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CONVERSION FROM CYCLOSPORINE TO SIROLIMUS IN KIDNEY TRANSPLANTATION - IS THERE RENOPROTECTION?

Artigo de revisão

Área científica de Farmacologia e Terapêutica

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List of abbreviations and acronyms

ApoB-100 - Apolipoprotein B-100

ApoC-III - Apolipoprotein C-III

AZA – Azathioprine

BMI – Body Mass Index

BCAR - Biopsy-Confirmed Acute Rejection

CAD - Chronic Allograft Dysfunction

CAN - Chronic Allograft Nephropathy

CrCl - Creatinine Clearance

CsA - Cyclosporine A

CNIs - Calcineurin Inhibitors

DGF – Delayed Graft Function

EMA - European Medicines Agency

FDA - Food and Drug Administration

GFR – Glomerular filtration rate

HLA - Human leukocyte antigen

IL-2 – Interleukin-2

LDH – Lactate Dehydrogenase

LDL – Low-Density Lipoprotein

MMF - Mycophenolate Mofetil

mTOR - Mammalian Target of Rapamycin

mTOR-Is - Mammalian target of rapamycin Inhibitors

RCT – Randomized Controlled Trial

rHuEPO – Recombinant Human Erythropoietin

RMR - Rapamune Maintenance Regimen

SRL - Sirolimus

ST - Corticosteroids

TAC – Tacrolimus

TGF-beta - Transforming Growth Factor - Beta

UPr/Cr - Urinary Protein-to-Creatinine Ratio

Resumo

Introdução: O aparecimento dos imunossupressores inibidores da calcineurina, em particular da ciclosporina A (CsA), revolucionou a transplantação de órgão, nomeadamente a nível renal, permitindo uma redução significativa das taxas de rejeição e garantindo uma maior sobrevida do enxerto a longo prazo. No entanto, os inibidores da calcineurina apresentam vários efeitos secundários, tais como a nefrotoxicidade, e o seu uso prolongado está associado a disfunção e nefropatia crónicas do enxerto, que limitam o tempo potencial de sobrevida que este teria caso fossem usados imunossupressores mais renoprotectores. Uma das principais estratégias terapêuticas de minimização da nefrotoxicidade induzida pela CsA é a conversão para fármacos menos nefrotóxicos, sendo o sirolimus (SRL), da classe dos inibidores da mTOR, uma das opções mais praticadas. Contudo, a sua utilização está associada ao aparecimento de proteinúria e as suas propriedades renoprotectoras têm sido alvo de amplo debate.

Objectivos e Métodos: Este trabalho de revisão faz uma breve análise das várias abordagens possíveis na utilização do sirolimus, dando um particular destaque aos resultados da conversão da CsA para SRL, com base nos principais ensaios clínicos aleatorizados e controlados. Pretende-se ainda abordar os possíveis efeitos renoprotectores resultantes da conversão de ciclosporina para sirolimus bem como discutir a indução de proteinúria relatada em alguns dos trabalhos.

Desenvolvimento e Conclusão: A conversão tardia de CsA para SRL está associada a taxas de rejeição aguda aceitáveis e a uma melhoria da função renal, sendo esta mais eficaz em doentes cuja deterioração renal é ainda moderada. No entanto, esta conversão pode originar alguns efeitos secundários (nomeadamente dislipidémias, anemia e aumento da proteinúria) que podem limitar a sua utilização em certos doentes. Por outro lado, quando a conversão de

ciclosporina para sirolimus é feita nos primeiros meses após o transplante, ocorrem melhorias significativas da função renal, apresentando este protocolo taxas de sobrevida do enxerto e taxas de rejeição aguda equiparáveis às da CsA. No entanto, esta abordagem está também associada ao aparecimento de dislipidémias, diabetes e proteinúria, devendo ser evitada em pacientes com risco metabólico elevado. Deve também ser considerada uma reconversão para ciclosporina sempre que os valores de proteinúria atinjam níveis considerados preocupantes. Em suma, a conversão de CsA para SRL é uma opção terapêutica claramente renoprotectora, que assegura um bom compromisso entre eficácia imunossupressora (prevenção de rejeição aguda) e efeitos secundários (prevenção da nefrotoxicidade). Contudo, devido ao risco de desenvolvimento de proteinuria a função renal deve ser vigiada de forma apertada, nomeadamente nos casos em que a conversão é promovida mais tardiamente e o enxerto apresente algum comprometimento prévio.

Palavras-chave

sirolimus, ciclosporina, transplantação renal, conversão precoce ou tardia, nefropatia crónica de enxerto, disfunção crónica de enxerto, renoprotecção, proteinúria.

Abstract

Introduction: The appearance of calcineurin inhibitors (CNIs), in particular of cyclosporin A (CsA), has revolutionized organ transplantation, namely at renal level, by enabling a significant reduction in rejection rates and ensuring increased long-term graft survival. However, CNIs have several side effects, such as nephrotoxicity, and its prolonged use is associated with chronic allograft nephropathy and dysfunction, which ultimately limit the potential survival time than it would have if a renoprotective immunosuppressant was used. One of the major therapeutic strategies used to minimize the nephrotoxicity induced by CsA is conversion to less nephrotoxic drugs, with sirolimus (SRL), an mTOR inhibitor, being one of the most used options. However, the use of SRL is associated with the onset of proteinuria, and its renoprotective properties have been under extensive debate.

Aims and Methodology: This review makes a brief analysis of several possible approaches in the use of sirolimus, giving special emphasis to the results of the conversion from cyclosporine to sirolimus, through a detailed analysis of randomized controlled trials. This work also addresses the putative renoprotective properties obtained with conversion to SRL, as well as discusses the reports of development of proteinuria.

