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De Novo Therapy with Everolimus and Low-Dose Calcineurin Inhibitors in Kidney Transplantation

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Abstract

At present, calcineurin inhibitor-based immunosuppression is considered the standard-of-care in kidney transplantation as a result of multiple studies indicating an advantage in preventing acute rejection, with scant data supporting a long-term graft survival advantage with this strategy. This is thought to be due to the inherent nephrotoxicity of these agents and dose-related side effects (cardiovascular risk factors) and complications (malignancy) that are associated with calcineurin inhibitor use that compromise graft survival. The use of mammalian target of rapamycin-based immunosuppression with everolimus together with minimization of calcineurin inhibitors has been proposed as a strategy to optimize the balance between potency and toxicity. The purpose of this review is to critically summarize the use of the mammalian target of rapamycin inhibitor everolimus in combination with low-dose calcineurin inhibitors in kidney transplantation. Randomized controlled trials suggest that* de novo *everolimus with calcineurin inhibitor minimization provides similar efficacy, with the potential to better preserve renal function compared to standard-dose calcineurin inhibitor regimens. Additional characteristics of everolimus have been viewed favorably (for example, its potential impact upon viral infection and malignancy) and unfavorably (for example, its impact upon wound healing and association with proteinuria) and thus an individualized approach to transplant immunosuppression and an understanding of the dose-dependent benefits and risks of this combination is necessary. **(Trends in Transplant. 2014;8:17-26)**

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Key words

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Introduction

Over the past 20 years, advances in immunosuppression in kidney transplantation have led to a remarkable reduction in the incidence of acute rejection, which in turn has led to dramatic improvements in short-term kidney allograft survival. Unfortunately, these short-term benefits have not translated into a similarly dramatic improvement in long-term graft survival. Graft attrition rates after the first year have remained fairly constant from 1989 to 2009, with graft attrition after the first year posttransplantation averaging 5-7% per year, and are identical from the third to fifth year and from the fifth to the tenth year posttransplantation[1]. While attrition rates are lower in living donor kidney transplants, similar trends persist. These findings raise important questions such as, what are the causes of later graft loss, and are there modifiable factors that can be targeted to reduce graft loss?

A number of recent studies have allowed the transplant community to reconsider the causes of later graft loss. A single-center study of 1,317 consecutive transplants over the period 1996-2006 reported the causes of graft loss with a rigorous follow-up schema including late biopsies to ascertain clinicopathologic causes of graft loss[2]. Of a total 330 subjects with graft loss, 138 (43.4%) were due to death with function, 39 (11.8%) were due to primary nonfunction, and 153 (46.3%) were due to graft failure. Of the latter category, 95% underwent biopsy at a mean 4.7 months prior to graft loss. Glomerular pathology (recurrent and *de novo* glomerulonephritis together with transplant glomerulopathy) was the predominant cause of graft loss, followed closely by interstitial fibrosis/tubular atrophy (IF/TA). When correlated with clinical events, many underlying causes of glomerulopathy (anti-HLA donor-specific antibodies [DSA]) and IF/TA (prior rejection, bradykinin [BK] virus infection, and/or preexisting donor disease) could be identified; thus it was rare that a cause of "pure" calcineurin inhibitor (CNI) nephrotoxicity as a cause of graft loss was identified. Another biopsy series examining causes of later graft loss following "for cause" biopsy suggested that the vast majority of patients who underwent biopsy and ultimately lost graft function had ongoing alloimmune injury (predominantly antibody-mediated) and were often suspected of medication nonadherence[3]. Finally, a protocol biopsy series of 574 patients who underwent 963 biopsies during the first year posttransplantation from 1991 to 2001 with much longer follow-up (mean 14.5 years) suggested that chronic lesions, particularly of transplant glomerulopathy (which are often but not always associated with DSA) and arteriolar hyalinosis (which is often but not always associated with CNI) were strong predictors of later graft loss[4]. On the basis of these biopsy studies, it is apparent that not only is indolent alloimmune injury a considerable problem even with modern immunosuppression and in the absence of "acute rejection", but also that later graft loss is strongly linked to chronic lesions that may be exacerbated or caused by current immunosuppression.

