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REVIEW

Calcineurin inhibitor-free immunosuppression in kidney transplantation

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Summary

The introduction of calcineurin inhibitors (CNI) revolutionized kidney transplantation (KTx). Exceptionally low acute rejection rates and excellent graft survival could be achieved with CNI-based (cyclosporine and tacrolimus) immunosuppressive protocols. However, despite short-term success, long-term graft attrition continues to be a significant problem, thus leaving clinicians looking for possible interventions. CNI nephrotoxicity is but one of numerous factors that may be contributing to long-term damage in transplant kidneys. Therefore, newer immunosuppressive agents such as mycophenolate mofetil and sirolimus (Rapa) have raised the possibility of withdrawing or avoiding CNIs altogether. Protocols exploring these options have gained greater attention over the last few years. Herein, we review studies addressing either CNI withdrawal or CNI avoidance strategies as well as discuss the risks versus benefits of these protocols. Given the accumulated experience to date, in our opinion, the use of CNIs as a part of immunosuppressive regimens remains the proven standard of care for renal transplant patients. The long-term safety and efficacy of CNI withdrawal and avoidance strategies need to be further validated in controlled clinical trials.

Immunosuppressive regimens for kidney transplantation (KTx) have evolved significantly over the last 45 years. The introduction of cyclosporine (CsA), a calcineurin inhibitor (CNI) in the early eighties, led to dramatically improved outcomes in all organ transplants and established KTx as the therapy of choice for end stage renal disease [1].

Current immunosuppressive maintenance regimens after KTx include typically a CNI, either CsA or tacrolimus (TAC), with the addition of an antiproliferative agent, most commonly mycophenolate mofetil (MMF), and corticosteroids [2]. CNI-based maintenance immunosuppression used in conjunction with increasingly efficacious induction regimens and MMF has been associated with marked decreases in acute rejection rates [2]. However, despite progressively decreasing acute rejection rates in recent years, the rate of long-term attrition in graft survival has remained surprisingly constant [3,4]. This apparent disconnect between short- and long-term outcomes can be explained by several factors. Probably most importantly, the increase in immunosuppression that has allowed for very low acute rejection rates, has also led to an incremental incidence of different opportunistic infections and malignancies. The recent epidemic of BK (polyoma) virus nephropathy is a disturbing example of a late complication leading to accelerated graft loss that may be a consequence of early over immunosuppression. BK virus nephropathy was virtually unknown 10 years ago when acute rejection rates were significantly higher; while presently it affects a significant proportion of patients and has become a significant cause for graft loss. Another factor affecting long-term graft attrition might be CNI nephrotoxicity. Even though CNIs have revolutionized KTx by significantly decreasing acute rejection rates, these medications might contribute in the long-term to graft loss through their intrinsic nephrotoxic effects. In fact, pathologic lesions that may be partially attributed to CNI injury exists almost universally in renal

allografts 10 years after transplantation [5]. On the other hand, the pathogenesis of chronic allograft nephropathy is certainly multifactorial [5]. Immunologic causes of progression of chronic renal failure include late episodes of acute cellular rejection, recurrent rejection, persistent smoldering rejection or humorally mediated rejection [6]. Nonimmunologic factors include donor age, the quality of the allograft, nephrotoxicity attributed to CNIs, and development of or persistence of illnesses afflicting kidney transplants such as hypertension, diabetes, dyslipidemia, obesity and metabolic syndrome [6]. While nonimmunologic factors involved in long-term graft loss might be exacerbated by toxic effects of CNIs, any attempt to reduce the burden of these factors by reducing the exposure to CNIs is probably accompanied by the risk of triggering alloimmune factors, which can in turn drive long-term graft loss.

Shortly after the introduction of CsA in 1984, both acute and chronic toxic effects attributable to CsA became increasingly apparent. The acute toxicities of CNIs include hypertension, renal dysfunction and neurologic disturbances such as tremors and seizures. CNI related acute renal dysfunction may manifest as distinct pathologic entities: (i) acute vasculopathy; (ii) acute tubulopathy; (iii) chronic vasculopathy; and (iv) chronic interstitial (striped) fibrosis [7,8]. While the pathologic lesions of CNI toxicity are well described, CsA also has an immediate pharmacodynamic effect on renal vasculature that consists of afferent arteriole constriction. CsA-mediated afferent arteriolar constriction may manifest clinically as reversible concentration dependent fluctuations in glomerular perfusion and consequently raise serum creatinine levels; in and of itself not necessarily a manifestation of irreversible nephrotoxicity [7].

Calcineurin inhibitors have also been implicated in the pathogenesis of post-transplant diabetes mellitus, hypertension, hyperlipidemia and cosmetic stigmata [5,9,10]. While the CNIs have often been discussed as a class, differences between CsA and TAC became evident shortly after the introduction of TAC in the mid 1990s. Hypertension, hyperlipidemia and cosmetic side effects such as hirsutism are more common with CsA whereas impaired glucose tolerance, neurotoxicity and alopecia are more common with TAC. Some clinicians also feel that TAC may exhibit less nephrotoxicity; supporting this opinion is one study where pathologic evidence of decreased fibrogenicity with TAC was noted [11]. However, such evidence remains controversial to date as other studies have not reported any differences in profibrogenic effects between CsA and TAC at the renal or molecular level [12].

Nankivell *et al.* [5] demonstrated anatomic lesions (vasculopathy, interstitial fibrosis, and glomerulosclerosis), which they attributed to CNI effects in serial protocol biopsies of kidney allografts. These lesions increased in prevalence over time in the lifespan of an allograft [5]. However, lesions of vascular sclerosis, interstitial fibrosis, and moreover, in this observational series, the cumulative incidence of subclinical rejection was significantly greater in patients on CsA and azathioprine (AZA) than in those on TAC and MMF. Thus the exact and exclusive contribution of CNIs alone to the development of these lesions and more importantly the role of these lesions as predominant contributors to failure of the renal allograft over time is not necessarily as unequivocal as has been portrayed.

Interestingly, despite the tremendous gains in renal allograft outcomes accrued in the CNI era, the side effects attributed to these agents prompted inquiry into strategies designed to minimize or avoid CNIs upfront. While the side effects are numerous, the principal driving force behind these regimens has been the quest to preserve renal allograft function.

