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REVIEW

Calcineurin inhibitor minimization, withdrawal and avoidance protocols after kidney transplantation

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Summary

A nonquantitative summary of the current evidence suggests that calcineurin inhibitor (CNI) minimization and also CNI-free protocols are safe and efficient when used after the initial 3 months post-transplantation. In fact, the largest study so far showed that low-dose CNI in combination with mycophenolate mofetil (MMF) and steroids performed better than standard dose cyclosporine A (CsA). If CsA is used in combination with a mammalian target of rapamycin-Inhibitor (mTOR-I) considerable dose reduction of both drugs is required. A better choice than using both drug groups in lower doses together may be the withdrawal of CsA from this combination after 3–12 months. Later withdrawals or conversions to an mTOR-I failed to show additional benefit in terms of graft function or survival but caused less post-transplant malignancies. With improved short- and medium-term outcomes, this entity will become more of an issue. In fact, in some areas of the world, nowadays malignancies are the leading cause of death.

Introduction

Compliance with immunosuppressive therapy is mandatory to maintain adequate long-term renal allograft survival. Clinical trials of patients weaned off their immunosuppressive therapy under close observation of the investigators of the immune tolerance network basically failed so far. Although some patients remained virtually free of immunosuppressive drugs, the majority of patients experienced a biopsy-confirmed acute rejection (BCAR) and had to be restarted on their immunosuppressive regimen (http://www.immunetolerance.org) [1].

Traditionally the maintenance immunosuppressive regimen consists of a triple therapy of a corticosteroid, an anti-metabolite and either a calcineurin inhibitor (CNI) or a mammalian target of rapamycin (mTOR) antagonist.

This triple strategy was mainly designed based on empirical observations and some clinical trial evidence. Reasons for the lack of rational approaches are manifold but the absolute low volume of renal transplantation, the lack of valid surrogate markers for long-term outcomes including patient and graft survival, unclear reference range plasma levels in therapeutic drug monitoring (TDM) of immunosuppressant combination therapy over time as well as the lack of biomarkers for the patients' humoral and cellular immune response status.

Recently, sequential adaptation of the immunosuppressive regimen has been advocated in an attempt to incorporate the changing risk profiles in terms of BCAR, immunosuppressant side-effects and co-morbidities into the decision process. As recently shown by Nankivell *et al.* in their protocol biopsy series, frank and subclinical BCAR is of importance in the initial months after transplantation [2]. Thereafter, morphological features suggestive of CNI toxicity were observed, but BCAR is rare. The patients of this series were mainly treated with cyclosporine A (CsA) (93%), from 1999 onwards; mainly tacrolimus (TAC) was used. Both CNIs were prescribed in combination with either azathioprine (AZA) (62% of cases) or mycophenolate mofetil (MMF). Whether these

findings apply also to modern immunosuppressive regimen containing TAC and MMF remains unknown.

In a recent protocol-biopsy study from Canada, David Rush *et al.* did not find similar high rates of overt and subclinical BCAR in the TAC -based study as they did 10 years ago in an identical trial but using CsA-based immunosuppression [3,4]. Thus whether the dose-dependent nephrotoxicity observed in CsA-treated subjects is similar in TAC based regimen of comparable CNI doses is unclear [5].

However, besides intrinsic renal damage, CNIs exhibit several other side-effects which may want to be avoided if possible [6]. Among clinical important effects is the increased risk of post-transplant malignancy, which in some areas of the world is the main cause of death nowadays [7,8]. Other researchers found accelerated cardiovascular co-morbidity and arterial hypertension when using CsA- based immunosuppression [9]. Cosmetic problems such as hirsutism and gingival hyperplasia are the main cause that in health-related quality of life studies, CsA-free regimen containing sirolimus (SRL) immunosuppression was favored by the majority of patients studied [10].

The main other CNI, TAC, on the other hand causes sometimes neurotoxicity leading to severe tremors as well as impaired glucose metabolism contributes to new onset diabetes after transplantation [11].