Beyond the causes of graft loss is graft loss due to patient death, i.e. "death with graft function". As above and as registry analyses indicate, this contributes to nearly 50% of all graft losses, primarily due to cardiovascular and infectious complications together with malignancies[5,6]. If causes of death can be attenuated via novel clinical or medication strategies, the utility and attrition rate of transplanted kidneys could be modified greatly.

Given this perspective, a simplified framework for posttransplant immunosuppression can be developed and current immunosuppressive strategies can be critiqued with

Table 1. Goals of immunosuppression

1. Control the immune response: low rejection rates, low rate of donor-specific antibody formation
2. Avoid infections and malignancies
3. Control comorbidities: cardiovascular risk factors, diabetes
4. Avoid nephrotoxicity

these underpinnings in mind (Table 1). Goals of immunosuppression should be first, to control the alloimmune response, both early and late markers of injury; second, to avoid infections and malignancies, particularly those that may be influenced by immunosuppression; third, to control the patient's comorbidities, with attention to those comorbidities that impact cardiovascular risk; and finally, to avoid nephrotoxicity, including nephrotoxicity of immunosuppressive agents alone and in combination. This review will follow this framework in reviewing the performance of everolimus/low-dose CNI (EVR/CNI [lo]).

Everolimus/low-dose calcineurin inhibitor

Efficacy assessment and control of alloimmunity

A comparison of EVR/CNI (lo) to standard maintenance immunosuppressive regimens is best interpreted from the multicenter, randomized, open-label A2309 trial comparing EVR/CNI (lo) to standard-dose CNI in combination with mycophenolate (MPA, either mycophenolate mofetil or mycophenolate sodium) over 24 months of follow-up[7]. The CNI used within this study was cyclosporine (CsA), with a planned reduction in exposure of CsA in the EVR arm from day 5 to month 6 that would result in ~ 50% less CNI exposure at each pre-specified study visit. Despite this reduction in CNI exposure, acute rejection incidence at one year was equivalent between groups (16.2% in the EVR/CNI (lo) vs. 17.0% in the MPA/CNI arm; p = ns). The overall efficacy of acute rejection, graft loss, death, and loss to follow-up was also equivalent between groups (25.3 vs. 24.2%, respectively). Acute rejection was inversely related to EVR exposure, with rejection rates optimized when EVR C0 is > 3 ng/ml (Fig. 1)[8]. Based upon these data, EVR/CNI (lo) can be considered equally potent to standard CNI/MPA immunosuppression in the context of CsA use and would suggest that EVR is more potent than MPA in the prevention of acute rejection.

There may be important differences in efficacy when using tacrolimus (TAC) rather than CsA as the CNI. Unfortunately, at the time of this manuscript a head-to-head comparison of EVR/TAC (lo) versus standard TAC/MPA-based immunosuppression has not been reported (see later discussion of US92 and TRANSFORM clinical trials). Currently, the best clinical trial data available using EVR/TAC (lo) are from two open-label trials comparing EVR with differing TAC dose regimens. A randomized, open-label, phase III trial (ASSET, n = 224) compared EVR (target C0 3-8 ng/ml) plus TAC (target C0 4-7 ng/ml for the first three months for all participants, then stratified to two groups: (i) TAC C0 1.5-3.0 ng/ml; vs. (ii) C0 4-7 ng/ml after three months posttransplantation) in *de novo* renal transplant recipients[9]. This comparison demonstrated similar biopsy-proven acute rejection rates (2.7 and 1.1%) and graft loss rates (1.3 and 1.1%) during months 4-12. A second randomized, open-label study