Given the excellent prophylaxis against acute rejection that CNIs afford in combination with induction agents, antiproliferatives and corticosteroids, any attempt at minimization or elimination of CNIs is necessarily accompanied by an increased risk of acute rejection. Thus, with the advent of newer, potent immunosuppressants, which do not have the intrinsic nephrotoxic potential of CNIs, such as MMF, sirolimus (Rapa), steroids, and most recently belatacept (LEA29Y), increasing attention has been directed to CNI avoidance/minimization protocols in recent years.

In this article, we will critically review different studies that have incorporated CNI minimization/withdrawal or upfront avoidance. We selected publications for inclusion in this review on the basis of a comprehensive search of the literature solely focusing on kidney transplants. We only included trials utilizing two or more immunosuppressive drugs in combination. Also, we used operational constructs to classify studies as follows: trials were classified as minimization studies when the intervention was restricted to CNI exposure reduction; CNI withdrawal trials involved initial CNI use with subsequent tapering off; and lastly, avoidance studies enrolled *de novo* recipients who either received CNI for less than one month or who were never initiated on CNIs. Particular attention has been directed to 'toxicity-sparing' regimens: CNI withdrawal with MMF/prednisone (pred) or AZA/prednisone; Rapa/MMF/prednisone; Rapa/prednisone; and belatacept/MMF/prednisone. The risks and benefits of CNI minimization, withdrawal, and avoidance in KTx are discussed based on review of published data both from clinical trials and registry-based studies.

CNI withdrawal/minimization

CNI withdrawal with MMF or AZA combined with corticosteroids

In the early experiences with CNI-based immunosuppression, CsA was used with AZA and corticosteroids [13]. With the aim of minimizing CsA nephrotoxicity, a few small single-center studies evaluated the safety and efficacy of withdrawing CsA shortly after transplantation while maintaining recipients on AZA and steroids. A meta-analysis of these numerous, small controlled trials (randomized and nonrandomized) revealed an 11% increase in incidence of acute rejection in recipients whose CsA was withdrawn compared to control recipients who remained on CsA ($P < 0.001$); graft survival did not differ significantly between the groups [14]. These findings led to the formulation of an alternative protocol incorporating CsA withdrawal from the CsA/AZA/prednisone regimen at 1-year post-transplantation in patients with stable allograft function. In this study, 165 out of 192 renal transplant recipients with no known acute rejection episodes at 1-year post-transplantation had CsA tapered gradually over twelve weeks, preceded by an increase in AZA and prednisone. For comparison, a historical control group of patients who remained on CsA or who had already undergone a CsA taper over 6 weeks at that center were evaluated. Acute rejection rates within 6 months were 9.1% with the gradual taper of CsA over 12 weeks when compared to 29.6% in recipients who underwent a rapid 6 week taper ($P < 0.01$). Importantly, 5-year graft survival did not differ significantly across the various groups (81.7% for patients on CsA, 88.9% for patients with a CsA taper over 6 weeks, and 81.5% with a 12 week CsA taper; $P > 0.05$). While CsA withdrawal did not impact long-term renal allograft survival in this setting, further evaluation of this study which employed historical controls warranted prospective trials (Table 1) [15].

Mycophenolate mofetil is an antiproliferative agent which, when used with CNIs and corticosteroids, affords excellent protection from acute rejection with no accompanying intrinsic nephrotoxicity [16,17]. In combination with a CNI, MMF is associated with a decreased relative risk of graft failure independent of its effects on acute rejection as opposed to AZA [18]. MMF also provides significant protection against long-term deterioration of renal allograft function [19] and prophylaxis against late rejection greater than a year out from transplantation [17]. Given the salutary effects of MMF on acute rejection, it was reasoned that MMF could potentially permit minimization or withdrawal of CNIs and thereby ameliorate or avoid CNIs' nephrotoxic effects.

In a prospective study conducted by Smak Gregoor *et al.*, individuals with stable renal graft function on maintenance CsA/prednisone regimen were randomized to undergo CNI withdrawal following conversion to either MMF or AZA. In this small study of 64 renal transplant recipients, as could be expected with a decrease in the overall immunosuppression, acute rejections did occur post-CNI withdrawal. Importantly, the incidence of acute rejection was significantly higher in patients taking AZA versus MMF (AZA: 36.7%; MMF: 11.8%; $P = 0.04$) (Table 1) [20]. This finding is in line with what we now know about the immunosuppressive efficacy of MMF with regard to both the prevention of acute rejection in *de novo* transplant recipients and prevention of late acute rejection [17,21–24]. As could be expected, interest in using AZA as primary immunosuppression after CsA withdrawal has waned based on the unacceptably high rates of acute rejection observed using this drug.

Several large prospective, controlled trials have subsequently investigated the impact of MMF regimens after CNI withdrawal. Abramowicz *et al.* [25] reported a multi-center trial involving 170 patients on CsA/pred maintenance with or without AZA at 12–30 months post-transplant. In this study, AZA was replaced by MMF over 3 months and all patients including those who were exclusively on CsA/prednisone at the beginning of the trial were then randomized to one of two treatment arms: MMF/prednisone or MMF/CsA/prednisone. With MMF treatment, renal function as measured by creatinine clearance, calculated glomerular filtration rate (GFR), and serum creatinine improved. Serum cholesterol levels decreased in the CsA withdrawal group as compared to recipients maintained on CsA. However, as could be expected, a moderate but statistically significant increase in acute rejection at 6 months occurred in the CsA withdrawal group versus controls (10.6% vs. 2.4%, respectively, $P = 0.03$) (Table 1) [25]. These patients were then followed-up for an additional 4 years; and the 5-year patient and graft survival rates were 93% and 88%, respectively, for the MMF group and 95% and 92%, respectively, for the CsA/MMF/prednisone group. Unfortunately acute rejection episodes increased to 16% in the CsA withdrawal group versus 1% in the control group ($P = 0.0029$) [26]. Similar findings were reported by Schnuelle *et al.* in 84 renal transplant recipients with stable graft function who were randomized after 3 months post-transplant to either CsA/prednisone or MMF/prednisone. The acute rejection rate observed in the CNI withdrawal group was 11%; yet both creatinine clearances and calculated GFRs had significantly improved in the MMF group.) However, follow-up time was limited only to 1 year (Table 1) [27].

In another trial, Land *et al.* also studied prospectively, 46 renal transplant recipients with stable graft function at 1-year post-transplantation and followed-up for a

Table 1. Calcineurin inhibitor withdrawal in patients with stable graft function.

