*, 349(10), 931-40 (2003)

87. J. F. Burke, Jr., J. D. Pirsch, E. L. Ramos, D. R. Salomon, D. M. Stablein, D. H. Van Buren and J. C. West: Long-term efficacy and safety of cyclosporine in renal-transplant recipients. *N Engl J Med*, 331(6), 358-63 (1994)

88. M. Pascual, R. D. Swinford, J. R. Ingelfinger, W. W. Williams, A. B. Cosimi and N. Tolkoff-Rubin: Chronic rejection and chronic cyclosporin toxicity in renal allografts. *Immunol Today*, 19(11), 514-9 (1998)

89. P. F. Halloran: Call for revolution: a new approach to describing allograft deterioration. *Am J Transplant*, 2(3), 195-200 (2002)

90. J. P. Vella, M. Spadafora-Ferreira, B. Murphy, S. I. Alexander, W. Harmon, C. B. Carpenter and M. H. Sayegh: Indirect allorecognition of major histocompatibility complex allopeptides in human renal transplant recipients with chronic graft dysfunction. *Transplantation*, 64(6), 795-800 (1997)

91. M. H. Sayegh: Why do we reject a graft? Role of indirect allorecognition in graft rejection. *Kidney Int*, 56(5), 1967-79 (1999)

92. T. P. Theruvath, S. L. Saidman, S. Mauiyyedi, F. L. Delmonico, W. W. Williams, N. Tolkoff-Rubin, A. B. Collins, R. B. Colvin, A. B. Cosimi and M. Pascual: Control of antidonor antibody production with tacrolimus and mycophenolate mofetil in renal allograft recipients with chronic rejection. *Transplantation*, 72(1), 77-83 (2001)

93. A. Vongwiwatana, A. Tasanarong, L. G. Hidalgo and P. F. Halloran: The role of B cells and alloantibody in the host response to human organ allografts. *Immunol Rev*, 196, 197-218 (2003)

94. P. I. Terasaki and M. Ozawa: Predicting kidney graft failure by HLA antibodies: a prospective trial. *Am J Transplant*, 4(3), 438-43 (2004)

95. P. C. Lee, P. I. Terasaki, S. K. Takemoto, P. H. Lee, C. J. Hung, Y. L. Chen, A. Tsai and H. Y. Lei: All chronic rejection failures of kidney transplants were preceded by the development of HLA antibodies. *Transplantation*, 74(8), 1192-4 (2002)

96. S. Mauiyyedi, P. D. Pelle, S. Saidman, A. B. Collins, M. Pascual, N. E. Tolkoff-Rubin, W. W. Williams, A. A. Cosimi, E. E. Schneeberger and R. B. Colvin: Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol*, 12(3), 574-82 (2001)

97. H. Regele, G. A. Bohmig, A. Habicht, D. Gollowitzer, M. Schillinger, S. Rockenschaub, B. Watschinger, D. Kerjaschki and M. Exner: Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol*, 13(9), 2371-80 (2002)

98. F. Cardarelli, S. Saidman, T. Theruvath, N. Tolkoff-Rubin, A. B. Cosimi and M. Pascual: The problem of late allograft loss in kidney transplantation. *Minerva Urol Nefrol*, 55(1), 1-11 (2003)

99. C. Legendre, V. Garrigue, C. Le Bihan, M. F. Mamzer-Bruneel, M. L. Chaix, P. Landais, H. Kreis and S. Pol: Harmful long-term impact of hepatitis C virus infection in kidney transplant recipients. *Transplantation*, 65(5), 667-70 (1998)

100. K. Solez, F. Vincenti and R. S. Filo: Histopathologic findings from 2-year protocol biopsies from a U.S. multicenter kidney transplant trial comparing tarolimus versus cyclosporine: a report of the FK506 Kidney Transplant Study Group. *Transplantation*, 66(12), 1736-40 (1998)