Development and Conclusion: Late conversion from CsA to SRL is associated with acceptable rates of acute rejection and improved renal function, which is more effective in patients with moderate renal impairment. However, this conversion is also associated with some adverse effects (such as dyslipidemia, anemia and increased proteinuria) that may limit its use in certain patients. On the other hand, when the conversion from cyclosporine to sirolimus is done during the first months after transplantation, there is a significant improvement in renal function and the rates of both graft survival and acute rejection are comparable to those of CsA. However, this approach is also associated with the appearance of dyslipidemia, diabetes and proteinuria, and should be avoided in patients with a high

metabolic risk. Re-conversion to cyclosporine should also be considered whenever the values of proteinuria reach levels considered worrisome. As summary, the conversion of CsA to SRL is a therapeutic option clearly renoprotective, which ensures a good balance between immunosuppressive efficacy (prevention of acute rejection) and side effects (prevention of nephrotoxicity). However, the development of proteinuria and changes in renal function should be monitored tightly, particularly in cases where the conversion is promoted later and the graft already has some prior commitment.

Key-words

sirolimus, cyclosporine A, renal transplantation, early or late conversion, chronic allograft nephropathy, chronic allograft dysfunction, renoprotection, proteinuria.

I - Introduction

Renal transplantation has become the treatment of choice for most patients with end-stage renal disease, by improving their quality of life and avoiding a lifetime of dialysis treatment.(1) However, even when high histocompatibility between the donor's organ and the recipient is assured, there are still allograft rejection phenomena occurring, mediated by the host's T-cells and triggered by foreign human leukocyte antigen (HLA) molecules.(2) This allograft rejection justifies the need of immunosuppressive therapies, with the aim of reducing the immune system's action and ensuring a lower risk of rejection and a consequent longer-term graft function.

Calcineurin inhibitors (CNIs), [such as cyclosporine A (CsA) or tacrolimus (TAC)], provide an effective immunosuppressive effect and have been historically viewed as one of the best choices in renal transplant patients, (1) due to a substantive reduction of graft rejection and improvement of survival. Cyclosporine A, the first CNI, is a macrolide with potent immunosuppressant properties that, through the inhibition of calcineurin in t-lymphocytes, prevents the production of interleukin-2 (IL-2), thus restricting the immune response over the transplanted tissue. (3) However, CsA is nephrotoxic, and prolonged exposure is associated with multiple chronic injuries that diminish graft function over time and contribute to the reduction of graft survival in the long term. (4)

One of the main strategies to reduce the nephrotoxicity induced by CsA is the conversion protocols to less nephrotoxic drugs, with Sirolimus (SRL) being one of the most promising options. (5) Sirolimus has lymphocyte specific features similar to those of calcineurin inhibitors, but a different mechanism of action. Some studies suggest that it is not only less nephrotoxic, but also appears to grant protection against chronic rejection, graft vascular disease (6) and delay the progression of chronic allograft lesions caused by CsA. (7)

However, other studies have reported a significant increase in proteinuria, the appearance of hyperlipidemia, hematological side effects (8) and a decrease in renal function, when combined with CsA. (9) Therefore, the balance between preventing allograft failures and avoiding serious adverse events is still an unresolved issue with SRL, which have been widely addressed during the last years, namely in more prolonged trials.

II – Aims and methodology of revision

The aim of this work is to review the literature concerning CsA sparing regimens based on sirolimus use, particularly highlighting the results obtained with protocols of conversion from CsA to SRL, through the data from the major randomized controlled trials. This work also addresses the putative renoprotective properties obtained after conversion to SRL, as well as discusses the reports of development of proteinuria.

The method used for the preparation of this paper consisted in a review of medical literature on the subject, using preferentially the randomized controlled trials available on this issue. The study was mainly supported by search medical/scientific articles in the PubMed database, using the following combination of terms in the title/abstract:

Search: (cyclosporin[Title/Abstract] OR cyclosporine[Title/Abstract] OR calcineurin[Title/Abstract]) AND (sirolimus[Title/Abstract] OR rapamycin[Title/Abstract]) AND (conversion[Title/Abstract] OR substitution[Title/Abstract] OR replacement[Title/Abstract] OR sparing[Title/Abstract]) AND (kidney[Title/Abstract] OR renal[Title/Abstract])

This search resulted in 59 articles. In addition, relevant articles mentioned in the references list of these articles were also considered. Other scientific databases were searched, including Medline, Biomednet, Scopus, Science Citation Index expanded and Web of Science.

III – Protocols of minimization of CsA-induced nephrotoxicity

Although the use of calcineurin inhibitors has increased the half life of kidney transplants, its success is not devoid of both acute and chronic toxicity phenomena, which are responsible for both functional and histological renal impairment. (4, 10) The acute toxicity of CNIs includes hypertension, renal dysfunction and neurologic disturbances such as tremors and seizures. (11) However, side effects such as impaired glucose tolerance and neurotoxicity appear to be more common with TAC, while CsA usage is more frequently associated with hypertension, hyperlipidemia, (12) hirsutism and gingival hyperplasia. (13) CsA also has an immediate effect on renal vasculature that consists of afferent arteriole constriction through upregulation of angiotensin II receptors and augmentation of angiotensin II-induced calcium responses (14, 15), and despite not being an irreversible side effect, it is responsible for concentration-dependent fluctuations in glomerular perfusion and for a consequent raise in serum creatinine. (6) Acute CsA toxicity is also responsible for a reduction in the glomerular filtration rate (GFR). (14, 15) CsA's long-term toxicity is a great contributor to the development of chronic allograft nephropathy (CAN), (4) which is characterized clinically by progressive renal dysfunction associated with hypertension and proteinuria (16) and accounts for about 50% of kidney graft loss in the long run. (17) Risk factors that are associated with CAN include both immune and non-immune mechanisms, including CNI-related nephrotoxicity, (18) which is histologically characterized by arterial hyalinization, glomerulosclerosis and tubulo-interstitial damage. (4) CAN is one of the major contributors to the appearance of chronic allograft dysfunction (CAD), with the other major cause being chronic rejection, (5) and since CAN is

the most prevalent cause of long term graft failure, (10) many attempts have been made to delay its progression. The major strategies used are either minimizing or avoiding CNI usage, but since they grant an excellent prophylaxis against acute allograft rejection, most attempts are accompanied by acute rejection and graft loss. (19) This work will briefly summarize some of the protocols of CsA avoidance, reduction and/or conversion to other immunosuppressive agents, namely azathioprine (AZA) and mycophenolate mofetil (MMF), and then review in more detail those based on SRL regimens, particularly the ones concerning CsA conversion to SRL, in the proper section.