Reference	No. of patients	Time of IS change	Induction	Maintenance	Acute rejection	Renal function improvement*	Comments
Heim-Duthoy *et al.* [15]	165	12 months	NR	AZA, Pred (CsA tapered over 12 or 6 weeks)	6 months: 9.1% with CsA tapered over 12 weeks vs. 29.6% when CsA tapered over 6 weeks	Yes	5-year graft survival: 81.7% on CsA 88.9% with CsA 6 week taper 81.5% with CsA 12 week taper
Smak-Gregoor *et al.* [20]	64	12 months	NR	MMF, AZA, Pred (CsA withdrawn)	AZA – 36.7% MMF – 11.8%	Yes	
Abramowicz *et al.* [25]	170	12–30 months	NR	MMF/Pred or CsA/MMF/Pred	6 months – 10.6% on no CsA vs. 2.4% on CsA 5 years – 16% on no CsA vs. 1% on CsA	Yes	Decrease in total and LDL CHL with CsA withdrawal 5-year patient and graft survival : no change b/n groups
Schnuelle *et al.* [27]	84	3 months	NR	CsA/Pred or MMF/Pred	5% on CsA 11% on MMF/Pred	Yes	Short F/U time of 1 year Improved BP after CsA w/ NSD in lipid profile
Land *et al.* [9]	46	12 months	NR	TAC/Pred or CsA/pred or MMF/Pred	11% on MMF/Pred	Yes	
Ekberg *et al.* (CAESAR Study) [28]	536	6 months	DZB (IL-2 blocker)	Low-dose CsA/MMF/Pred Standard dose CsA/MMF/Pred MMF/Pred (CsA taper over 2 months)	Low-dose CsA: 25.4% Standard dose CsA: 27.5% No CsA: 38%	No	NSD with BP and chol. b/n groups NSD in infections between the two groups
Oberbauer et al. (RMR Study) [46,47]	430	3 months	NR	Rapa/CsA/Pred or Rapa/Pred	Rapa/CsA: 6.5% Rapa/Pred: 10.2% ($P = 0.223$)	Yes	36 months: decrease in CAN index with Rapa/Pred 48 months death censored graft survival: 96.1% Rapa/Pred
Pearson *et al.* (Spare-the-Nephron Study) [51]	254	30–180 days	NR	CNI/MMF/Pred or Rapa/MMF/Pred	CNI: 12.3% vs. 6.3% on Rapa	Yes	Graft loss after 12 months: 5.3% on CNI vs. 2.1% on Rapa

W/, withdrawal; DZB, daclizumab; BXB, basilizimab; ATG, thymoglobulin; NR, not reported; CNI, calcineurin inhibitor; CsA, cyclosporine; TAC, tacrolimus; MMF, mycophenolate mofetil; Pred, prednisone; Rapa, sirolimus; belatacept, LEA29Y; mo, months; wk, week; b/n, between; BP, blood pressure; CHL, cholesterol; TG, triglycerides; sub, substitution; F/U, follow-up; pt, patient; SD, significant difference; NSD, no significant difference; plts, platelets; vs., versus; AZA, Azathioprine; IS, Immunosuppression.

*Renal function as measured by each individual study revealed improved outcome with change in IS regimen.

minimum of 2 years after conversion. They converted their patients from a CNI-based immunosuppressive regimen (TAC or CsA)/prednisone to a MMF/prednisone regimen. Follow-up was for a minimum of 2-year post-conversion. Acute rejections did occur in 11% of patients. Despite this incidence of acute rejection upon conversion from CNI/prednisone to MMF/prednisone, there was a decreased rate of graft loss compared to this center's historical control group. Importantly, renal function improved over the reported follow-up period (Table 1) [16]. These promising results are however derived from a relatively small group of patients from a single center and a relatively homogenous study population (European) devoid of a concurrent control group. Thus conclusions from this study are difficult to apply readily to more diverse groups of transplant patients.

Recently, the CAESAR (cyclosporine Sparing with MMF, daclizumab and corticosteroids in Renal Allograft

Recipients) trial investigated the possibility of maintaining recipients on a CNI for an abbreviated course (no longer than 6 months) or in reduced doses to avoid the unacceptable acute rejection rates found with the sole use of MMF/prednisone [25] and yet minimize CNI nephrotoxicity. Recipients of kidney transplants ($n = 536$) were either randomized in a 1:1:1 ratio to low-dose CsA/MMF/prednisone (target trough level of 50–100 ng/ml for 12 months), standard dose CsA (target trough level of 150–300 ng/ml up to month 4 and then 100–200 ng/ml thereafter)/MMF/prednisone, or CsA withdrawal (CsA taper starting at month 4 post-transplant and completed by month 6 post-transplant; remaining only on MMF and prednisone). All patients received an IL-2 receptor blocker (daclizumab) for induction. GFR did not differ significantly across the three groups at 12 months post-transplantation. Despite withdrawal of CsA at 6 months after transplantation along with an extended taper over a few months, acute rejection rates remained significantly higher in the CsA withdrawal group (38%) compared with the low-dose CsA (25.4%) and standard-dose CsA groups (27.5%), ($P < 0.05$) (Table 1) [28]. This study also did not achieve the hoped improvement in renal function that might have been expected in the low CsA exposure group or the CsA withdrawal group. Thus, the potential advantage of minimizing nephrotoxicity was probably offset by the deleterious impact on allograft function of the higher acute and or subclinical rejection rates.

Taken together, the two multicenter trials summarized above initially underscore the cardinal limitation to CsA withdrawal from MMF regimens: increased risk of acute rejection episodes [25,28]. Given, the potential deleterious effects of acute rejection episodes on graft survival and long-term graft function, extreme caution in patient selection and follow-up is advised when attempting these strategies [28].

Thus far, we have reviewed studies that focus on subjects with stable allograft function. However, as CNIs do have both acute and chronic deleterious effects on allograft function, interventions directed on patients with deteriorating renal function are of immediate interest to the clinician.

On the premise that MMF could make CSA sparing a safe approach in renal transplant recipients with known chronic allograft dysfunction, Ducloux *et al.* studied 31 kidney transplant recipients with known deterioration in renal function. These subjects had AZA substituted for MMF and also underwent concomitant CSA withdrawal. Over the follow-up period (average: 27 months), serum creatinine levels stabilized in the conversion group following an initial decrease ($P < 0.0005$). The final serum creatinine after conversion was significantly lower than the initial serum creatinine. Acute rejection episodes only occurred in two patients (6.5%) after 13 and 24 months of CSA withdrawal (Table 2) [29].