101. J. M. Campistol and J. M. Grinyo: Exploring treatment options in renal transplantation: the problems of chronic allograft dysfunction and drug-related nephrotoxicity. *Transplantation*, 71(11 Suppl), SS42-51 (2001)

102. B. J. Nankivell, R. J. Borrows, C. L. Fung, P. J. O'Connell, R. D. Allen and J. R. Chapman: The natural history of chronic allograft nephropathy. *N Engl J Med*, 349(24), 2326-33 (2003)

103. M. Hojo, T. Morimoto, M. Maluccio, T. Asano, K. Morimoto, M. Lagman, T. Shimbo, M. Suthanthiran: Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature,* 397(6719), 530–534 (1999)

104. J. Dantal, M. Hourmant, D. Cantarovich, M. Giral, G. Blancho, B. Dreno and J. P. Soulillou: Effect of long-term immunosuppression in kidney-graft recipients on cancer incidence: randomised comparison of two cyclosporin regimens. *Lancet*, 351(9103), 623-8 (1998)

105. B. Kaplan, J. D. Schold and H. U. Meier-Kriesche: Long-term graft survival with neoral and tacrolimus: a paired kidney analysis. *J Am Soc Nephrol*, 14(11), 2980-4 (2003)

106. D. Cantarovich, M. Renou, A. Megnigbeto, M. Giral-Classe, M. Hourmant, J. Dantal, G. Blancho, G. Karam and J. P. Soulillou: Switching from cyclosporine to tacrolimus in patients with chronic transplant dysfunction or cyclosporine-induced adverse events. *Transplantation*, 79(1), 72-8 (2005)

107. I. Matl, O. Viklicky, L. Voska, A. Lodererova and S. Vitko: The effect of different immunosuppressive regimens on TGF-beta1 expression in kidney transplant patients. *Transpl Int*, 18(6), 668-71 (2005)

108. J. Minquillon, B. Morancho, S. J. Kim, M. Lopez-Botet, J. Aramburu: Concentrations of cyclosporin A and FK506 that inhibit IL-2 induction in human T cells do not affect TGF-beta1 biosynthesis, whereas higher doses of cyclosporin A trigger apoptosis and release of preformed TGF-beta1. *J Leukoc Biol*, 77(5), 748-758 (2005)

109. M. C. Roos-van Groningen, E. M. Scholten, P. M. Lelieveld, A. T. Rowshani, H. J. Baelde, I. M. Bajema, S. Florquin, F. J. Bemelman, E. de Heer, J. W. de Fijter, J. A. Bruijn and M. Eikmans: Molecular comparison of calcineurin inhibitor-induced fibrogenic responses in protocol renal transplant biopsies. *J Am Soc Nephrol*, 17(3), 881-8 (2006)

110. M. Meier, M. Nitschke, B. Weidtmann, W. J. Jabs, W. Wong, S. Suefke, J. Steinhoff and L. Fricke: Slowing the progression of chronic allograft nephropathy by conversion from cyclosporine to tacrolimus: a randomized controlled trial. *Transplantation*, 81(7), 1035-40 (2006)

111. J. D. Pirsch, J. Miller, M. H. Deierhoi, F. Vincenti and R. S. Filo: A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. FK506 Kidney Transplant Study Group. *Transplantation*, 63(7), 977-83 (1997)

112. R. Margreiter: Efficacy and safety of tacrolimus compared with ciclosporin microemulsion in renal transplantation: a randomised multicentre study. *Lancet*, 359(9308), 741-6 (2002)

113. T. R. McCune, L. R. Thacker, II, T. G. Peters, L. Mulloy, M. S. Rohr, P. A. Adams, J. Yium, J. A. Light, T. Pruett, A. O. Gaber, S. H. Selman, J. Jonsson, J. M. Hayes, F. H. Wright, Jr., T. Armata, J. Blanton and J. F. Burdick: Effects of tacrolimus on hyperlipidemia after successful renal transplantation: a Southeastern Organ Procurement Foundation multicenter clinical study. *Transplantation*, 65(1), 87-92 (1998)