1 – Avoidance of Cyclosporine use with AZA or MMF

There were many attempts to minimize the CsA-induced nephrotoxicity, including protocols of complete avoidance using another immunosuppressant drug, such as AZA or MMF combined with corticosteroids (ST) and antibodies.

One of those studies, conducted by Grimbert *et al.* (2002), reported the results of a 12-year CNI-free regimen in 117 patients. After induction therapy with anti-lymphocyte globulins, they were randomly assigned to either start a therapy of CsA/AZA/prednisone (group CsA, n=59) or a maintenance therapy of AZA and prednisone (group "No CsA", n=58). After a 12 year follow-up period, the results showed that patients in the "NoCsA" group had lower serum creatinine levels than those in the CsA group (121 micromol/l vs. 168 micromol/l; P=0,006), but there were no statistical significant changes in creatinine clearance and biopsy proven acute rejection rate was 56% in the "NoCsA" group. (20)

In another trial, Asberg *et al*. (2006) described a prospective, open-label and randomized study, which compared the 1 year results of a regimen of daclizumab/MMF/prednisone

(n=27) versus a regimen of CsA/MMF/prednisone (n=27) in a low immunogenic risk population. Glomerular filtration rate (GFR) was significantly lower in the noCsA group than in the CsA group (52±20 ml/min vs. 69±29 ml/min, P=0.029); Graft survival did not differ between groups but the overall acute rejection rate was 70.4% in the noCsA group vs. 29.6% in the CsA group (P=0,006).(21)

The results from these studies, which are in agreement with several others of identical type, suggest that the combination of either AZA/prednisone or MMF/prednisone and the complete avoidance of CsA after transplant are associated with high acute rejection rates. Since CNIs offer a great prophylaxis against acute rejection when in combination with induction agents, any attempt in avoiding them must be followed by the usage of an immunosuppressant powerful enough to, at least, equal its capabilities to prevent acute rejection. (19) Therefore, other combinations and/or newer immunosuppressive agents must be used in order to obtain a safe avoidance of CNIs. SRL has been used as one of the choices in CsA avoidance protocols. Some of the trials addressing its use will be referred in the respective chapter of this review.

2 – Cyclosporine dose reduction and/or conversion with AZA or MMF

There were also some attempts to minimize CsA-evoked nephrotoxicity through dose reduction and addition of another immunosuppressant drug, such as AZA or MMF, combined with corticosteroids.

In a meta-analysis of both randomized and non-randomized trials, Kasiske *et al.* (1993), concluded that early withdrawal of CsA in patients treated with regimens of both AZA and prednisone had no impact on the 1-year patient and graft survival and that the rate of acute graft rejection increased significantly by 11% ($P < 0.001$). (22) Some years later, Gallagher *et al* (2004) reported the 15 year follow-up results of a CsA-withdrawal with AZA introduction

trial in a total of 489 patients. They observed no significant patient-survival differences when using regimens with either CsA or AZA, but they observed a higher kidney-graft loss and a higher risk of developing CsA-induced nephrotoxicity when compared with the group not using it. In the group which had CsA withdrawal 3 months after transplantation significant lower creatinine levels were also observed. (23)

There were also studies that tried to achieve the reduction of CsA nephrotoxicity by adding MMF. One of the largest studies performed was the *CAESAR* (*Cyclosporine sparing with MMF, daclizumab and corticosteroids in renal allograft recipients*) trial involving 536 kidney transplant recipients and that consisted in reducing the time of the CNI-based therapy after transplantation to a maximum of 6 months, after which the patients were randomized into either a therapy with low-dose CsA (target trough level of 50–100 ng/ml for 12 months) /MMF/prednisone, 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 reduction starting at month 4 post-transplant and completed by month 6 post-transplant; remaining only on MMF and prednisone). All patients also received an IL-2 receptor blocker (daclizumab) for induction. Even with a 6-month progressive CsA reduction, acute rejection rates were still high, mainly in the CsA withdrawal group (38%), but also in both the low dose CsA group (25.4%) and the Standard-dose CsA group (27.5%), ($p<0.05$). There were also no differences in renal function improvement, patient survival and graft survival in the three groups after 12 months post transplantation. (24)

These studies showed that early CsA withdrawal leads to high rates of acute rejection when using the previously mentioned combinations, but suggests that reduction of the nephrotoxicity evoked by CsA can potentially be achieved with at least similar patient survival rates and renal function. Therefore, to minimize the risk of rejection, many recent trials have used MMF in combination with SRL after conversion from CsA, which grants an

additional protection against rejection. Since this issue is the core of this review, those trials will be discussed in detail in the proper section of this review.

IV – mTOR inhibitors as a new option to replace calcineurin inhibitors

Mammalian target of rapamycin (mTOR) inhibitors (mTOR-Is) are a group of macrolide agents that include sirolimus (also known as rapamycin) and a sirolimus analog - 40-O-(2-hydroxyethyl)-rapamycin or everolimus. (25) Sirolimus was approved for use in renal transplantation in 1999 by the Food and Drug Administration (FDA) and in 2000 by the European Medicines Agency (EMA). (5) mTOR-Is have been proposed as an alternative therapy to CNIs because of their immunosuppressive properties, as a mean to reduce CNI-related nephrotoxicity.