A potential alternative to the CNI-based regimens are mTOR-inhibitor therapies. So far SRL and everolimus (ERL) are the two available compounds of this class. Although lacking intrinsic nephrotoxicity and exhibiting sufficient immunosuppressant activity, other side-effects have been described that may limit the usage of this drug. On the other hand, some of the nonimmunosuppressant effects of this compound may actually turn out to be clinically most useful. Especially the strong anti-growth effect is currently being studied in patients with renal cell cancer in their native kidneys [12]. Furthermore, recent experimental data suggest that mTOR-I may cause less glucose utilization problems. Michael Hall *et al.* who actually discovered mTOR in 1991 and pursued basic science in that field ever since, showed that the same pathway that is blocked by the antidiabetic drug metformin is inhibited downstream by the mTOR-I SRL (unpublished data and [13]).

Besides CNI avoidance or minimization protocols using mTOR-I, other studies on immunosuppression have been conducted to evaluate safety and efficacy of co-stimulation blockade in human renal transplantation [14].

The introduction of CNI-based regimens in the early 1980s has dramatically improved short-term outcomes after renal transplantation [15]. Immunological causes of graft failure within the first year are the rare exceptions nowadays but graft loss thereafter remained virtually unchanged over the last decades [16]. Although it is evident that chronic deterioration of allograft function is a multifactorial process, immunosuppressant nephrotoxicity contributes to this enigma. As a consequence, many recent studies evaluated the safety and efficacy of CNI minimization or free immunosuppressive regimens after renal transplantation. The idea is to find equally potent and well-tolerated regimens that are CNI-free or only with minimal CNI exposure and thus potentially minimizing side-effects such as nephrotoxicity.

This review will critically discuss and summarize only results derived from randomized controlled trials (RCT) of CNI-sparing or -minimization protocols (Table 1). Retrospective, nonrandomized or compound analyses and experimental data were not considered in this overview.

Calcineurin inhibitor avoidance protocols

Versus mTOR-I

The largest RCT in renal transplantation was recently published by Ekberg *et al.* [17]. The ELITE-SYMPHONY study (Efficacy Limiting Toxicity Elimination – Symphony) was designed to evaluate the effect and safety of four immunosuppressive regimens. The authors equally randomized 1645 renal transplant recipients to either standard-dose CsA (150–300 ng/ml trough for 3 months, then 100–200 ng/ml), MMF and corticosteroids (S), or daclizumab induction, MMF and S in combination with low-dose CsA, low-dose TAC, or low-dose SRL. Low-dose CsA was considered a trough serum concentration of 50–100 ng/ml, low-dose TAC troughs were 3–7 ng/ml and low-dose SRL troughs 4–8 ng/ml. The mean glomerular filtration rate (GFR) at 1 year was significantly higher in the TAC arm (65 ml/min) when compared with all others. BCAR averaged 12% in the TAC stratum, 24% in the low-CsA group, 26% in the standard CsA patients and 37% in the SRL arm. Allograft survival was significantly better in TAC patients (94%) when compared with standard dose CsA (89%) and low-dose SRL (89%). Based on these data the author concluded that low-dose TAC is the preferred therapy in the first year after renal transplantation.

The conclusion is certainly right and supports what has become now the standard immunosuppressive regimen for the initial months after kidney allografting in many transplant centers. However, one point of criticism was that the defined SRL trough levels were too low. In fact, so far all SRL studies in *de novo* patients over the last decade used SRL troughs of 10–20 ng/ml when no induction with a lymphocyte-depleting antibody was used. This may explain the considerably high BCAR rates in SYMPHONY. On the other hand, the low-dose SRL arm

Table 1. Summary of CNI avoidance, withdrawal, minimization and conversion RCTs in renal transplantation. Some trials would fit in more than one category but are listed only in one. (a) CNI-avoidance trials; (b) CNI-withdrawal trials; (c) calcineurin inhibitor minimization trials; and (d) conversion trials.