114. M. A. Artz, J. M. Boots, G. Ligtenberg, J. I. Roodnat, M. H. Christiaans, P. F. Vos, P. Moons, G. Borm and L. B. Hilbrands: Conversion from cyclosporine to tacrolimus improves quality-of-life indices, renal graft function and cardiovascular risk profile. *Am J Transplant*, 4(6), 937-45 (2004)

115. S. Baid-Agrawal, F. L. Delmonico, N. E. Tolkoff-Rubin, M. Farrell, W. W. Williams, V. Shih, H. Auchincloss, A. B. Cosimi and M. Pascual: Cardiovascular risk profile after conversion from cyclosporine A to tacrolimus in stable renal transplant recipients. *Transplantation*, 77(8), 1199-202 (2004)

116. M. R. First: Improving long-term renal transplant outcomes with tacrolimus: speculation vs evidence. *Nephrol Dial Transplant*, 19 Suppl 6, vi17-vi22 (2004)

117. F. Vincenti, S. Firman, E. Scheuermann, L. Rostaing, T. Jenssen, J. M. Campistol, K. Uchida, M.D. Pescovitz, P. Marchetti, M. Tuncer, F. Citterio, A. Wiecek, S. Chadban, M. El-Shahawy, K. Budde, N. Goto on behalf of the DIRECT Investigators. Results of an International, randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. *Am J Transpl*, 7(1), 1-9 (2007)

118. C. Ponticelli: Chronic allograft nephropathy. *N Engl J Med*, 350(12), 1254-6; author reply 1254-6 (2004)

119. M. Mengel, W. Gwinner, A. Schwarz, R. Bajeski, I. Franz, V. Brocker, T. Becker, M. Neipp, J. Klempnauer, H. Haller and H. Kreipe: Infiltrates in protocol biopsies from renal allografts. *Am J Transplant*, 7(2), 356-65 (2007)

120. M. Mengel, J. R. Chapman, F. G. Cosio, M. W. Cavaille-Coll, H. Haller, P. F. Halloran, A. D. Kirk, M. J. Mihatsch, B. J. Nankivell, L. C. Racusen, I. S. Roberts, D. N. Rush, A. Schwarz, D. Seron, M. D. Stegall and R. B. Colvin: Protocol biopsies in renal transplantation: insights into patient management and pathogenesis. *Am J Transplant*, 7(3), 512-7 (2007)

121. J. H. Helderman: Long-term medical management of the renal transplant recipient: a consensus. *J Am Soc Nephrol*, 4(8 Suppl), S1 (1994)

122. M. R. Weir, L. Anderson, J. C. Fink, K. Gabregiorgish, E. J. Schweitzer, E. Hoehn-Saric, D. K. Klassen, C. B. Cangro, L. B. Johnson, P. C. Kuo, J. Y. Lim and S. T. Bartlett: A novel approach to the treatment of chronic allograft nephropathy. *Transplantation*, 64(12), 1706-10 (1997)

123. M. Pascual, W. W. Williams, A. B. Cosimi, F. L. Delmonico, M. L. Farrell and N. Tolkoff-Rubin: Chronic renal allograft dysfunction: a role for mycophenolate mofetil? *Transplantation*, 69(8), 1749-50 (2000)

124. I. Houde, P. Isenring, D. Boucher, R. Noel and J. G. Lachanche: Mycophenolate mofetil, an alternative to cyclosporine A for long-term immunosuppression in kidney transplantation? *Transplantation*, 70(8), 1251-3 (2000)

125. M. R. Weir, M. T. Ward, S. A. Blahut, D. K. Klassen, C. B. Cangro, S. T. Bartlett and J. C. Fink: Long-term impact of discontinued or reduced calcineurin inhibitor in patients with chronic allograft nephropathy. *Kidney Int*, 59(4), 1567-73 (2001)

126. B. Nashan: Maximizing the clinical outcome with mTOR inhibitors in the renal transplant recipient: defining the role of calcineurin inhibitors. *Transpl Int*, 17(6), 279-85 (2004