1 – A novel mechanism of action

Sirolimus is a lipophilic macrocyclic lactone that was isolated from the organism *Streptomyces hygroscopicus* in 1975. Its mechanism of action is different from that of the CNI's, since it doesn't interact with calcineurin and, consequently, does not affect the transcription of proinflammatory cytokines such as IL-2. (3) Sirolimus binds to a member of the FK binding protein family (FKBP12) forming a complex (SRL/FKBP12) that binds to the protein kinase mTOR and consequently blocks its function. (3) mTOR is a serine-threonine kinase that is central in an intracellular signaling pathway involving cell growth and proliferation, metabolism, autophagy and angiogenesis. (26) There are two types of mTOR (complexes 1 and 2) but the complex SRL/FKB12 exclusively binds to mTOR-1, (27) causing arrest of cell-cycle in G1 phase. (3) This effect is what grants sirolimus its

immunosuppressive properties, since it affects the second phase of T-cell activation (i.e. signaling from the IL-2 and other cytokines' receptors) and blocks the progression of cytokine-stimulated T-cells into the S phase, therefore suppressing T-cell interleukin-induced proliferation and clonal expansion. (28) Sirolimus also inhibits B-cell proliferation, differentiation and antibody production. (26) Some studies also suggest that the combination of CsA and sirolimus acts synergistically in the inhibition of T-Cell proliferation, (9, 29) but this combination can also result in an increase in the nephrotoxicity of CsA. (9, 30)

Sirolimus also inhibits the proliferation of many transformed cell lines (namely from lymphoid, melanocytic, hepatic, osteoblastic, myogenic, renal and connective tissue origin) (31) granting sirolimus (and its derivatives) an anti-tumoral activity against certain types of tumors. (32-35) Sirolimus also inhibits growth-factor-induced proliferation and migration of vascular smooth muscle, (36) endothelial cells and both mesangial and renal tubular cells (36, 37) which can help prevent chronic rejection, (38) *de novo* malignancies (32) and CAN in renal transplant patients. (3) Regarding CAN, Sirolimus has been shown to be able to reduce transforming growth factor beta, (TGF-beta) which appears to be a mediator of the fibrogenic process associated with the development of CAN. (39) Sirolimus also promotes the default way of apoptosis, by blocking the survival effects of the same growth factors. However, its antiproliferative and apoptotic effects on renal tubular cells can also contribute to some adverse effects observed with sirolimus therapy. (28)

2 – Side effects of sirolimus

The first experiments made using animal models (Sprague-Dawley rats) showed no significant functional renal impairment, no renal histopathology and a reduction in gain of body weight, when using doses well above therapeutic levels (peak blood levels of 79 ng/mL)

for 14 days. (40, 41) However, once the clinical trials started using SRL, its adverse effects were responsible for a high dropout rate (above 40%) (9, 42), although this number has diminished in recent trials. (43) The main causes of sirolimus discontinuation include aphtous ulceration, cutaneous rashes, proteinuria and SRL-related pneumopathy. (43-45)

Cutaneous adverse effects are a very common side effect. In a systematic evaluation of cutaneous adverse events in 80 renal transplant recipients receiving SRL-based therapy, Mahé *et al.* (2005) reported that 99% of the patients experienced cutaneous adverse effects, namely acne-like eruptions (46%), scalp folliculitis (26%) and hidradenitis suppurativa (12%). They also reported mucous membrane disorders, namely aphtous ulceration (60%), epistaxis (60%), chronic gingivitis (20%) and chronic fissure of the lips (11%). Nail disorders were also observed, such as chronic onychopathy (74%) and periungeal infections (16%). Other frequent complaints were of edematous nature, namely chronic edemas (55%) and angioedema (15%). (46) Other studies also reported a relatively high prevalence of lymphedema. (47) In a study of 150 kidney transplant recipients that were converted from CNIs to SRL, Garrouste *et al.* (2012) concluded that the independent predictive factor for cutaneous adverse effects after conversion was increased age [OR=1.03 (1.002 to 1.06); P = 0.04]. (48)

Aphtous ulceration is one of the most frequent causes of SRL discontinuation, (49) although it appears to be dose-related and in many cases can be resolved with the discontinuation of therapy. (50) A study by Chuang *et al*. (2007) also suggested that a direct application of a high potency topical steroid (Clobetasol) can resolve the ulceration caused by SRL-based immunosuppression in kidney-transplant patients without the risk of acute rejection associated with SRL dose reduction. (50)

Among the most common non-cutaneous side effects is hyperlipidemia. In a systematic review of 17 randomized controlled trials, Kasiske *et al.* (2002) reported high incidence of hypercholesterolemia and hypertrigliceridemia, which lead to a high number of patients treated with lipid-lowering agents (approximately 60%). (51) SRL also increased total cholesterol, LDL, triglycerides, apoB-100 and apoC-III, although these effects appear to be dose-dependent and reversible. (52)

Sirolimus is also associated with wound-healing complications due to its anti-proliferative properties, which commonly occur in the early stages post-transplantation. In a prospective trial, Dean *et al.* (2004) compared the rates of wound healing complications in two groups of kidney transplant patients: one randomized to a regimen of SRL/MMF/CS (n=64) and another, randomized to a regimen of TAC/MMF/CS (n=59). The results showed a higher incidence of complications in the SRL group when comparing with the TAC group (47% vs. 8% respectively, $p< 0.0001$). There was also an increase in the rates of perigraft fluid, superficial wound infection and incisional hernias in the SRL group. (53) Another study, by Knight *et al.* (2007) concluded that the independent risk factors for the development of wound complications were recipients over the age of 40 (odds ratio 2.536, $p = 0.011$), increased body mass index (BMI) [BMI > 26 (odds ratio 2.498, $p = 0.027$); BMI > 30 (odds ratio 3.738, $p = 0.007$)], use of thymoglobulin for induction immunosuppression (odds ratio 3.627, $p = 0.002$) and a cumulative dose of sirolimus of at least 35 mg by day 4 after transplant (odds ratio 2.694, $p = 0.023$). (54)

Hematological complications following Sirolimus use are also relatively common. The myelosuppressive effect of SRL is a consequence of its antiproliferative activity, and frequently results in thrombocytopenia, leukopenia and anemia. In the *CONVERT* trial, leukopenia occurred in 13.4% of the SRL patients vs. 4.4% in the CNI group ($p<0.001$) and

thrombocytopenia occurred in 14% of the patients taking SRL, while it only occurred in 3.3% of the patients taking CNI's ($p<0.001$). (43)