Author	Publication	Study design	Results
(a)			
Flechner ORION	2008	450 pts SRL + MMF + S TAC + MMF + S SRL + TAC-elimination at 12 weeks + S	No difference in patients and graft survival or GFR, but SRL + MMF + S arm due higher rate of BCAR discontinued
Ekberg SYMPHONY	2007	1645 pts standard dose CsA low-dose CsA low-dose SRL low-dose TAC	TAC group with highest GFR, lowest BPAR rate
Wyeth 0468H1-318-EU	discontinued	SRL + MMF + S	Higher rate of BCAR
Büchler	2007	145 pts 5 days of ATG induction SRL + MMF + S CsA + MMF + S	GFR not different at 12 months (60 ± 27 vs. 57 ± 21 ml/min)
Flechner	2007	61 pts Basiliximab induction SRL + MMF + S CsA + MMF + S	SRL group with longer graft survival, higher GFR
Martinez-Mier	2006	41 pts Basiliximab induction SRL + MMF + S CsA + MMF + S	No difference in patients and graft survival and GFR at 1 year
Vincenti	2005	218 pts Basiliximab induction CsA + MMF + S intensive Belatacept + MMF + S less intensive Belatacept + MMF + S	Belatacept groups with higher GFR and less chronic allograft injury at 1 year
(b)			
Ekberg CEASAR	2007	536 pts at 3 months after transplantation CsA withdrawal CsA low-dose CsA standard dose in combination with daclizumab induction, MMF + S	No difference in GFR, CsA withdrawal with higher rates of BCAR, best results with low-dose CsA
Legendre RMR study	2007	430 pts at 3 months after transplantation CsA withdrawal and increased SRL dose from CsA + SRL + S	Increase in BCAR in the first year, higher GFR, less allograft injury in biopsies at 3 years after CsA withdrawal, at 48 months longer graft survival rates
Hazzan	2006	108 pts at 3 months after transplantation CsA withdrawal MMF withdrawal from a triple therapy	CsA withdrawal with higher GFR and increased BCAR, biopsies at 1 year suggest ongoing humoral alloimmune response
Dudley C Creeping creatinine study	2005	122 pts with CAN 62 CsA withdrawal + MMF remained on CsA	58% vs. 32% pts stabilized their creatinine at 6 months after randomization
Baboolal	2003	87 pts at 3 months after transplantation on CsA + SRL + S CsA withdrawal CsA dose reduction	Higher GFR at 6 months after transplantation in CsA-withdrawal group
Abramowicz	2002	187 pts at 12–30 months after transplantation CsA tapered over 3 months	Lower serum creatinine at 6 months after withdrawal

Table 1. continued

Author	Publication	Study design	Results
(c)			
Tedesco-Silva 2306 & 2307	2007	493 pts low-dose CsA + 1.5 mg ERL + S low-dose CsA + 3 mg ERL + S 2307 rial with basiliximab induction	Less BCAR in 2307 trial, lower serum creatinine in low-dose ERL groups
Vitko B201	2005	588 pts CsA + MMF + S full dose CsA + 1.5 ERL + S full dose CsA + 3 ERL + S → CsA dose reduced	Both ERL groups with higher serum creatinine at one year → protocol amended Highest graft loss rates in 3 mg ERL group after 3 years; 1.5 mg ERL group similar to standard protocol
Lorber B251	2005	583 pts CsA + MMF + S full dose CsA + 0.5 mg ERL + S full dose CsA + 3 mg ERL + S → CsA dose reduced	Similar results as B201
Nashan B156	2004	111 pts CsA + MMF + S low-dose CsA + 3 mg ERL	Higher GFR in reduced dose CsA group
(d)			
Schena FP CONVERT	2008 In press	830 pts between 6 months and 10 yrs after transplantation on triple CNI-based therapy SRL conversion CNI maintenance	Similar rates of BCAR, graft survival, and patient survival; lower malignancy rates in SRL patients
Mulgaonka SNT	2008 (abs)	305 pts between 1 and 6 months after transplantation on CNI + MMF + S SRL conversion CNI maintenance	Increased GFR in SRL group, similar rates of BCAR
Lebranchu CONCEPT	2008 (abs)	235 pts at 12 weeks after transplantation CNI withdrawal SRL conversion CNI maintenance	Higher GFR in CNI withdrawal and SRL group
Watson	2005	40 pts between 6 months and 8 years after transplantation on CNI-based therapy SRL conversion CNI maintenance	Improved GFR after CNI withdrawal in long-term pt
Gallagher	2004	489 pts AZA + S CsA alone AZA + S conversion after short time CsA	No difference in patient and graft survival at 15 years, but in case of events occurring during first yr graft survival higher in CsA-withdrawal group, lowest serum creatinine in CsA-withdrawal group

experienced also a higher rate of adverse events and withdrawals.