127. B. Suwelack, U. Gerhardt and H. Hohage: Withdrawal of cyclosporine or tacrolimus after addition of mycophenolate mofetil in patients with chronic allograft nephropathy. *Am J Transplant*, 4(4), 655-62 (2004)

128. G. Bohmig and H. Regele: Diagnosis and treatment of antibody-mediated kidney allograft rejection. *Transpl Int*, 16(11), 773-87 (2003)

129. A. V. Mulay, S. Cockfield, R. Stryker, D. Fergusson and G. A. Knoll: Conversion from calcineurin inhibitors to sirolimus for chronic renal allograft dysfunction: a systematic review of the evidence. *Transplantation*, 82(9), 1153-62 (2006)

130. D. Abramowicz, D. Manas, M. Lao, Y. Vanrenterghem, D. Del Castillo, P. Wijngaard and S. Fung: Cyclosporine withdrawal from a mycophenolate mofetil-containing immunosuppressive regimen in stable kidney transplant recipients: a randomized, controlled study. *Transplantation*, 74(12), 1725-34 (2002)

131. D. Abramowicz, M. Del Carmen Rial, S. Vitko, D. del Castillo, D. Manas, M. Lao, N. Gafner and P. Wijngaard: Cyclosporine withdrawal from a mycophenolate mofetil-containing immunosuppressive regimen: results of a five-year, prospective, randomized study. *J Am Soc Nephrol*, 16(7), 2234-40 (2005)

132. P. J. Smak Gregoor, T. van Gelder, N. M. van Besouw, B. J. van der Mast, I. J. JN and W. Weimar: Randomized study on the conversion of treatment with cyclosporine to azathioprine or mycophenolate mofetil followed by dose reduction. *Transplantation*, 70(1), 143-8 (2000)

133. P. Schnuelle, J. H. van der Heide, A. Tegzess, C. A. Verburgh, L. C. Paul, F. J. van der Woude and J. W. de Fijter: Open randomized trial comparing early withdrawal of either cyclosporine or mycophenolate mofetil in stable renal transplant recipients initially treated with a triple drug regimen. *J Am Soc Nephrol*, 13(2), 536-43 (2002)

134. M. Hazzan, M. Labalette, M. C. Copin, F. Glowacki, F. Provot, F. R. Pruv and C. Noel: Predictive factors of acute rejection after early cyclosporine withdrawal in renal transplant recipients who receive mycophenolate mofetil: results from a prospective, randomized trial. *J Am Soc Nephrol*, 16(8), 2509-16 (2005)

135. H. Ekberg, J. Grinyo, B. Nashan, Y. Vanrenterghem, F. Vincenti, A. Voulgari, M. Truman, C. Nasmyth-Miller and M. Rashford: Cyclosporine sparing with mycophenolate mofetil, daclizumab and corticosteroids in renal allograft recipients: the CAESAR Study. *Am J Transplant*, 7(3), 560-70 (2007)

136. W. Wong, J. P. Venetz, N. Tolkoff-Rubin and M. Pascual: 2005 immunosuppressive strategies in kidney transplantation: which role for the calcineurin inhibitors? *Transplantation*, 80(3), 289-96 (2005)

137. M. Pascual, J. Curtis, F. L. Delmonico, M. L. Farrell, W. W. Williams, Jr., R. Kalil, P. Jones, A. B. Cosimi and N. Tolkoff-Rubin: A prospective, randomized clinical trial of cyclosporine reduction in stable patients greater than 12 months after renal transplantation. *Transplantation*, 75(9), 1501-5 (2003)

138. W. Wong, N. Tolkoff-Rubin, F. L. Delmonico, F. Cardarelli, S. L. Saidman, M. L. Farrell, V. Shih, W. C. Winkelmayer, A. B. Cosimi and M. Pascual: Analysis of the cardiovascular risk profile in stable kidney transplant recipients after 50% cyclosporine reduction. *Clin Transplant*, 18(4), 341-8 (2004)