Table 2. Calcineurin inhibitor withdrawal in patients with declining graft function.

Reference	No of patients	Time of IS change	Induction	Maintenance	AR	Renal function improvement*	Comments
Ducloux *et al.* [29]	31			MMF/Pred (CsA W/ and MMF sub of AZA	6.5%	Yes	27 months of F/U
Afzali *et al.* [30]	89	>12 months	NR	MMF/Pred (CsA W/ or red. And MMF sub AZA)	<1%	Yes	17 patients had graft loss – 6 died with functioning graft
Weir *et al.* [31]	105	29 months	ATG or OKT3	Red. CsA or TAC/MMF/ Pred or MMF/Pred	<1%	NR	37.5% graft loss with reduced CSA 32% graft loss with reduced TAC 7.7% graft loss on MMF/Pred
Dudley *et al.* (creeping creatinine study) [32]	143	6 months	NR	CSA/MMF/Pred or MMF/Pred	NSD	Yes	
Suwelack *et al.* [33]	39	1 month	NR	CSA or TAC/MMF/Pred or MMF/Pred	No rejection	Yes	SD in BP at week 35: higher on triple regimen
Frimat *et al.* (reference study) [34]	103	12 months	NR	CsA/MMF/Pred or 50% decrease in CsA/MMF/Pred	No rejection	Yes	SD of TG in group with reduction group

W/, withdrawal; DZB, daclizumab; BXB, basilizimab; ATG, thymoglobulin; NR, not reported; CNI, calcineurin inhibitor; CsA, cyclosporine; TAC, tacrolimus; MMF, mycophenolate mofetil; Pred, prednisone; Rapa, sirolimus; belatacept, LEA29Y; mo, months; wk, week; b/n, between; BP, blood pressure; CHL, cholesterol; TG, triglycerides; sub, substitution; F/U, follow-up; pt, patient; SD, significant difference; NSD, no significant difference; plts, platelets; vs., versus; AZA, Azathioprine.

*Renal Function as measured by each individual study revealed improved outcome with change in IS regimen.

A similar observational study was conducted by Afzali *et al.* where 89 renal transplant recipients with either biopsy proven CAN or with declining graft function over several years after transplantation underwent conversion from AZA to MMF along with CsA withdrawal or reduction over 3 months. Once again, renal function improved significantly after conversion to MMF and CsA withdrawal/reduction as evidenced by the slopes of mean reciprocal serum creatinines. Acute rejection occurred in only one patient (Table 2) [30]. Unfortunately however, attribution of these results exclusively to the salutary effects of MMF on graft function versus the beneficial effects of CsA withdrawal is not a straightforward task [29,30].

The significant findings from the above studies have bolstered the growing awareness of the utility of MMF as an agent that affords sufficient acute rejection prophylaxis in the context of CNI withdrawal and in turn helps improve both short and long-term graft function. Thus, in the last decade, focus has shifted to the possibility of using MMF/prednisone regimens in patients with established renal dysfunction with the intent to improve graft function and survival. Weir *et al.* reported an observational cohort study where 105 renal transplant recipients with impaired kidney function (baseline creatinine of approximately 3 mg/dl or biopsy proven CAN) either had their CNI dose reduced or discontinued altogether while remaining on MMF and corticosteroids for maintenance immunosuppression. This protocol was applied on average 29 months after transplantation and follow-up after initiation of the protocol varied between 41 and 75 months. There were 24 graft failures (24 of 64; 37.5%) in the reduced CsA group, 9 in the reduced TAC group (9 of 28; 32%), and only one graft loss (1 of 13; 7.7%) in the CNI withdrawal group. The incidence of acute rejection did not differ significantly among the groups, occurring only in 6 out of the 105 patients, all of whom responded to pulse steroid therapy. These results are indeed encouraging. Based on solely these findings, the safety and efficacy of CNI withdrawal in recipients with impaired graft function can not be made because of the inevitable selection bias inherent in any nonrandomized study (Table 2) [31].

In a randomized clinical trial reported by Dudley *et al.* (MMF 'Creeping Creatinine Study Group') 143 patients who had a significant deterioration in renal function (by serial reciprocal values of serum creatinine) more than 6 months post-transplantation were either maintained on their CsA-based immunosuppressive regimen or withdrawn from CsA and maintained on only MMF and corticosteroids. A significant improvement in renal function was noted in patients maintained only on MMF and prednisone compared with those who continued to receive CsA. Most remarkably, there was no increase in acute rejection rates after withdrawal of CsA (Table 2) [32]. This study suggests that, in renal transplant patients with worsening renal function, CsA withdrawal with the addition of MMF confers a significantly better renal function and possibly improved graft survival compared with CsA maintenance therapy. In a smaller randomized trial reported by Suwelack *et al.* [28], MMF was used with or without CNI withdrawal in long-term renal transplant recipients who had biopsy-proven chronic allograft nephropathy and progressive deterioration of renal function. Calculated creatinine clearance and blood pressure measurements improved significantly in patients who underwent withdrawal of CsA or TAC compared to those that remained on CsA (or TAC)/MMF/prednisone. Notably, no acute rejections were noted for up to 35 weeks of follow-up [33]. The absence of acute rejection in this study could represent both small sample size and a relatively short follow-up period.

Similar findings have been noted even with CNI dose reduction while continuing MMF and prednisone in the 'Reference' study. In this study, reduction of CsA by 50% led to an increase in creatinine clearance by approximately 11% during 2 years of investigation. No episodes of biopsy-proven acute rejection occurred (Table 2) [34].

In summary, MMF-based CNI sparing studies in patients with chronic graft dysfunction show some potential for a beneficial impact on renal function and possibly delaying graft failure.

CNI withdrawal with Rapa/prednisone

Inhibitors of m-TOR (sirolimus and everolimus), which block proliferation signal provided by T-cell growth factors to T-cells have been used as immunosuppressive agents in renal transplantation [35]. The anticipated benefits of m-TOR inhibitors were many and included the ability of sirolimus (Rapa) to inhibit smooth muscle proliferation, inhibit antibody synthesis and promote tolerogenic immune responses [36]; however, to date, these benefits remain largely speculative.