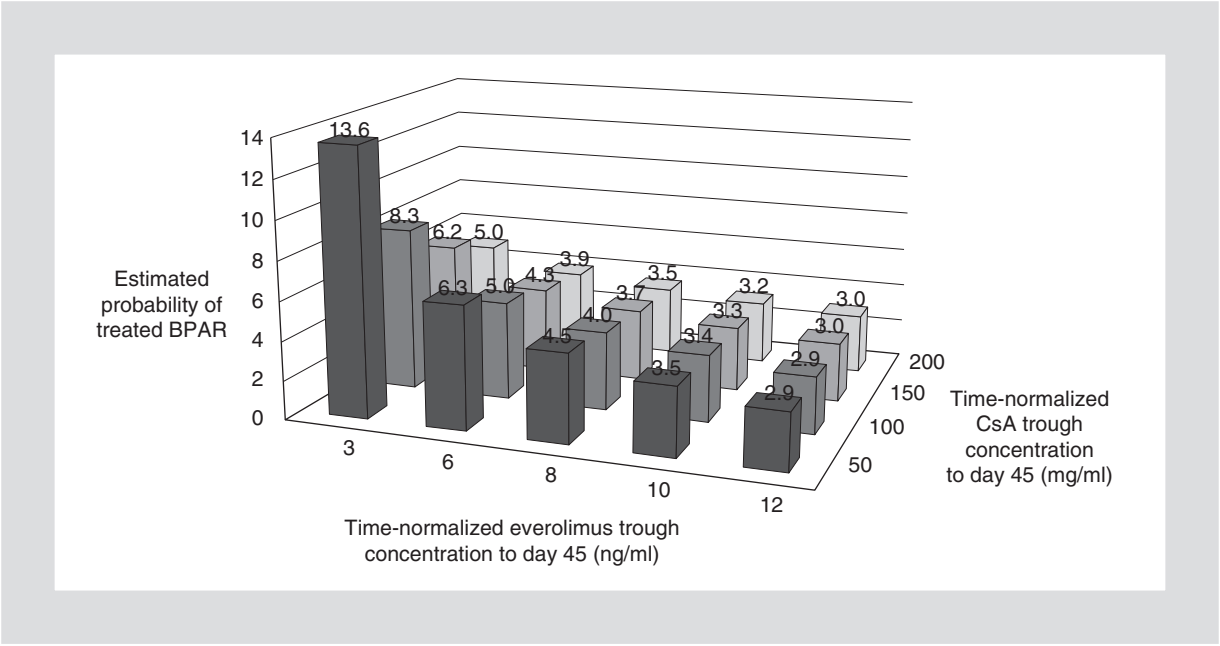

Figure 1. *Probability of acute rejection based upon time-normalized everolimus and cyclosporine trough concentrations to day 45* (Reprinted with permission from Shihab, et al.[8]).
BPAR: biopsy-proven acute rejection; CsA: cyclosporin A.

(US09) evaluated EVR/TAC (target TAC C0 4-7 ng/ml vs. 8-11 ng/ml from month 0-3 with planned TAC reduction in both groups from month 4-6) in patients also receiving basilixi-mab and steroids (n = 92)[10]. At month 6, biopsy-proven acute rejection rates were similar between the TAC minimization and standard-dose groups (14 vs. 16%), with a pooled analysis demonstrating significantly lower rates of biopsy-proven acute rejection associated with higher trough levels of EVR (≥ 3 vs. < 3 ng/ml; p = 0.03). Together, these three studies form the best available data to support the concept that EVR with goal C0 3-8 ng/ml provides protection from rejection at a range of CNI exposures, including very low CNI exposure below current treatment recommendations.

With respect to prevention of chronic alloimmune injury, questions remain regarding the strategy of EVR/CNI (lo) in the prevention of DSA formation, currently considered the best available marker for future immunologic graft loss. None of the above studies prospectively collected data regarding DSA development; thus, data regarding DSA formation must be extrapolated from alternative EVR-based studies. A recent single-center analysis suggested that following transition from CsA to EVR (EVR/MPA-based immunosuppression) there is a higher rate of DSA formation (14 of 61 patients, 23.0%) than in patients remaining on CsA/MPA (7 of 65,10.8%), with higher antibody-mediated rejection rates and antibody-mediated rejection-related graft loss[11]. Unfortunately, differences in dosing and exposure of the secondary immunosuppressive agents (MPA and prednisone) may have contributed to these differences as well[12]. Experimentally, HLA Class I-induced allograft vasculopathy has been shown to involve mammalian target of rapamycin (mTOR) signaling pathways and is inhibited by rapamycin, suggesting a potential advantage of mTOR inhibition in minimizing HLA antibody-driven injury[13]. Larger clinical trials will be necessary to clarify the effect of EVR in combination with CNI upon HLA antibody formation and HLA-mediated injury.