139. M. Cantarovich, J. S. Barkun, J. I. Tchervenkov, J. G. Besner, L. Aspeslet, P. Metrakos : Comparison of neural dose monitoring with cyclosporine through levels versus 2-hr postdose levels in stable liver transplant patients. *Transplantation*, 66(12), 1621-1627 (1998)

140. E. Gotti, N. Perico, A. Perna, F. Gaspari, D. Cattaneo, R. Caruso, S. Ferrari, N. Stucchi, G. Marchetti, M. Abbate

and G. Remuzzi: Renal transplantation: can we reduce calcineurin inhibitor/stop steroids? Evidence based on protocol biopsy findings. *J Am Soc Nephrol*, 14(3), 755-66 (2003)

141. F. Moreso, D. Seron, M. Carrera, S. Gil-Vernet, J. M. Cruzado, M. Hueso, X. Fulladosa, R. Ramos, M. Ibernon, A. M. Castelao and J. M. Grinyo: Baseline immunosuppression is associated with histological findings in early protocol biopsies. *Transplantation*, 78(7), 1064-8 (2004)

142. T. A. Gonwa, D. E. Hricik, K. Brinker, J. M. Grinyo and F. P. Schena: Improved renal function in sirolimus-treated renal transplant patients after early cyclosporine elimination. *Transplantation*, 74(11), 1560-7 (2002)

143. R. W. Johnson, H. Kreis, R. Oberbauer, C. Brattstrom, K. Claesson and J. Eris: Sirolimus allows early cyclosporine withdrawal in renal transplantation resulting in improved renal function and lower blood pressure. *Transplantation*, 72(5), 777-86 (2001)

144. H. Kreis, R. Oberbauer, J. M. Campistol, T. Mathew, P. Daloze, F. P. Schena, J. T. Burke, Y. Brault, M. Gioud-Paquet, J. A. Scarola and J. F. Neylan: Long-term benefits with sirolimus-based therapy after early cyclosporine withdrawal. *J Am Soc Nephrol*, 15(3), 809-17 (2004)

145. A. Teutonico, P. F. Schena and S. Di Paolo: Glucose metabolism in renal transplant recipients: effect of calcineurin inhibitor withdrawal and conversion to sirolimus. *J Am Soc Nephrol*, 16(10), 3128-35 (2005)

146. C. G. Groth, L. Backman, J. M. Morales, R. Calne, H. Kreis, P. Lang, J. L. Touraine, K. Claesson, J. M. Campistol, D. Durand, L. Wramner, C. Brattstrom and B. Charpentier: Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. *Transplantation*, 67(7), 1036-42 (1999)

147. H. Kreis, J. M. Cisterne, W. Land, L. Wramner, J. P. Squifflet, D. Abramowicz, J. M. Campistol, J. M. Morales, J. M. Grinyo, G. Mourad, F. C. Berthoux, C. Brattstrom, Y. Lebranchu and P. Vialtel: Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. *Transplantation*, 69(7), 1252-60 (2000)

148. H. T. Tran, M. K. Acharya, D. B. McKay, M. H. Sayegh, C. B. Carpenter, H. J. Auchincloss, R. L. Kirkman and E. L. Milford: Avoidance of cyclosporine in renal transplantation: effects of daclizumab, mycophenolate mofetil, and steroids. *J Am Soc Nephrol*, 11(10), 1903-9 (2000)

149. F. Vincenti, E. Ramos, C. Brattstrom, S. Cho, H. Ekberg, J. Grinyo, R. Johnson, D. Kuypers, F. Stuart, A. Khanna, M. Navarro and B. Nashan: Multicenter trial

exploring calcineurin inhibitors avoidance in renal transplantation. *Transplantation*, 71(9), 1282-7 (2001)