Patients with thrombocytopenia usually respond to SRL dose reduction or temporary suspension. (55) Regarding anemia, Augustine et al. (2004) performed a study about its prevalence in patients with *de novo* kidney transplant or kidney-pancreas transplants (n=214) that were submitted to either a SRL or an MMF-based therapy. The prevalence of anemia in the SRL group was 57% (vs. 31% in the MMF group; $p<0.001$) after 12 months. They concluded that SRL was an independent predictor of anemia and that the patients treated with it required significantly more recombinant human erythropoietin (rHuEPO) administration. Other predictive factors associated with anemia were higher recipient age, female gender, older donor age, chronic infection and decreased renal function at 12 months. (56)

Studies made in patients submitted to late conversion from CNIs to SRL also observed a decrease in hemoglobin in the first months after conversion, although this anemia stabilized with rHuEPO administration. (43, 48) When studies compare CsA withdrawal followed by SRL immunotherapy with therapies consisting of SRL/CsA/ST, the combination of CsA with SRL appears to be responsible for a greater decrease in hemoglobin levels after five years of follow-up. (57)

Some studies also suggested that paralleled with CD4+ T-cells suppression, SRL appears to stimulate memory CD8+ T-lymphocytes. (58) This immunostimulatory effect seems to be dose dependent, and can be attenuated when higher doses of SRL are administered (blood levels 40-100 ng/ml). (58) Since the doses used in renal transplant are usually lower, the data appears to suggest that in clinical practice, SRL therapy may enhance the response of T-memory cells. However, the actual effects of this boost in transplant patients are not yet fully understood.

Another metabolic effect of Sirolimus is post-transplant diabetes. In an analysis of 20124 kidney transplant recipients from the US Renal Data System database, Johnston *et al.* (2008) found an increased risk of new-onset diabetes among patients treated with SRL, when compared with patients receiving other immunosuppressive therapies. (59) Another study, by Teutonico *et al.* (2005) also found an association between the conversion from CsA or TAC to SRL and not only a decrease in insulin sensitivity (both P=0.01) but also a defect in the compensatory beta cell response (P=0.004 and P=0.02, respectively). These researchers also found an association between the increase of insulin resistance and the change of serum triglyceride concentrations after the conversion to SRL ($R(2) = 0.30$, $P = 0.0002$; and $R(2) = 0.19$, $P = 0.004$, respectively). (60)

Interstitial pneumonitis is another side effect of SRL, although rare. Clinical symptoms begin days to months after the start of SRL therapy and resolve only after discontinuation of the therapy. (61) Some studies also suggest that SRL has an impact in fertility. SRL decreases testosterone levels (62) and may affect spermatogenesis in a non-reversible way. (63)

Apart the adverse effects above described, SRL has been associated with proteinuria development. Even though, the drug is viewed as renoprotective. The hypothetical renoprotection conferred by SRL, namely after CsA use, and its induction of proteinuria will be highlighted in this paper in the coming sections.

V – Cyclosporine sparing protocols through the use of sirolimus.

Sirolimus has been shown to be a potent immunosuppressant in different transplanted recipients, including liver, kidney, heart and lung. (64) The question that remains is whether it can substitute CNIs, namely in kidney transplant patients, and if that replacement can be done

immediately after transplant, (by completely avoiding CNI exposure), if it can be done after a period of treatment with CNIs (either long or small) or if it can be done by associating CNIs in a lower dose with Sirolimus. Table 1 summarizes the main RCTs in each type of protocol analyzed in this review.

1. Combinations of cyclosporine and sirolimus

Two of the most important studies trying to address this topic were the "*Rapamune US study group*" and the "*Rapamune global study group*". A total of 1268 patients were included in both tests. In the "*Rapamune US study group*", patients were randomly assigned to one of three groups: one with a regimen of CsA ("full dose")/ prednisone/ Azathioprine and the other two with a regimen of CsA ("full dose")/ prednisone and SRL in either a dose of 2 mg or a dose of 5 mg, daily. The "*Rapamune global study group*" used the same design structure, but instead of using AZA, used a placebo. An analysis of 24 month data from both studies reveals no differences in graft and patient survival between treatment groups in both trials, whilst the frequency of biopsy-confirmed acute rejection (BCAR) was lower in the SRL-treated arm (mainly in the SRL 5mg/d group), compared with those receiving AZA or placebo (although the difference between SRL 2mg/d and AZA wasn't statistically significant). Regarding renal function, a comparison of the mean 24-month serum creatinine reveals that the aggregate values of all patients in the 2 and 5 mg/d study groups were higher than those of patients in the AZA (1.5mg/dL; $P<0.05$) or placebo cohorts (1.6 mg/dL; $P<0.05$), while the calculated creatinine clearance was lower in the SRL-treated arm in both studies ($P<0.05$) (Table 1). (9) The decrease in renal function observed in these studies can be explained by the combination of SRL with full dose CsA (blood trough levels 150-350 ng/ml), which is known to lead to nephrotoxicity, namely through the appearance of higher levels of serum creatinine. (9, 30)