Impact upon viral infections and malignancies

Provocative experimental data suggests that the mTOR signaling pathway is involved in a number of intracellular signaling processes that can permit viral replication, oncogenesis, and prolferation[14-16]. Clinically, mTOR inhibition with EVR has been successfully applied to the treatment of a variety of malignancies, including renal cell carcinoma, neuroendocrine tumors, and breast cancer[17-19]. In transplantation, transition to an mTOR inhibitor from CNI-based therapy has been shown to reduce recurrent skin cancer, reduce the incidence of *de novo* malignancies, and is the treatment of choice for Kaposi's sarcoma in the posttransplant setting[20-22]. When used *de novo*, patients placed on EVR/CsA (lo)-based therapy had less neoplasms at 12 months than standard CsA/MPA-based therapy (3.3 vs. 5.9%)[7], but these differences were not apparent at 24-month follow-up (8.0 vs. 8.8%)[23]. Questions still unanswered are whether the antineoplastic potential of mTOR inhibition requires either a more prolonged period of observation, the absence of CNI, or higher doses of EVR than is employed in transplantation. These questions are part of a planned prospective trial (TRANSFORM study, described later).

Not only is cytomegalovirus (CMV) disease a significant clinical problem following transplantation and associated with high morbidity, but CMV infection (viremia) itself is associated with an increased risk of graft loss independent of mortality[24]. The mTOR signaling facilitates CMV replication in the early phases of infection, while late infection appears to be mTOR independent[25]. Interestingly, mTOR inhibition may enhance antiviral CD8 memory T-cell generation while inhibiting alloimmunity[26]. Clinically, this may be expected to translate into fewer CMV-related complications. In the aforementioned A2309 study, CMV infection was seen less frequently in the EVR/CsA (lo) arm than the standard CsA/MPA arm (1.5 vs. 6.2% at 24 months)[27]. When these data are combined with results of two other randomized controlled trials with similar EVR dosing but higher CsA goals, this difference was still apparent and was statistically significant (the hazard for CMV events was 1.8-fold higher in the CsA/MPA arms than the EVR/CsA arms in those receiving CMV prophylaxis, and 2.81 higher in those who did not receive routine CMV prophylaxis; $p = 0.0063$ and < 0.0001, respectively)[28].

Similarly, BK virus (BKV) infection and reactivation following kidney transplantation has emerged as a significant cause of infection-related graft dysfunction that can ultimately lead to graft loss. The degree of immunosuppression is the strongest risk factor for BKV reactivation, with infection particularly noted after the introduction and widespread use of TAC/MPA-based immunosuppression. *In vitro* data suggest less inhibition of BKV-specific T-cell responsiveness with mTOR versus CNI[29], and registry data suggests a reduced risk of treated BKV in patients receiving mTOR[30], raising the possibility that the EVR-based regimens may be associated with reduced BKV-related events. Clinical data is mixed in this regard since it is difficult to determine whether there is a protective effect of one agent versus another without controlling for differences in overall immunosuppression burden. In the A2309 trial, rates of reported BKV infection were less in EVR/CsA (lo)-treated patients than CsA/MPA-treated patients (2.5 vs. 5.1%), while in the ASSET study, BKV infection was higher with EVR plus TAC 1.5-3 ng/ml (4.2%) than with EVR plus TAC 4-7 ng/ml (0.8%). These studies did not comprehensively or prospectively monitor for BKV, and thus these results could be different if routine screening were incorporated.