150. S. N. Sehgal: Rapamune (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. *Clin Biochem*, 31(5), 335-40 (1998)

151. A. Asberg, K. Midtvedt, P. D. Line, J. Narverud, H. Holdaas, T. Jenssen, A. V. Reisaeter, L. F. Johnsen, P. Fauchald and A. Hartmann: Calcineurin inhibitor avoidance with daclizumab, mycophenolate mofetil, and prednisolone in DR-matched de novo kidney transplant recipients. *Transplantation*, 82(1), 62-8 (2006)

152. F. Vincenti: Interleukin-2 receptor monoclonal antibodies in renal transplantation: current use and emerging regimens. *Transplant Proc*, 33(7-8), 3169-71 (2001)

153. S. M. Flechner, D. Goldfarb, C. Modlin, J. Feng, V. Krishnamurthi, B. Mastroianni, K. Savas, D. J. Cook and A. C. Novick: Kidney transplantation without calcineurin inhibitor drugs: a prospective, randomized trial of sirolimus versus cyclosporine. *Transplantation*, 74(8), 1070-6 (2002)

154. S. M. Flechner, S. M. Kurian, K. Solez, D. J. Cook, J. T. Burke, H. Rollin, J. A. Hammond, T. Whisenant, C. M. Lanigan, S. R. Head and D. R. Salomon: De novo kidney transplantation without use of calcineurin inhibitors preserves renal structure and function at two years. *Am J Transplant*, 4(11), 1776-85 (2004)

155. S. M. Flechner, D. Goldfarb, K. Solez, C. S. Modlin, B. Mastroianni, K. Savas, D. Babineau, S. Kurian, D. Salomon, A. C. Novick and D. J. Cook: Kidney transplantation with sirolimus and mycophenolate mofetil-based immunosuppression: 5-year results of a randomized prospective trial compared to calcineurin inhibitor drugs. *Transplantation*, 83(7), 883-92 (2007)

156. S. M. Flechner, M. Glyda, S. Steinberg, M. B. Harler, for the ORION Trial Investigators: A Randomized, Open-Label Study To Compare the Safety and Efficacy of Two Different Sirolimus (SRL) Regimens with a Tacrolimus (Tac) and Mycophenolate Mofetil (MMF) Regimen in De Novo Renal Allograft Recipients: Renal Function Results from the ORION Study. *American Transplant Congress* Abstract #1141 (2007)

157. A. F. Hamdy, A. E. El-Agroudy, M. A. Bakr, A. Mostafa, M. El-Baz, M. El-Shahawy el and M. A. Ghoneim: Comparison of sirolimus with low-dose tacrolimus versus sirolimus-based calcineurin inhibitor-free regimen in live donor renal transplantation. *Am J Transplant*, 5(10), 2531-8 (2005)

158. T. R. Srinivas, J. D. Schold, G. Guerra, A. Eagan, C. M. Bucci and H. U. Meier-Kriesche: Mycophenolate mofetil/sirolimus compared to other common immunosuppressive regimens in kidney transplantation. *Am J Transplant*, 7(3), 586-94 (2007)

159. S. J. Swanson, D. A. Hale, R. B. Mannon, D. E. Kleiner, L. C. Cendales, C. E. Chamberlain, S. M. Polly, D. M. Harlan and A. D. Kirk: Kidney transplantation with rabbit antithymocyte globulin induction and sirolimus monotherapy. *Lancet*, 360(9346), 1662-4 (2002)

160. J. M. Grinyo, S. Gil-Vernet, J. M. Cruzado, A. Caldes, L. Riera, D. Seron, I. Rama and J. Torras: Calcineurin inhibitor-free immunosuppression based on antithymocyte globulin and mycophenolate mofetil in cadaveric kidney transplantation: results after 5 years. *Transpl Int*, 16(11), 820-7 (2003)

161. T. S. Larson, P. G. Dean, M. D. Stegall, M. D. Griffin, S. C. Textor, T. R. Schwab, J. M. Gloor, F. G. Cosio, W. J. Lund, W. K. Kremers, S. L. Nyberg, M. B. Ishitani, M. Prieto and J. A. Velosa: Complete avoidance of calcineurin inhibitors in renal transplantation: a randomized trial comparing sirolimus and tacrolimus. *Am J Transplant*, 6(3), 514-22 (2006)