Similar trials sponsored by Wyeth such as the ORION trial (Optimizing Renal Transplant Immunosuppression to Overcome Nephrotoxicity) and the '318 study' found also BCAR rates above 30%. Consequently the data safety monitor board suggested discontinuation of the SRL + MMF study arm in ORION and stopped the '318 trial'. Inadequate study performance and monitoring in many centers in the ORION trial are a valid explanation for the resulting observations (see below).

The ORION trial has only been presented in abstract form so far [18]. It is a three-arm study of 450 *de novo* patients evaluating a SRL + MMF + S combination, a SRL + TAC-elimination at 12 weeks + S versus a standard regimen consisting of TAC + MMF + S. All patients received daclizumab induction therapy. At 2 years, patient and graft survival and GFR were not different between groups. The urinary proteinuria to creatinine ratio (UPr/Cr) was significantly higher in both SRL-containing arms when compared with the TAC group.

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Retrospective analyses of the data showed a strong center inhomogeneity in terms of study administration. Of the 56 study sites, the 15 centers where more than 50% of patients were discontinued observed also the highest BCAR rates. Furthermore, 43 centers enrolled less than five patients in this study. When investigators looked into center details, it was obvious that protocol violations had occurred. Specifically, SRL trough levels were below the reference range in 43% of cases with BCAR and in a considerable number of patients with BCAR, trough levels were not measured for weeks after transplantation!

A similar study showed also a higher rate of BCAR in 319 *de novo* patients randomized to basiliximab induction, MMF + S and SRL versus CsA immunosuppression (18% vs. 3%) [19]. The Wyeth-sponsored study 0468H1-318-EU was discontinued in 2006 as a consequence of this finding. Despite the fact of a higher rejection rate in the SRL arm, the GFR at 1 year was not inferior to the CsA group patients. A similar finding was observed in the study of the SPIESSER group.

Büchler *et al.* randomized 145 *de novo* patients after 5 days of polyclonal antibody induction to SRL + MMF + S or CsA + MMF + S [20]. Corticosteroids were withdrawn at month 6 in subjects with more than one BCAR or steroid-resistant rejection. At 1 year, patient and graft survival averaged 97% vs. 97% and 93% vs. 90% respectively. The rates of BCAR were 14% and 9% respectively and steroids could be withdrawn in 83% and 84% of the patients. Also, similar good results could be achieved in a single center study from the Cleveland clinic [21].

Flechner *et al.* randomized 61 patients to the same regimen as above with the exception that basiliximab instead of antithymocyte globuline (ATG) induction was used. The authors followed their patients up to 5 years and managed to perform protocol biopsies in 87% of cases at baseline and year 2. Patients treated with the SRL regimen exhibited longer functional graft survival (96% vs. 77%, $P = 0.027$), higher GFR (67 vs. 51 cc/min, $P = 0.008$), and fewer graft losses from chronic allograft injury. The BANFF biopsy scores at year 2 were an independent predictor of GFR at year 5. Based on these long-term findings the authors concluded that SRL use in *de novo* patients of low to moderate risk is safe and effective.

Martinez-Mier *et al.* conducted a similar RCT as Flechner in 41 live donor renal transplant recipients [22]. This single center study from Mexico found a numerically higher rate of BCAR in the first year in the SRL arm, which however was not statistically significant (17% vs. 5%). Furthermore, no patient who was maintained within SRL target levels between 10 and 15 ng/ml experiences BCAR. Patient and graft survival and GFR were not different between study arms at 1 year.

In summary, SRL may be used for selected *de novo* patients in combination with MMF and steroids if a lymphocyte depleting antibody induction is used. The trough levels within the first year should not be lower than 10 ng/ml. Whether CNI-free regimens using mTOR-I maintenance therapy is efficacious in the long term will be discussed in the paragraphs below where withdrawal and conversion studies are presented.