Another study that analyzed the combination of CsA with SRL was the *Rapamune Maintenance Regimen (RMR)* study. In this multicenter study, 525 kidney transplant patients received a dose of SRL (blood trough levels > 5 ng/mL), CsA (150–400 ng/ml) and steroids for 3 months after transplantation. At month 3, eligible patients (n=430) were randomized to one of two treatment groups: CsA withdrawal and SRL/ST continuation (SRL trough levels 15-25 ng/mL) (Group 1; n=215), or maintenance of the original CsA/SRL/ST immunosuppressive regimen (Group 2; n=215). Right after randomization, there was a greater incidence of biopsy-proven acute rejection in the SRL/ST group (9.8%) when compared with the CsA/SRL/ST group (5.1%). After 3 years, the calculated GFR was significantly better with SRL/ST (59.4 vs. 47.3 ml/min; p<0.001) and histological damage (assessed by the Chronic Allograft Damage Index) was also significantly lower in this group (P=0.003). There was also a decrease in tubular atrophy in the SRL/ST group between 12 and 36 months. (65) At 4 years, graft survival was superior in the SRL/ST group (91.5% vs. 84.2%; p=0.024) and there were no differences in mortality and biopsy-proven acute rejection rates between the groups. It should also be noted that non-adherence with the study protocol was higher in the SRL/ST group after 4 years (60.9 vs. 44.2%) (Table 1). (42) Another study also analyzed the 4-year outcomes of the *RMR* trial and concluded that the improvement in GFR was most marked in patients with a baseline calculated GFR ≤ 45ml/min. (66) Five-year follow-up results of this study show that 50.2% of the patients in the group 1 remained on therapy (vs. 12.1% in the CsA/SRL/ST group), with Kaplan-Meier estimates of graft survival being 88.0% vs. 82.6% (SRL/ST vs. CsA/SRL/ST) when censoring for loss to follow-up. Calculated GFR was also significantly higher with SRL-ST (60.3 vs. 47.1 mL/min; P <0.001), when including values from discontinued patients. (67) Despite the better results obtained with the SRL/ST regimen, we must not forget that the association of CsA and SRL is responsible for an increase of CsA's nephrotoxicity. Therefore, this improved renal function may only reflect the

avoidance of the synergistic nephrotoxicity, and not a real improvement in GFR provided by SRL. Nonetheless, these studies have shown that a combination of CsA and SRL can grant an immunosuppressive efficacy against acute rejection.

Other studies were made with the intent of analyzing the combination of SRL with a lower dose of CsA, namely when in comparison with CsA withdrawal. In one of those studies, Baboolal *et al.* (2003) analyzed the conversion of 133 kidney transplant patients from a therapy of "standard-dose" CsA/ SRL/ ST to either "low-dose" CsA (50-100 ng/ml)/SRL/ST or "CsA elimination" /SRL/ST, 3 months after transplant. After 6 months of follow-up, there were no differences in serum creatinine, patient and graft survival or biopsy-proven acute rejection rates, but calculated creatinine clearance was higher in the CsA elimination arm (65 mL/min vs. 57 mL/min; P=0.027) (Table 1). (68) Another study, by Tedesco-Silva *et al.* (2010) analyzed 207 kidney transplant patients who were randomized at 3 months after transplant from a therapy of "standard-dose" CsA/SRL/ST to either a therapy of "low-dose" CsA(50-75 ng/ml)/SRL/ST or "CsA elimination"/SRL/ST. After a follow-up of 12 months, they found no differences in serum creatinine, GFR, BCAR and patients and graft survivals between the groups (Table 1). (69) Both studies concluded that any of these approaches can be used as a treatment option in renal transplantation, although longer follow-up times are needed to conclude the long-term efficacy of both approaches.

2. Cyclosporine avoidance by using sirolimus regimens

Many studies have tried to address the efficacy of avoiding CNIs right after transplantation by using a sirolimus based therapy, in an attempt to minimize acute rejection rates. Some studies used a combination of MMF with SRL, in the hope that the synergistic combination of both drugs could provide a better outcome than the isolated use of SRL. (70) In some of those

studies, this protocol has obtained positive results, with one year patient and graft survival above 90% and a stable renal function. (71-73) When comparing CsA-based regimens with protocols using basiliximab induction and a combination of MMF/SRL/ST, some trials show no significant differences in terms of acute rejection rates, (72, 73) and a significant increase in GFR after 5 years of follow-up. (73) One of the broadest studies investigating the combination of MMF with SRL was the *ELITE-SYMPHONY* study, in which 1645 renal-transplant recipients were randomly assigned to receive standard-dose cyclosporine/ MMF/ ST (group 1) or daclizumab induction/ MMF/ ST in combination with either cyclosporine in a low dose (group 2), tacrolimus in a low dose (group 3) or SRL in a low dose (group 4). After 12 months of follow-up, biopsy-proven acute rejection was 37.2% in SRL/MMF patients vs. 24% in the CsA/MMF and 12.3% in the TAC/MMF (P<0.01) (Table1). Calculated GFR was also lower with SRL/MMF (57.3 ml/min vs. 65.4 ml/min with TAC/MMF, P<0.0001) and graft survival was also significantly inferior in SRL/MMF patients (89.3% vs. 94.2% with TAC/MMF; P=0.02). (74)

Although the SRL doses used in this study were low, similar results have been reported by Srinivas *et al.* (2007), in a retrospective analysis of the outcomes of a therapy of SRL/MMF (independently of the dose used) in solitary kidney transplant patients, by analyzing the data reported in the *Scientific Registry of Renal-Transplant Recipients* (2000-2005). They concluded that 6-month acute rejection rates were higher with SRL/MMF (16%) vs. other regimens (11.2%; P<0.001), that overall allograft survival at 5-year post transplantation was significantly lower with SRL/MMF and that delayed graft function (DGF) rates were significantly higher in SRL/MMF recipients from deceased donors (47% vs. 27%; P<0.001). (75) In a meta-analysis examining 11,337 patients from 56 randomized controlled trials, Sharif *et al.* (2011) reached some different conclusions: the combination of mTOR-Is with MMF was also associated with increased overall graft failure (OR 1.43 [95% CI 1.08–

1.90], P = 0.01, I^2 = 19%), but they found no differences in rejection rates when compared with CNI-based regimens (OR 1.46 [95% CI 0.86–2.46], P = 0.16, I^2 = 62%). (76)

These studies suggest that the efficacy of the combination Sirolimus/MMF, when used for *de novo* kidney transplants is subject to contradictory results. Therefore, more studies are needed in order to find the perfect combination of immunosuppressants and antibodies that can confirm this regimen as an alternative to calcineurin inhibitors.

3. Conversion from cyclosporine to sirolimus

3.1 Early conversion to sirolimus

Several studies have been performed in order to understand if an initial CsA regimen, followed by a conversion to SRL few months after transplant, would have a better outcome than the one obtained with the previously mentioned strategies.