162. M. H. Sayegh, L. A. Turka: The role of T-cell costimulatory activation pathways in transplant rejection. *N Engl J Med,* 338(25), 1813–1821 (1998)

163. A. D. Kirk, D. M. Harlan, N. N. Armstrong, T. A. Davis, Y. Dong, G. S. Gray, X. Hong, D. Thomas, J. H. Fechner, Jr. and S. J. Knechtle: CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci U S A*, 94(16), 8789-94 (1997)

164. C. P.Larsen, E. T. Elwood, D. Z. Alexander, S. C. Ritchie, R. Hendrix, C. Tucker-Burden, H. R. Cho, A. Aruffo, D. Hollenbaugh, P. S. Linsley, K. J. Winn, T. C. Pearson: Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature,* 381(6581), 434–438 (1996)

165. J. S. Crippin: What's new--what's hot in clinical science: American Transplant Congress 2004. *Am J Transplant*, 4(11), 1747-53 (2004)

166. P. J. Morris and N. K. Russell: Alemtuzumab (Campath-1H): a systematic review in organ transplantation. *Transplantation*, 81(10), 1361-7 (2006)

167. C. J. Watson, J. A. Bradley, P. J. Friend, J. Firth, C. J. Taylor, J. R. Bradley, K. G. Smith, S. Thiru, N. V. Jamieson, G. Hale, H. Waldmann and R. Calne: Alemtuzumab (CAMPATH 1H) induction therapy in cadaveric kidney transplantation--efficacy and safety at five years. *Am J Transplant*, 5(6), 1347-53 (2005)

168. A. Vathsala, E. T. Ona, S. Y. Tan, S. Suresh, H. X. Lou, C. B. Casasola, H. C. Wong, D. Machin, G. S. Chiang, R. A. Danguilan and R. Calne: Randomized trial of Alemtuzumab for prevention of graft rejection and preservation of renal function after kidney transplantation. *Transplantation*, 80(6), 765-74 (2005)

169. A. K. Sundberg, J. A. Roskopf, E. L. Hartmann, A. C. Farney, M. S. Rohr and R. J. Stratta: Pilot study of rapid steroid elimination with alemtuzumab induction therapy in kidney and pancreas transplantation. *Transplant Proc*, 37(2), 1294-6 (2005)

170. P. Tryphonopoulos, D. Weppler, D. M. Levi, S. Nishida, J. R. Madariaga, T. Kato, N. Mittal, J. Moon, G. Selvaggi, V. Esquenazi, P. Cantwell, P. Ruiz, J. Miller and A. G. Tzakis: Transplantation for the treatment of intra-abdominal fibromatosis. *Transplant Proc*, 37(2), 1379-80 (2005)

Abbreviations: CNI: calcineurin inhibitor; CsA: cyclosporine; MMF: mycophenolate mofetil; TAC: tacrolimus; SRL: sirolimus; IL2r-mAb: anti–interleukin-2 monoclonal antibody; DAC: daclizumab; Bsx: basiliximab; CAN: chronic allograft nephropathy; US: United States; GFR: glomerular filtration rate; HLA: human leukocyte antigen; LDL: low-density lipoprotein; SPKTx: simultaneous pancreas-kidney transplantation.

Key Words: Immunosuppression minimization, Kidney transplantation, Corticosteroid free, Calcineurin inhibitor sparing, Calcineurin inhibitor avoidance, Cyclosporin, Tacrolimus, Monoclonal antibodies, Tolerance, Review

Send correspondence to: Diego Cantarovich, MD, PhD, ITERT - Institut de Transplantation et de Recherche en Transplantation, Service de Nephrologie et d'Immunologie Clinique, Nantes University Hospital , 30 Boulevard Jean Monnet 44000, Nantes, France, Tel: 33-2-400-87440, Fax: 33-2-403-0962, E-mail : diego.cantarovich@chu-nantes.fr