For a brief period of time, Rapa was used in combination with CNIs, initially CsA and then TAC. The synergistic immunosuppression provided by the CsA/Rapa combination afforded acute rejection prophylaxis superior to the then standard regimen of CsA, AZA and corticosteroids [37]. However, serum creatinine levels were higher in CsA/Rapa/prednisone treated patients compared to those on CsA/AZA/prednisone. These findings were attributed at that time to the effects of full dose CsA [38]. Subsequently, in both prospective clinical trials and retrospective analyses, Rapa in combination with CNIs has been associated with inferior graft survival and renal

function compared to CsA or TAC with MMF and corticosteroids in KTx likely because of the potentiation of CNI nephrotoxicity by Rapa [35,39–42]. Over the years, in comparison with other commonly used CNI-based regimens in renal transplantation, the CNI/Rapa combination has fared the worst [43]. Thus, focus has shifted to investigating the use of Rapa with other immunosuppressive agents such as MMF and/or corticosteroids with the ultimate aim of withdrawing or avoiding CNIs altogether in transplantation [40–42,44].

In a phase II study and a subsequent, larger phase III trial, renal transplant patients were randomized at 3 months to discontinue CsA from their Rapa/CsA/prednisone regimen which resulted in a significantly better renal function at 6, 9, and 12 months in the CNI withdrawal group [45]. To date, the largest randomized, prospective study investigating this approach is the Rapamune Maintenance Regimen (RMR) trial. The RMR trial at three months randomized patients on triple therapy, Rapa-CsA-corticosteroids, to either continue this initial regimen unchanged or to a CsA withdrawal group with higher targeted SRL levels. The overall graft survival after 48 months was significantly better in the Rapa-corticosteroid arm compared with the triple therapy control arm, both when including death with a functioning graft (84.2% vs. 91.5%, $P = 0.024$) and when censoring for it (90.6% vs. 96.1%, $P = 0.026$). Also, the calculated GFR was significantly higher with the withdrawal of CsA (54.5 vs. 68.6 ml/min, $P < 0.001$). However, these results are not altogether unexpected as removal of CsA eliminates its contribution to the synergistic nephrotoxic effects observed with CsA/Rapa and by extension improved GFR. The incidence of biopsy-proven acute rejection was similar in the CsA maintenance (6.5%) and withdrawal groups (10.2%) ($P = 0.223$). As one may expect, between 3 and 6 months into the study, more acute rejections occurred in the CNI withdrawal group (Table 1) [46,47]. Analysis of protocol biopsies at 36 months revealed a significantly lower chronic allograft damage index in the Rapa-corticosteroid group. Tubular atrophy and inflammation were also lower at 12 and 36 months in the CsA withdrawal group [48].

A similar study, albeit on a smaller scale used the same strategy as the RMR trial [49]. Protocol biopsies performed on these patients revealed less chronic allograft nephropathy with the withdrawal of CNI and the severity of these lesions was significantly worse in the Rapa/CsA/prednisone arm compared with the CNI withdrawal group (90% in Rapa/CsA/prednisone versus 32% in Rapa/prednisone; $P < 0.05$) [49].

Taken together, initial results of these trials appear promising. However, as we noted above, the association of improved renal function with the elimination of CsA from the CsA/Rapa/prednisone regimen may merely reflect elimination of known synergistic nephrotoxic effects of CsA and Rapa [7,50]. Further validation of long-term efficacy for CNI elimination with Rapa maintenance will thus need evaluation trials that compare this strategy with a standard control group of a CNI combined with MMF.

CNI withdrawal with Rapa/MMF/prednisone

Most recently, there has been a growing drive to withdraw CNI with the use of Rapa/MMF/prednisone as triple-based immunosuppressive regimen. In the ongoing Spare-The-Nephron Trial, Pearson *et al.* have reported early results on the effects of substituting CNI with Rapa in stable renal transplant recipients on CNI, MMF and prednisone. 254 of 340 recipients on MMF, CsA or TAC and prednisone were randomized 30–180 days post-transplantation to discontinue their CNI and switch to an MMF/Rapa/prednisone regimen or to continue their current immunosuppressive regimen (CNI/MMF/prednisone). The primary endpoint of this trial was the percentage change in measured GFR 12-month postrandomization. Preliminary results were as follows: iothalamate GFRs increased by approximately 20% (19.2 mean percent +/– 42.6) from baseline in the MMF/Rapa group whereas those remaining on MMF/CNI only exhibited a 4.4% increase (including individuals taking TAC) (Table 1) [51]. Thus, preliminary data for MMF/Rapa regimen appeared promising in this study as well.

CNI avoidance

CNI avoidance with Rapa/MMF/prednisone

A few clinical trials have explored the possibility of CNI avoidance in an attempt to avoid altogether potential toxic effects of CNIs. The most extensively studied strategy has been to employ Rapa combined with MMF and corticosteroids *de novo* after KTx. Two open-labeled, randomized parallel-group trials were conducted in over 19 centers throughout Europe (Table 3) [52,53]. In these trials, the patients were randomized to receive either Rapa or CsA, in combination with AZA and steroids or MMF and steroids; follow-up being for 2 years. Calculated GFRs were significantly higher in Rapa versus CsA-treated patients (69.3 vs. 56.8 ml/min at 2 years, $P = 0.004$) [54].

Thus, following these trials several prospective trials have evaluated the outcome of Rapa/MMF/pred on graft survival with a view to mitigating immunologic graft loss while avoiding CNI nephrotoxicity. Flechner *et al.* performed a prospective study where 61 *de novo* renal transplant recipients receiving basiliximab for induction, MMF 2 g/day and corticosteroids, were randomized to either

Table 3. Calcineurin inhibitor avoidance in patients with stable graft function.