One of the largest multicenter, prospective, open-label, randomized trials tried to understand if the conversion from CsA to SRL 3 months after transplantation was associated with a positive risk-benefit balance was the *CONCEPT* Study, which enrolled 192 kidney transplant patients that received an induction therapy of daclizumab in addition to CsA/MMF and a corticosteroid for 12 weeks after transplant. At 12 weeks, they were randomized to be either kept on CsA/MMF-based immunosuppression (n=97) or to be converted from CsA to SRL/MMF-based immunosuppression (n=95) (SRL blood trough levels 8-15 ng/mL). By year 1 post-randomization, mean Creatinine Clearance (CrCl) was significantly improved in the SRL group (68.9 ml/min vs. 64.4 ml/min in the CsA group; P=0.017), but BCARs were also higher in that group (17% vs. 8% in the CsA group; P=NS), although not significantly different and occurring mainly during the period of steroid withdrawal (by month 8 after randomization). The major adverse events that lead to drug discontinuation in the SRL group

(n=6) were skin lesions and mouth ulcers, which the researchers associated with an excessive SRL loading dose. At week 52, proteinuria, hemoglobin levels, total cholesterol and LDL values were similar in both groups, although triglycerides were higher in the SRL group ($P<0.01$). They concluded that the introduction of SRL after 3 months is associated with an improvement in renal function but advised some caution because of the high dropout rate and the increased percentage of acute rejection. (44)

A later 4 year follow-up of the *CONCEPT* study, named *POSTCONCEPT* trial, analyzed the outcomes of 162 patients (n=77 in the SRL group and n=85 in the CsA group). Death censored graft and patient survival rates were 97.4% and 97.4% in the SRL group vs. 100% and 97.6% in the CsA group, respectively. Renal function was significantly better in the SRL group than in the CsA one (CrCl 62.6 vs. 57.1 mL/min; P=0.013) and two BCAR episodes occurred in each group. The percentage of patients with proteinuria $\geq$ 0.5 g/d was 29.7% in the SRL group vs. 11.9% in the CsA group (P=0.035) and the incidence of new-onset diabetes was numerically increased in the SRL group. The researchers concluded that conversion to SRL is associated with an improvement in renal function that can be maintained for at least 4 years. (77)

Recently, the two-year follow-up results of the *SPARE-THE-NEPHRON* trial have been published: in this multicenter, open-label, randomized trial, 299 patients that were on either a therapy of Tacrolimus/MMF (n=239) or CsA/MMF (n=60) were randomized, 30-180 days (average time: 115 days) after transplantation, to discontinue their CNI and switch to either a regimen of MMF/SRL (mean concentrations 8-10 ng/mL)/Prednisone (n=148) or to continue their current immunosuppressive regimen (TAC/MMF, n=120; CsA/MMF, n=31). After 12 months, the mean percentage change from baseline in measured GFR was significantly higher in MMF/SRL group (24.4% vs. 5.2% for MMF/CNI; P=0.012), but this difference was reduced after 24 months (8.6% in the MMF/SRL vs. 3.4% in the MMF/CNI; P=not

significant). Biopsy-confirmed acute rejection at 12 months was 7.4% for the MMF/SRL group and 6.0% for the CNI/MMF group (P=NS). Late BCAR (occurring > 12 months after randomization) was even lower for the SRL/MMF group when compared with the CNI/MMF group (2.9% vs. 8.0%, respectively). The rates of serious adverse events and opportunistic infections were similar between the two groups and the most common adverse effect was hyperlipidemia, occurring in 81% of patients in the MMF/SRL group, compared with 63% of patients in the CNI/MMF group. There was also an increase in urinary protein to creatinine ratio (UPr/Cr) in the MMF/SRL group when compared with the MMF/CNI group (it increased from 0.2±0.4 at baseline to 0.6±1.9 at 24 months in the MMF/SRL group, whereas there were no changes in the MMF/CNI group). There were also no differences in the dropout rate between the groups. The researchers concluded that maintenance immunosuppression with MMF/SRL provides an improvement in renal function, graft survival and patient survival for at least two years after conversion, when compared with a CNI/MMF regimen. However, these results must be balanced with the detected increases in lipids and proteinuria. (45)

Another CsA conversion study recently published its 36 months follow-up observational results: In the *SMART* trial, 132 patients with low immunological risk were started on CsA, MMF and steroids for 2-3 weeks after kidney transplantation, after which were randomized to be converted to SRL (blood trough levels 5-7 ng/ml)/MMF/ ST or to continue the regimen of CsA/MMF/ST. After 36 months, 59.4% of the SRL patients discontinued the treatment (vs. 42.3% in the CsA group), but the renal function was superior for the SRL/MMF group (ITT-eGFR=60.88 vs. 53.72 ml/min; P=0.031). There were also no statistical differences between the groups in late rejection episodes, BCARs, proteinuria and graft and patient survival during the 36 months follow-up of the study. However, patients in the SRL/MMF group had a statistically-significant increase in lipid values (P=0.0269) and a decrease in the incidence of

de-novo malignancies (P=0.026). In a multivariate analysis, the researchers concluded that donor age > 60 years, S-creatinine at conversion > 2mg/dl, CMV naïve (negative) recipients and immunosuppression with CsA were predictive of an impaired renal function at 36 months. This study also concluded that conversion to a SRL-based immunosuppression provides a sustained improvement in renal function up to 36 months after transplantation. However, future early conversion protocols should be made after a longer time post-transplant, in order to avoid the high dropout rate observed. The researchers suggest that patients with good quality organs, good initial renal function and patients who are at risk for CMV infections can be good candidates for said trials. (78) A summary of these RCTs can be seen in (Table 1).

3.2 Late conversion to sirolimus

Late conversion from CNIs to SRL in kidney transplantation has emerged as one possible therapeutic strategy mainly because of CNI-related nephrotoxicity, or in patients where CAN has already developed, as a mean to preserve renal function. By late CsA conversion, we also consider all the studies in which conversion occurred more than 6 months after transplant.