Based on these *in vitro* mechanistic studies and emerging clinical data, the potential of EVR/CNI (lo)-based therapy to influence

malignancy rates and viral infections remains provocative. Longer-term studies together with dedicated viral monitoring should better define the role of EVR/CNI (lo) for these important clinical outcomes.

Cardiovascular measures

Cardiovascular events are the leading cause of graft loss (death with a functioning graft) following kidney transplantation. Traditional cardiovascular risk factors such as diabetes, hypertension, and dyslipidemia are highly prevalent in patients with chronic kidney disease undergoing transplantation, and these factors can be unmasked or exacerbated following transplantation as a result of untoward effects of immunosuppression.

Hypertension in particular is highly prevalent in kidney transplant recipients, identified in 75-90% of all recipients following transplantation[31]. Calcineurin inhibitor therapy has been implicated in the development of hypertension via mechanisms including increased sympathetic nerve activation, oxidative stress, and afferent arteriolar vasoconstriction[32]. Minimizing CNI with concurrent mTOR-based therapy could theoretically reduce the severity and/or incidence of hypertension, but multicenter clinical trials have failed to demonstrate differences in this regard. For example, EVR/CsA (lo)-treated patients had a reported incidence of hypertension as an adverse event of 29.6% compared to 30.0% in CsA/MPA-treated patients[7]. While differences in hypertension prevalence will be difficult to demonstrate due to its widespread prevalence, the end-organ effects of hypertension manifested by left ventricular hypertrophy and its regression may be an important surrogate marker of cardiovascular events and differential effects of mTOR- vs. CNI-based therapy[33]. In experimental setting, mTOR inhibition results in regression of cardiac hypertrophy, postulated to be due to inhibition of signaling via mTORC1. A small single-center study of 39 patients suggests that this may be relevant clinically; a transition from CNI- to mTOR-based therapy was associated with a reduction in left ventricular mass index[34].

New-onset diabetes after transplantation (NODAT) is commonly ascribed to CNI use, and is considered to be more prevalent with TAC than CsA via mechanisms involving both direct pancreatic β-cell toxicity as well as insulin resistance[35]. Experimentally, high-dose mTOR inhibitors also have been shown to induce a direct β-cell toxic effect[36]. Clinically, registry data suggest a rate of new-onset diabetes with mTOR use that is comparable to CsA[37]. The combination of mTOR and CNI, particularly sirolimus/tacrolimus (SRL/TAC) in combination, was associated with the highest rates of NODAT. Minimization of CsA with EVR did not reduce the incidence of NODAT compared to CsA/MPA in the 2309 trial, and minimization of TAC to very low levels (1.5-3 vs. 4-7 ng/ml) in the ASSET trial did not alter the incidence of NODAT (17.8 vs. 20.5%)[7,9].

Dyslipidemia is commonly identified and treated following kidney transplantation, and may be exacerbated with use of mTOR inhibition. A retrospective comparison between TAC/SRL and TAC/MPA suggest an incidence of dyslipidemia of 62% in the former and 51% in the latter, highlighting the frequent occurrence of dyslipidemia following transplantation and additional risk with mTOR use[38]. When minimizing CNI using EVR and using investigator-identified adverse events as a criterion for comparison, EVR/CsA (lo)-treated patients had a reported incidence of hypertension of 20.8% compared to 15.8% in standard-dose CsA/MPA-treated patients, despite a reported incidence of lipid-modifying agent use of 64.6 vs. 57.5%[7]. This reflects the perceptions of investigator-interpreted severity of the problem of hyperlipidemia, which is indeed higher with EVR/CsA (lo) but is perhaps

manageable with traditional lipid-lowering therapy in the majority.

In summary, when considering traditional risk factors such as hypertension, NODAT, and hyperlipidemia, EVR/CNI (lo)-based immunosuppression has a neutral-to-increased risk for the occurrence of these surrogate cardiovascular endpoints. Other nontraditional cardiovascular endpoints (such as left ventricular hypertrophy regression and atherosclerotic plaque regression) are provocative but less well defined in the kidney transplant population (perhaps more definitive in the interventional cardiology literature). Longer-term follow-up and dedicated trials to determine the rates of "hard" cardiovascular endpoints such as cardiovascular events, interventions, and mortality are needed to better define these risks and benefits. At present, it may be prudent to risk-stratify patients prior to initiation of EVR/CsA (lo)-based therapy to reduce the rate of NODAT, and monitor and aggressively treat hyperlipidemia.