Reference	No of patients	F/U time	Induction	Maintenance	AR	Renal function improvement*	Comments
Vincenti *et al.* [62]	98	12 months	DZB	MMF/Pred	48% at 6 mo; 53% at 12 mo	Yes	Patients and graft survival – 97% and 96%
Flechner *et al.* [55]	61	12 months	BXB	Rapa/MMF/Pred or CsA/MMF/Pred	NSD	Yes	NSD in pt and graft survival NSD in lipid profile, but Rapa has increased TG
Kreis *et al.* [53]	78	12 months	NR	Rapa/MMF/Pred or CsA/MMF/Pred	Rapa – 27.5% vs. CSA – 18.4%	Yes	NSD in patients and graft survival NSD in lipid profile Rapa with higher incidence of low plts and diarrhea
Larson *et al.* [57]	165	13–47 months	ATG	Rapa/MMF/Pred or TAC/MMF/Pred	Rapa – 19% vs. TAC – 14%	NSD by 2 years	Higher incidence of chronic vascular changes with TAC vs. Rapa
Ekberg *et al.* (symphony study) [61]	1645	12 months	DZB	Low-dose CsA/MMF/Pred or Standard dose CsA/MMF/Pred or TAC/MMF/Pred or Rapa/MMF/Pred	CSA – 22% vs. TAC – 11% vs. Rapa – 33%	No (GFR red with Rapa)	Graft survival inferior with Rapa vs. TAC
Vincenti *et al.* (belatacept study) [64]	218	12 months	BXB	Intensive LEA29Y/MMF/Pred or Less intensive LEA29Y/MMF/Pred or CsA/MMF/Pred	Intensive LEA29Y 7% vs. Less LEA29Y 6% vs. CSA – 8%	Yes	Less CAN with LEA29Y Improved outcome of BP & chol with LEA29Y NSD rate of infection

W/, withdrawal; DZB, daclizumab; BXB, basilizimab; ATG, thymoglobulin; NR, not reported; CNI, calcineurin inhibitor; CsA, cyclosporine; TAC, tacrolimus; MMF, mycophenolate mofetil; Pred, prednisone; Rapa, sirolimus; belatacept, LEA29Y; mo, months; wk, week; b/n, between; BP, blood pressure; CHL, cholesterol; TG, triglycerides; sub, substitution; F/U, follow-up; pt, patient; SD, significant difference; NSD, no significant difference; plts, platelets; vs., versus.

*Renal Function as measured by each individual study revealed improved outcome with change in IS regimen.

Rapa ($n = 31$) or CsA ($n = 30$). The primary goal of this study was to compare the efficacy of Rapa/MMF/prednisone with a CsA/MMF/prednisone regimen as reflected in the incidence of acute rejection rates and renal function over a year. Patient and graft survival rates along with acute rejection incidence were not significantly different between the CsA and Rapa treated groups at both 6 and 12 months (Table 3) [55]. Furthermore, at 1 year, the authors noted both lack of deterioration of renal function and less histologic evidence for chronic renal injury by protocol biopsy in the Rapa/MMF/prednisone group. Comparable acute rejection rates and excellent short-term allograft and patient survival were noted both in recipients taking Rapa/MMF/prednisone and CSA/MMF/prednisone [55,56].

Another small prospective trial randomized *de novo* renal transplant patients into two arms: Rapa/MMF/prednisone ($n = 40$) or CSA/MMF/prednisone ($n = 38$). After evaluating these patients for 12 months, graft survival (92.5% Rapa; 89.5% CsA), patient survival (97.5% Rapa; 94.7% CsA), and the incidence of acute rejection (27.5% Rapa; 18.4% CsA) did not demonstrate any statistically significant difference between the groups, a result similar to those reported by Flechner *et al.* [53,56].

More recently, Larson *et al.* conducted a prospective trial wherein *de novo* renal transplant recipients were randomized to Rapa/MMF/prednisone ($n = 81$) or TAC/MMF/prednisone ($n = 84$). Follow-up ranged from 13 to 47 months. Graft function was comparable in the two groups at 1 and 2 years. At the end of the study, protocol biopsies revealed no pathologic difference in interstitial, tubular or glomerular findings between the two groups; however, there existed a higher incidence of chronic vascular changes with the TAC group compared with those on Rapa (43% vs. 26%, $P = 0.03$). Also, minimal variations in renal function existed between the two groups.

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In fact, glomerular filtration decreased slightly over 1 year in the TAC/MMF group with a greater decline observed in the Rapa/MMF group. However, at 1–2 years, there was no significant difference in mean GFR between the two groups. Acute rejection rates were comparable (Table 3) [57]. These results are encouraging; however, the trend towards nonimprovement of GFR at 1 year in the Rapa/MMF group in this study is against the prevailing mantra that CNI free immunosuppression will result in an improved renal function. Thus, it is difficult to extrapolate from these short-term results the long-term renal allograft prognosis in recipients on Rapa/MMF/prednisone. At this juncture, one must contrast the difference in renal function noted in the study of Larson *et al.* with that reported by Flechner *et al.* The CsA/MMF comparator in the study of Flechner *et al.* [58] could well have tilted their results towards inferior renal function as studies do suggest that the CsA/MMF combination may be associated with allograft function slightly inferior to the TAC/MMF combination.

It should be noted that each of the clinical trials alluded to above has limitations in terms of the number of patients enrolled and the accrued follow-up time. These constraints limit the widespread application of data from these studies to analyses directed at endpoints such as graft and patient survival which demand a greater number of subjects to afford sufficient statistical power to detect small differences in graft and patient survival. In this regard, analysis of large transplant databases such as the Scientific Registry of Renal Transplant Recipients (SRTR) becomes relevant [59].

Such analysis became feasible given the accrual of data as regard to Rapa/MMF usage throughout multiple centers over 7 years. With a view to evaluate outcomes with Rapa/MMF in renal transplantation, a comparison of that regimen with other commonly used regimens in renal transplantation was performed. Data used in this analysis were reported to the SRTR database between 2000 and 2005. This retrospective analysis assessed the impact of different immunosuppressive medications, in particular Rapa/MMF, on renal graft outcome in the USA. In deceased donor transplant recipients, Rapa/MMF versus TAC/MMF or CsA/MMF at 6 months post-transplantation was associated with a significantly lower graft survival at 5-year post-transplantation (64%, 78%, and 78%, respectively). Rapa/MMF was associated with a 75% increased risk for patient death (AHR = 1.75, 95% CI, 1.53–2.00; $P < 0.01$) relative to the TAC/MMF discharge regimen. Among both living and deceased donor transplants, 6-month acute rejection rates were the highest for individuals on the Rapa/MMF regimen (16.4% in living donor transplants and 15.8% in the deceased donor transplants) as compared to CsA or TAC/MMF and CsA or TAC/Rapa (approximately 10%) [43]. Thus, the Rapa/MMF regimen, in the context of clinical practice, appeared to be associated with outcomes inferior to other commonly used maintenance regimens in KTx. The inferior graft survival noted in the Rapa/MMF group possibly stemmed from varying contributions of high acute rejection rates and overall poor tolerability of this regimen. Importantly, interpretation of the results of this retrospective study should necessarily be tempered by the following caveats [60]. Firstly, this finding reflected the use of particular drug combinations in the relatively uncontrolled setting of clinical practice as opposed to a clinical trial conducted in a carefully predefined population. Secondly, many biases operate in the choice of immunosuppressive agents. For instance, it is entirely possible that higher risk transplants were selected to receive SRL/MMF or that this regimen was used preferentially for kidneys perceived to be at higher risk for nephrotoxic insults, such as kidneys from older or extended criteria donors. In this regard, multivariate statistical analysis does correct for some but not all the selection biases using such measures as on-treatment analysis of outcomes and analysis of outcomes across all patient subgroups. Lastly, the SRTR database does not contain any dosing or drug concentration data that can help interpret the effects of drug doses and exposure on transplant outcomes. Thus, any associations derived in such analyses, should only be extrapolated to the pattern of clinical use of particular drug combinations during the historic timeframes analyzed. Therefore, to establish causality between a drug and an outcome, one must necessarily integrate the results of retrospective studies with the results from randomized clinical trials.