Versus co-stimulation blockers

An alternative to using mTOR-I as substitute for CNIs is the administration of a co-stimulation blocker in *de novo* patients. Vincenti *et al.* studied the short-term safety and efficacy of belatacept in 193 low- and 25 high-risk patients in a three-arm trial [14]. Two different belatacept doses were used together with MMF and steroids, the comparator arm was CsA + MMF + S. All patients received basiliximab induction therapy. The rates of clinically suspected and BCARs at 6 months were 7%, 6% and 8% respectively. The measured GFRs in a subset of patients at 12 months were significantly higher in the belatacept groups (66, 62 ml/min) when compared with the CsA group (54 ml/min). Protocol biopsies at 1 year in roughly 50 out of the 70 patients in each group showed less chronic allograft injury in the belatacept patients. Thus, based on these findings the authors concluded that belatacept is not inferior to CsA-based therapy and may lead to less allograft injury. These promising results led to the initiation of two larger RCTs, the BENEFIT and BENEFIT-EXT study (Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial). Both trials are currently performed in roughly 100 and 80 centers worldwide respectively. Sample size in BENEFIT is 667 and 540 in the extension study. The last patient was enrolled in mid 2007 and first results are expected to be presented in mid 2009.

Calcineurin inhibitor withdrawal studies

No dose increase of concomitant immunosuppressants

Hazzan *et al.* recently published the 2-year findings of 108 stable transplant recipients that were randomized to either CsA or MMF withdrawal from a triple immunosuppressive therapy at 3 months after transplantation [23]. CsA withdrawal caused an increase of BCAR which was statistically significant, the overall numbers were low however (10 vs. 3 patients). Protocol biopsies obtained at 1 year showed more diffuse and/or focal C4d staining suggestive of ongoing humoral alloimmune response. Despite this fact, calculated GFR was higher without CsA than without MMF (46 vs. 38 ml/min). Within a period of 2 years, 12 out of 54 patients in the CsA-withdrawal

group were restarted on CsA and 18 out of 54 in the MMF-withdrawal group needed MMF subsequently. Based on this small study with 2 years of follow up it remains unclear whether an early CsA withdrawal is safe or promotes chronic allograft injury by activated humoral alloimmunity.

Another study that evaluated CsA withdrawal in *de novo* patients after renal transplantation was conducted by the CAESAR investigators (Cyclosporine Avoidance Eliminates Serious Adverse Renaltoxicity; [24]). The researchers randomized 536 patients to either CsA withdrawal over months 4–6 or CsA low- or CsA standard dose, all in combination with daclizumab induction, MMF and steroids and followed the study participants for 12 months. GFRs were not different between groups, although the CsA-withdrawal group exhibited a higher rate of BCAR (38%) than the low (25%) and the standard dose CsA (27%). From this trial, it may be concluded that low-dose CsA resulted in slightly better outcome, although reduction of the CsA dose did not translate into an improvement of renal function.

The CsA withdrawal in maintenance patients 12–30 months after transplantation was investigated by Abramowicz *et al.* [25]. CsA was tapered over a period of 3 months and the patients followed for another 6 months. In the intention to treat (ITT) analysis, the serum creatinine at 6 months after withdrawal was numerical lower, in the per protocol analysis which excluded patients with BCAR, this difference was also statistically significant (126 vs. 136 μmol/l, $P = 0.015$ vs. baseline). Although the trial was randomized, GFR was unequal at baseline between groups and no statistical information is provided on the direct comparison of GFR between the two groups at 6 months after CsA was withdrawn. The 5-year follow up study was published in 2005 [26]. The authors reported that CsA withdrawal from a MMF-containing regimen resulted in improved renal function but also in a higher rate of graft losses (12% vs. 8%).

In summary, withdrawal of CsA without increased concomitant immunosuppression may not be wise. Therefore, a potential alternative may be the increase of nonCNI-based concomitant therapy after CsA withdrawal, which will be discussed below.

Dose increase of concomitant immunosuppressants

The RMR study (Rapamune Maintenance Study) was designed to evaluate whether CsA could be withdrawn from stable patients 3 months after transplantation from a regimen consisting of CsA + SRL + S [27]. Patients with adequate renal function and absence of BANFF III BCAR were randomized 1:1 to CsA withdrawal over a period of 4 weeks and a concomitant increase of the SRL dose to reach trough levels above 20 ng/ml using an immunoassay. This trough corresponds to roughly 15 ng/ml determined by HPLC assays. About 93% of subjects randomized to CsA withdrawal were CsA-free at 3 months after random allocation. Although CsA withdrawal caused an increase in BCAR in the first year, calculated GFR was higher throughout the remaining 4 years of the study. Morphology at 3 years in the subset of subjects that received protocol biopsies showed less allograft injury when compared with the patients that remained on the CsA + SRL combination. At 48 months after enrollment, the rate of graft survival was significantly higher in CsA-free subjects.