Avoiding nephrotoxicity

A great deal of research has focused upon preservation of renal function over time, and one factor that is implicated in the lack of improvement in long-term graft survival is the use of CNI. While many factors contribute to graft loss (as detailed throughout this review), CNI use likely does have an additive and independent impact upon graft attrition over time, perhaps best confirmed by 15-year outcomes from a randomized trial of CsA vs. azathioprine-based immunosuppression in which graft survival was inferior, beginning only after at least five years of follow-up[39]. Minimizing CNI with use of EVR rather than MPA is one strategy that has been proven to be efficacious in the short-term (efficacy, above) and may minimize the contribution of CNI to later ischemia, glomerular injury, and fibrosis[40]. In the 2309 study, estimated glomerular filtration rate (eGFR) at 24 months was numerically but not statistically higher in the EVR/CsA (lo) arm than the standard CsA/MPA arm, with differences in median eGFR ranging from 2.5-4.5 ml/min/1.73 m^2 vs. MPA at all timepoints[7]. In the ASSET trial, the EVR/TAC 1.5-3 ng/ml arm had a higher eGFR than the EVR/TAC 4-7 ng/ml arm (57.1 vs. 51.7 ml/min/1.73 m^2) at 12 months, but again did not reach statistical significance, perhaps due to inadequate separation of TAC exposure throughout the study period[9]. Thus, with current reported literature, there remain hints of improvement of renal function using an EVR/CNI (lo) strategy but no definitive evidence.

This potential benefit in GFR must be weighed against the recognition that mTOR use is associated with higher rates of proteinuria, which is associated with progressive renal disease when caused by distinct pathological entities. The mechanisms for mTOR-associated proteinuria are likely multifactorial, and may involve release of CsA-induced afferent arteriolar vasoconstriction, antagonism of vascular endothelial growth factor, and/or loss of nephrin expression with mTOR[41-43]. In the most detailed analysis of EVR/CNI (lo) vs. standard CNI/MPA, the risk for proteinuria (defined as spot urine protein/creatinine ratio > 300 mg/g) was higher with EVR/CNI but was dose-dependent, not universal (less than 1/3 of patients developed proteinuria), not progressive over 24 months, and was treatable with angiotensin-converting enzyme/angiotensin receptor blocker therapy. Importantly, proteinuria was not statistically higher with EVR/CNI (lo) compared to standard CsA/MPA when EVR was dosed with a C0 goal of 3-8 ng/ml (HR: 1.20; p = 0.19; 95% CI: 0.92-1.57)[44]. Thus, while proteinuria is a concern when using mTOR, an EVR/CNI (lo) strategy that includes close EVR therapeutic drug monitoring together with screening and treatment of proteinuria can reduce the potential (and as-yet undefined) untoward effects of proteinuria upon graft function and survival.