Recently, the Symphony study was designed to evaluate low toxicity immunosuppressive regimes that could potentially both preserve adequate renal allograft function and achieve excellent graft survival. In the Symphony trial, standard-dose CsA-based regimens were compared with low-dose CsA, TAC or Rapa in combination with MMF, daclizumab and corticosteroids in renal transplantation. At 1 year, biopsy-proven acute rejection in Rapa/MMF patients was 33% vs. 11% with TAC/MMF ($P < 0.01$) and 22% with CsA/MMF. The calculated GFR was 57.3 ml/min with Rapa/MMF versus 65.4 ml/min with TAC/MMF ($P < 0.0001$). Lastly, 1-year graft survival was significantly inferior in Rapa/MMF patients (TAC/MMF: 94%; Rapa/MMF: 89%; $P = 0.017$) (Table 3) [61].

The Symphony study taken together with the preceding summary of the SRTR analysis does suggest that the Rapa/MMF immunosuppressive regimen when used in *de novo* renal transplant recipients falls short of preserving renal allograft function and improving survival.

CNI avoidance with MMF/prednisone

The MMF/prednisone regimen has also been investigated in *de novo* renal transplant recipients to a limited extent. One such study included 98 renal transplant patients with known low immunologic risks. Recipients received an IL-2 receptor blocker, daclizumab, as induction along with MMF (3 gm/day for the first 6 months and 2 gm/day thereafter) and corticosteroids. Over a 12-month period, patient and graft survival were excellent (97% and 96%, respectively). Disturbingly, the incidence of acute rejection at 1-year post-transplant was significantly high at 53%; in fact, 62% of patients were started on a CNI (Table 3) [62]. Such findings underscore the necessity of maintaining *de novo* renal transplant recipients (even those on potent agents such as MMF) on a CNI to minimize the risk of acute rejection. The question of how best to diminish CNI nephrotoxicity and in turn effect further improvement in long-term graft function remained unanswered. In summary, the MMF/prednisone regimen (with complete avoidance of CNI) in *de novo* transplant recipients has not had a favorable effect on graft survival and results to date do not support its use in renal transplant recipients.

CNI avoidance with belatacept/MMF

Thus far, we have discussed CNI avoidance in the context of relatively well established drug combinations. Recently, a novel approach to CNI avoidance was pursued in a multinational, multicenter randomized trial using belatacept (LEA29Y), which is not an FDA approved agent yet and currently in phase II trials in the US. Belatacept is a selective co-stimulation blocker that binds to surface costimulatory ligands (CD80 and CD86) of antigen presenting cells. Blockade of signal 2 inhibits T-cell activation [63] unlike CNI where T-cell activation is solely diminished. In a phase II trial, different doses of belatacept were administered along with MMF and steroids; the control group received CsA/MMF/prednisone. Similar acute rejection rates were observed between the belatacept and CsA groups (19% on LEA29Y vs. 18% on CsA). At 1-year, GFR was significantly higher with belatacept than with CsA and CAN was less commonly noted in protocol biopsy in belatacept treated patients (belatacept: 29%; CsA: 44%; $P < 0.05$). Favorable trends with regard to cardiovascular risk factors such as blood pressure, total cholesterol, and nonhigh-density lipoprotein cholesterol were also noted with belatacept [64]. Thus far, these promising preliminary results with belatacept suggest its safety and efficacy in *de novo* renal transplant recipients. However, these results do need to be validated in a phase III trial.

Discussion

The primary impetus to eliminate CNIs from immunosuppressive protocols in KTx has stemmed from concerns about their intrinsic nephrotoxic effects. However, even after careful consideration of the major studies directed at CNI minimization/avoidance, the question still remains: are we ready to give up completely on CNIs? We submit that based on the data thus far, the answer at this time is no.

Granted, CNIs may be associated with functional and morphologic manifestations that accompany progressive allograft failure [5,7]. However, one must also keep in mind that the best outcomes to date in renal transplantation have been realized in the CNI era. Certainly, studies reported by Nankivell *et al.*, showing evidence of anatomic lesions consistent with CNI effect in serial protocol biopsies of kidney allografts, offer a point of concern. However, by the same token, such studies are observational and therefore do not establish these CNI related lesions as the immediate and predominant cause of progressive renal dysfunction. In a recent report, Nankivell *et al.* have shown that lesions with morphology similar to CNI toxicity are less common in MMF treated subjects receiving CsA and corticosteroids as opposed to those on AZA in a CsA-based triple therapy regimen [65]. As may be noted in a previous publication from this group, the cumulative burden of subclinical rejection was also more common in AZA treated patients [5,66]. Therefore, we submit, attribution of all vascular and fibrotic lesions in an allograft biopsy entirely to CsA nephrotoxicity is too simplistic and probably incorrect. Fibrointimal changes in the vasculature and fibrotic/atrophic changes in the tubulointerstitial compartment may be secondary to nonspecific pathologic manifestations of repair following injury by the alloimmune, metabolic or the toxic effects of a drug [6,67]. Importantly, observational studies such as those of Nankivell *et al.* do not test the hypothesis that graft function, histology and survival would be superior had the treatment regimen been a CNI free regimen. These questions can only be answered in adequately powered controlled trials.