This trial has been criticized years after it started, because evidence from other trials arose that a combination of full dose CsA plus even a reduced dose of SRL, as was used in the RMR study, might potentiate the CsA nephrotoxicity. Therefore, other investigator evaluated whether a reduced dose of CsA together with an mTOR-I might deliver better results (see ERL studies in the CsA minimization studies paragraph below).

Dudley *et al.* investigated whether CsA withdrawal and concomitant addition of MMF in patients with chronically progressing renal allograft failure will result in stabilization of serum creatinine 6 months after CsA withdrawal [28]. The authors found that 58% of the 62 patients randomized to CsA withdrawal stabilized their creatinine but only 32% of the 60 patients randomized to be maintained on CsA. As the incidence of BCAR was not different between the groups the authors concluded that CsA withdrawal and MMF initiation might be beneficial for patients with chronic allograft nephropathy.

Conversion trials

Between 1983 and 1986, colleagues from Australia randomized patients receiving their first renal allograft to either AZA + S (which was the standard therapy at that time) or to CsA alone or to AZA + S conversion after short-time CsA use. The study was intended to evaluate CsA which was just introduced as immunosuppressant for renal transplantation. In 2004, the investigators reported the 15-year follow up data [29]. No differences in patient and graft survival were found, but when events occurring in the first year after engraftment were censored, graft survival was significantly higher in the CsA-withdrawal group when compared with the AZA + S or CsA only groups respectively (70% vs. 58% vs. 51%, $P = 0.01$). The CsA-withdrawal group exhibited also the lowest serum creatinine values between 3 months and 10 years after transplantation. Therefore, the authors concluded that long-term CsA use reduces long-term graft survival.

In 2005, colleagues from Cambridge performed a small RCT in 40 patients between 6 months and 8 years after

transplantation [30]. The investigators randomized the subjects who all were on a CNI-based protocol to SRL conversion or CNI maintenance. Median GFR that was around 37 ml/min at baseline significantly improved by almost 10 ml/min in the SRL group but decreased by 4 ml/min in the CNI group 1 year after randomization ($P = 0.004$ between groups). At this point, this small study found an impressive increase in GFR after CNI withdrawal in long-term patients. The next paragraph discusses whether the finings could be confirmed in the largest conversion study so far.

The prime study evaluating a conversion from a CNI-based triple regimen to a SRL-based triple regimen has recently been completed [31]. In this trial, 830 stable patients 6 months to 10 years after transplantation were randomized in a 2:1 ratio to have their CNI stopped and SRL started or to be maintained on their CNI regimen. Protocol biopsies at study entry and at 2 years after randomization were performed. The ITT showed no difference in GFR but the on-therapy analysis of the stratum with baseline GFR above 40 ml/min revealed a significantly higher GFR (63 vs. 60 ml/min, $P = 0.009$). The median UPr/Cr increased significantly after SRL conversion (0.87 vs. 0.48, $P < 0.001$). Rates of BCAR, graft survival, and patient survival were similar between groups but malignancy rates were significantly lower after conversion (4% vs. 11%, $P < 0.001$).

Incorporating the enormous amount of information provided by this trial into one conclusion is almost impossible. Our personal summary of this study however is that SRL and CNI-based regimens performed equally in many aspects but the statistically lower incidence of malignancy is certainly a benefit that needs to be considered when treating long-term maintenance patients because post-transplant malignancy is among the leading causes of death in this population [32].

A study evaluating an earlier conversion from CsA to SRL with concomitant MMF + S therapy is the SNT (Spare the Nephron Trial), which has only been presented in abstract form so far [33]. Investigators randomized 305 subjects on a CNI + MMF + S-based regimen 1–6 months after transplantation to SRL or continuation of their CNI protocol. The SRL trough target ranges were defined as 10 ng/ml in combination with 1.5 g of MMF and steroids. At final 2-year results of renal function will be presented at the ATC 08. In previous presentation, the authors showed that BCAR was below 10% in both arms and that converted patients showed an increase in GFR over the next 2 years but the patients maintained on the CNI-based protocol exhibited a drop in GFR over this time period.