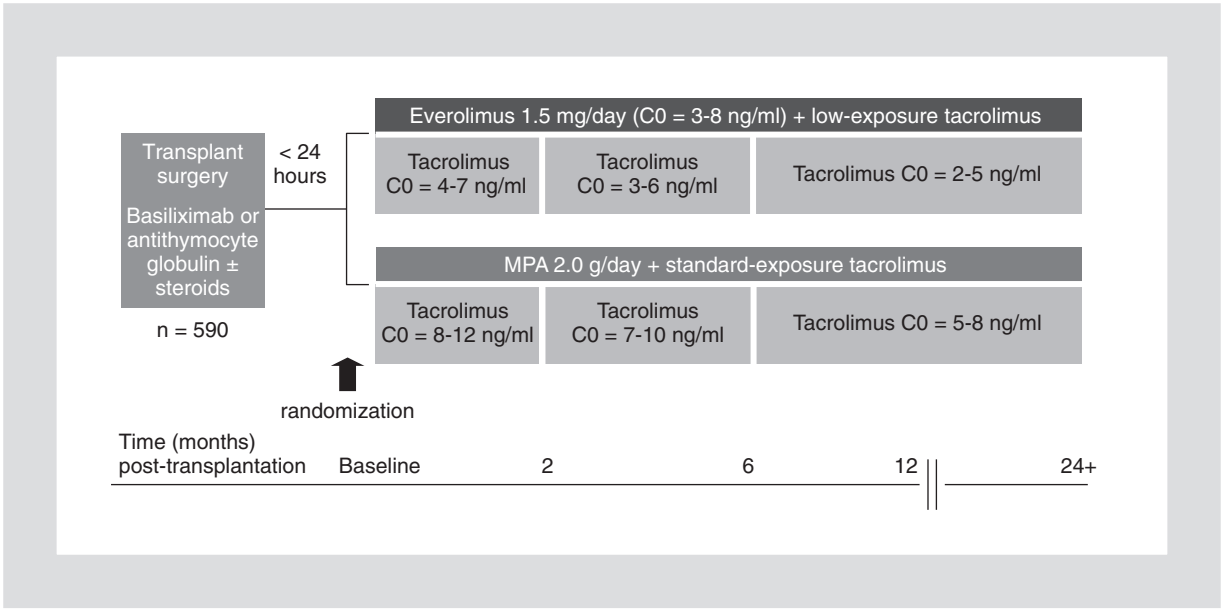

Figure 2. *Study design for the US92 and A2433 (TRANSFORM) multicenter, randomized clinical trials. Standard tacrolimus/mycophenolate-based immunosuppression compared to everolimus/low-dose tacrolimus-based immunosuppression.*

Safety and tolerability

Beyond the goals of immunosuppression from a graft survival and patient survival standpoint are the individual side effect profiles of these agents that influence quality of life. The EVR/CNI (lo) combination has the potential advantages of minimizing some of the CNI-related side effects such as hirsutism (CsA) or alopecia (TAC), neurologic effects of TAC such as tremor and insomnia, or hyperuricemic effects of CsA. By replacing MPA with EVR, common side effects such as diarrhea and leucopenia seen with MPA may be minimized. These advantages are counterbalanced with side effects that are more commonly noted with mTOR therapy, such as stomatitis (typically in prednisone-sparing regimens) and impaired wound healing complications, including postsurgical lymphocele, lymph leakage, incisional hernia, and dehiscence[45]. The latter complication is a dose-dependent antiproliferative effect of mTOR inhibition; similar to proteinuria, its prevalence and significance is significantly reduced in studies of EVR/CsA in which EVR C0 is maintained between 3-8 ng/ml[46].

Ongoing and future studies

Currently, the emerging evidence supports the use of EVR/CNI (lo), given its efficacy in the prevention of acute rejection and excellent short-term graft survival, together with its potential impact upon malignancy and viral infection rates. As alluded to throughout this review, gaps in our knowledge regarding the potential benefits of an immunosuppressive strategy using EVR and low-dose CNI exist, including the influence upon *de novo* donor-specific antibody formation, potential improvements in renal function and graft loss, and effects upon cardiovascular events, compared to a present-day standard TAC/MPA regimen. To address these questions, one study (US92 trial) of 508 kidney transplant recipients has been completed with 12-month data forthcoming at the time of this publication, and will provide more comprehensive data regarding the early (12 month) renal function and infection-related outcomes, while a larger and longer 24-month trial of 2,040 subjects with expected extension to five years (TRANSFORM trial) will use the same study design to address the potential differences in

malignancy rates and donor-specific antibody formation and more fully address the potential effects upon GFR, proteinuria, and graft loss (Fig. 2). Everolimus use together with low-dose CNI appears to be optimally applied in terms of efficacy and tolerability when everolimus C0 is maintained between 3-8 ng/ml, and this combination holds promise in maximizing graft survival beyond their immunosuppressive effects.

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