With regard to nephrotoxicity, all CNIs are probably not alike and therefore CNI avoidance or withdrawal studies can have a different meaning for regimens containing different CNIs. Certainly, clinical experience and many studies including the one reported by Baboolal *et al.* [11] support the notion that TAC may be less nephrotoxic than CsA. In fact, CsA is additionally associated with more hypertension, hypertrichosis and dyslipidemia compared with TAC while TAC exhibits a higher risk for diabetes, neurotoxicity and alopecia when compared with CsA. With regard to interactions with MPA, CsA and

TAC are also different. MPA exposure is lower in CsA treated patients than in those receiving TAC. The higher MPA exposure in TAC treated recipients may have in part mediated the lower acute rejection rates associated with TAC/MMF compared with CsA/MMF and enabled lower TAC targets probably mitigating nephrotoxic effects [66,68].

From an empiric standpoint, removal of a CNI and especially CsA from immunosuppressive regimens usually results in lower creatinine levels. This may be interpreted by the transplant physician as a manifestation of improved renal function. However, this improvement in the creatinine probably signifies nothing more than an intrarenal hemodynamic effect. In fact, the removal of CsAs constrictor effect on the afferent arteriole causes an acute increase in GFR. One must be cautious in not being forced into complacency on the basis of this phenomenon as this might not necessarily have an impact on preexistent histologic lesions that can continue to progress over time and additionally the decreased levels of immunosuppression may manifest many months later as overt rejection or even worse as subclinical rejection not readily apparent on cursory and sporadic follow-up of serum creatinine levels. Also to be noted from the practical standpoint is that any patient on a minimization regimen who encounters even the most transient interruption of their dosing be it due to intercurrent illness or frank noncompliance is at a much greater risk for an acute rejection episode. Furthermore, the incidence of acute rejection rates upon withdrawal of CNIs is not negligibly small; and unfortunately, the effects of such rejection episodes on attrition of graft function and in turn patient survival still remain largely unknown. In addition, even though the incremental rates of acute rejection in many CNI withdrawal studies are small, the underlying risk might be under appreciated because of the under diagnosis of acute rejection when only for cause biopsies are performed. The higher rates of clinically overt acute rejections might be a marker of a much greater increase in subclinical rejections that ultimately could have a significant impact on long-term graft survival.

To a large extent, transplant physicians now practise in an era where very low acute rejection rates are the norm with standard immunosuppression [43]. On the other hand, such rejection episodes as manifest in the recent era are less likely to respond to treatment and have a far greater deleterious impact on long-term graft survival [4].

Even with very low acute rejection rates, long-term results have not significantly changed and long-term CNI toxicity could be certainly one of the reasons. On the other hand, there are several other very visible and recently emerging causes that hamper long-term success after KTx. The more sophisticated immunosuppression that has allowed for these very low acute rejection rates has brought on new and more numerous long-term complications like BK virus nephropathy, other opportunistic infections and malignancies [69,70].

The large scale applicability of *de novo* CNI free immunosuppression with Rapa/MMF has been questioned by recent clinical trials and registry data. The failure of the combination of Rapa/MMF to provide a safe platform for CNI avoidance might be in part related to the poor tolerability of this regimen with potentially additive or even synergistic side effects between the two drugs. Leucopenia and diarrhea are rate limiting toxicities for both Rapa and MMF. Rapa has also been associated with proteinuria, pneumonitis and renal insufficiency in both native and transplant kidneys with ongoing injury. This lack of tolerability with certain immunosuppressive regimens used for CNI avoidance can trigger suboptimal compliance, breakdown in immunosuppression and ultimately decreased patient and allograft survival. Thus, with the currently available armamentarium, the goal of avoiding CNIs altogether is not a casual exercise.

The use of therapeutic drug monitoring for MPA could potentially allow optimal delivery of MMF in a concentration-controlled manner as CNIs are withdrawn or avoided. This hypothesis however needs to be tested in adequately powered trials [71]. However, it has also been shown that the mere substitution of MMF for AZA in CsA-based triple therapy regimen or its introduction into a double therapy regimen with CsA and corticosteroids stabilizes renal allograft function [72]. This leaves open the question of whether it is possible to separate clearly the effects of superior rejection prophylaxis afforded by MMF from the effect of CNIs on renal function [17,19,66].

Investigational agents such as belatacept carry future promise of the delivery of immunosuppression without the use of a CNI and in the coming years we will probably see several other new immunosuppressants that will be developed and tested for CNI-free protocols. On the other hand, the development of CNIs with potentially less nephrotoxicity and improved tolerability compared to the traditional CNIs, such as ISA 247, may provide yet another avenue in securing excellent prophylaxis against acute rejection and yet maintain allograft function in the context of CNI containing regimens [73].

To date, data in *de novo* renal transplant recipients indicate a less than acceptable outcome when using CNI free immunosuppression with currently FDA-approved agents. The effects of CsA withdrawal in patients with chronic allograft nephropathy appear to be associated with favorable outcomes over a one to 2-year follow-up period [31,32,74]; however, studies where recipients with

stable graft function who have had CsA withdrawn overtime do have an increased risk of acute rejection, of which the long-term impact on graft function is not entirely clear [20,28]. When considering CNI minimization and or withdrawal strategies it is important to assess the risks and benefits for each individual patient. In patients with advanced chronic allograft dysfunction the potential downside of reducing or withdrawing CNIs is probably significantly less compared to a patient with excellent and stable renal function. These patients face imminent allograft failure and anything to delay this process by some time is going to be a significant success. On one hand, for a patient with excellent renal function, any risk for acute rejection might outweigh the potential long-term benefits.

On the other hand, the potential long-term gain could be also significantly more in a patient with excellent renal function, but unfortunately to date this is still hypothetical and no regimes have been shown to fulfill this wisdom.

Conclusions

CNI free regimens were developed and promoted with the promise of improving graft function and ultimately long-term graft survival. However, results to date have indicated varying efficacy and studies with long-term follow-up are not available. The lack of long-term follow-up is the greatest limiting factor in the ability of the transplant physician to select CNI free regimens in deciding who would benefit the most from such regimens and more importantly who could be harmed. Future prospective studies will therefore need to direct their endeavors to the realization of these goals.

Authorship

GG, TRS and H-UM-K wrote the paper. TRS and H-UM-K supervised the study.

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