A similar study also sponsored by Roche is the CONCEPT study, a French multicenter trial, which has also been presented only in abstract form so far [34]. In this trial, stable renal transplant recipients were randomized at 12 weeks, as was the case with SNT, to CNI withdrawal and SRL introduction or to CNI continuation. The primary efficacy endpoint included GFR at 1 year, secondary safety endpoints patient and graft survival and histology at 1 year. The interim analysis presented showed that CNI withdrawal and SRL introduction caused a significantly higher GFR at 1 year when compared with CNI maintenance patients (62 vs. 55 ml/min, $P = 0.01$).

The SNT and the CONCEPT studies suggest that if CNIs are withdrawn early enough, i.e. within months 1–6 after engraftment and substituted with a mTOR-I, the kidney graft has the potential to increase its GFR by more than 10% over the next few months. This benefit does not seem to be counterbalanced by a potentially increased risk of BCAR. Although the Watson paper found improved GFR in converted long-term maintenance patients, the considerably larger CONVERT trial did not confirm this finding. These findings suggest that conversion of subjects more than a year after transplantation is not as effective in increasing GFR as early exchange.

Conversion from a CNI to SRL may also be considered to reduce the high rate of post-transplant malignancies in patients on CsA.

Calcineurin inhibitor minimization studies

In combination with mTOR inhibitors

Most of the trials investigating CsA minimization in combination with a mTOR-I used ERL and were performed mainly for registration purposes.

As the initial registration studies of ERL using full dose CsA Neoral (RAD B201 and B251) with 1.5 or 3 mg ERL and steroids showed higher serum creatinine levels at 1 year than the reference arm on CsA + MMF + S, the protocol was amended and CsA doses reduced. The 3-year data of these studies have recently been published by Vitko *et al.* and Lorber *et al.* [35,36]. In the B201 trial, graft loss occurred in 7%, 17% and 11% of patients in the ERL 1.5, 3 mg, and MMF groups, respectively ($P = 0.005$). Based on these findings, the authors concluded that 1.5 mg of ERL but not the 3-mg group performed equally well as MMF in combination with CsA and steroids.

In the B251 trial, Lorber *et al.* reported equal incidences of primary efficacy failure (including BCAR, graft loss or death), which were in the range of 31–34% in all groups but discontinuation of patients from assigned therapy was more frequent in the ERL arms. On the basis of the findings in B201, the authors concluded that CsA-induced nephrotoxicity will require judicious lowering of CsA exposure with close TDM of ERL troughs.

In the RADB156 trial, 3 mg ERL with reduced dose CsA, defined as roughly half the regular troughs, showed a significantly higher GFR at 1 year when compared with regular dose CsA (62 vs. 51 ml/min, $P < 0.05$) [37].

Last year, Tedesco-Silva Jr *et al.* reported the compound analysis of the two Novartis registration trials 2306 and 2307 [38]. The study design was identical in both trials with the exception of basiliximab induction in 2307 and therefore lower CsA C2 target levels (600 vs. 1200 ng/ml). The two-arm studies compared efficacy and safety of 1.5–3 mg of ERL both with adjacent low-dose CsA and steroids. Low-dose CsA was defined as C2 TDM aim of 600 ng/ml until month 3 and then 400 ng/ml. The incidence of BCAR in the first year was 25% in the low-dose ERL arm and 15% in the 3 mg ERL stratum in the 2306 study. The 2307 trial, which used IL2-Ab induction found roughly 14% rejections in both arms within the first year. Both studies found lower serum creatinine concentrations at 1 year in the low-dose.

In summary, these trials showed that a combination of low-dose CsA and 1.5–3 mg ERL after induction therapy with an IL2-antibody was effective in prevention of BCAR and provided adequate 1- to 3-year graft function and survival. The lower ERL dose however was better tolerated.

CsA minimization in combination with SRL

Now if it turned out that a minimal dose of CsA in combination with mTOR-I is better than full dose CsA plus mTOR-I, the obvious legitimate question is how a complete withdrawal of CsA from this combination performs in comparison to a combo of minimal CsA plus mTOR-I. Baboolal *et al.* from the UK investigated whether CsA elimination from a SRL + S combination at 3 months yielded a higher 1-year GFR than a dose minimization of CsA (troughs of 50 ng/ml) in a combination with SRL [39]. The authors of this trial which randomized 87 patients found that a complete withdrawal caused a higher GFR at 6 months after transplantation (65 vs. 57 ml/min; $P = 0.03$).

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