Many non-controlled studies were performed to understand the efficacy of late conversion to SRL, reaching different conclusions: Diekmann *et al.* (2004) studied 59 renal transplant patients with chronic allograft dysfunction and histological evidence of calcineurin toxicity, who were on CNIs for a mean time of 88 months post-transplant. After 12 months, 54% of the patients had an improved or stable renal function, lower proteinuria and better histological grade of CAN than at baseline. In a multivariate analysis, proteinuria at conversion below 800 mg/day was found to be the only independent predictor for improved graft function in patients with CAN (positive predictive value of 90%). The main adverse events observed were anemia

(which improved after rHuEPO therapy) and hyperlipidemia. (79) The same predictor for graft function was obtained by Cardinal *et al.* (2009) in an uncontrolled study of 193 kidney transplant patients converted to SRL from CsA: from multivariate analyses, proteinuria ≥ 1 g/L at baseline was the only predictor for deteriorating GFR at 1 year post-conversion. (80)

Another study, by Saurina *et al.* (2006), followed 14 patients that had clinical signs of CAD after more than a year of treatment with CNIs (CsA n=12; TAC n=2). No significant differences were found in serum creatinine before and after abrupt conversion to SRL, but afferent arteriolar resistance and renal functional reserve decreased (34.84 vs. 13.47%; P=0.019) while effective renal plasma flow, intraglomerular pressure and proteinuria (predominantly of a glomerular source) increased (338 vs. 1146 mg/24h; P=0.006). The researchers concluded that conversion to SRL in patients with CAD is associated with hyperfiltration (caused by CNI withdrawal), which can partially explain the increase of proteinuria in already damaged kidney grafts. Therefore, they recommend that conversion to SRL in patients with CAD should be performed as early as possible, preferably when renal insufficiency is still moderate. (6)

In a study by Bumbea *et al.* (2005), 43 patients who had either CAN or CNI-related nephrotoxicity, were followed for 27 months after conversion to SRL. Kidney biopsies showed no differences in Banff scores when compared with biopsies made at baseline, no acute rejections occurred and 67% of patients had a stable or increased renal function by the end of the follow-up. 30.2% of patients discontinued treatment and 28% of the cohort developed proteinuria greater that 1g/day, although at baseline 82% had proteinuria lower than 150 mg/day. A biopsy was made at the time of developing this proteinuria and showed *de novo* lesions of focal and segmental hyalinosis. Another adverse effect found was hemoglobin decrease (50% of patients), which stabilized after the administration of rHuEPO. Three independent predictive factors for a positive response were identified: absence of

proteinuria at conversion, use of antihypertensive drugs at conversion and a level of LDH ≤ 559 IU/I at month 1 after conversion. (81)

One of the largest uncontrolled studies, by Garrouste *et al.* (2012), followed 150 patients for a median of 2.8 years (maximum 5 years) after a late conversion to SRL. There was a mean net gain in eGFR of 3 to 4 mL/min and BCAR occurred in 5.3% of the patients. 33% of the patients presented with proteinuria, this being *de novo* in 27% of the cohort. 37 patients stopped SRL therapy and were re-converted to CNIs, mainly because of cutaneous adverse events, heavy proteinuria or early degradation of renal function. The main adverse events detected were anemia and hyperlipidemia, which have improved in those patients that were re-converted to CNIs. The independent predictive factors associated with improved eGFR were: the graft being from a living donor, absence of anti-HLA alloantibodies at conversion and pre-conversion treatment with cyclosporine rather than tacrolimus. The researchers concluded that conversion to SRL improves renal function but is associated with a high discontinuance rate (43.3%), although reintroduction of CNIs is safe and grants a stable renal function for at least 1 year. (48)

Although these studies have shown some potential benefits from conversion, a randomized-controlled trial was needed to confirm these assumptions.

One of the first published randomized trials on late CNI withdrawal was made by Stallone *et al.* (2005). In this single-center study, 84 CNI-treated kidney-transplant patients that had been graft recipients for 12 to 36 months and had biopsy-proven CAN lesions, were randomized into two groups: group 1 was given a 40% reduction in CNI dose and had MMF added to the regimen and group 2 had CNI withdrawn and SRL started (trough levels between 6-10 mg/mL). Two years after randomization, the allograft survival rate was significantly higher in the SRL group (P=0.03) and kidney biopsies showed a stabilization of CAN lesions in patients of group 2, while CAN lesions in group 1 were significantly increased. The

expression of interstitial and vascular α-smooth muscle actin protein, a marker of fibroblast activation linked to the appearance of interstitial fibrosis, was also significantly reduced in group 2, whereas it was increased in biopsies from the CNI-treated group (P=0.005). Therefore, the researchers suggested that SRL could be able to slow the progression of chronic allograft lesions in kidney-transplant patients with CAN. (82)

Another randomized controlled trial, by Watson *et al*. (2005), involved 38 renal transplant recipients who were randomly assigned, 6 to 96 months after transplantation, to remain on their CNI-based regimen or to be converted to SRL (trough levels 5-15 ng/mL). The concomitant immunosuppression made before conversion (such as prednisone, AZA or MMF) was maintained. The conversion was abrupt, and the results after 12 months show not only that none of the patients in the study suffered acute rejection episodes during the trial, but also that there was a mean improvement in GFR of 8.5mL/min in patients switching to SRL, while patients that remained on CNIs had a mean GFR fall of 4.3 mL/min. The researchers also observed a positive correlation between baseline GFR and GFR at 12 months, suggesting that the better the initial renal function, the greater the improvement in GFR after conversion to SRL. There were no significant changes in 24h proteinuria between groups at 12 months, although all patients who switched to SRL had low levels of proteinuria at baseline. The principal adverse events following conversion were rashes (68%), and mouth ulcers (32%), that were mostly resolved with dose reduction. The researchers concluded that conversion to SRL improves GFR in patients with impaired graft function caused by CNI-based immunosuppression. (83)

One of the largest late-conversion trials made was the *CONVERT* trial, in which 830 CNI-based kidney-transplant patients were randomized, 6-120 months post transplant, either to be maintained on the same regimen or to be converted to SRL (trough levels 8-20 ng/mL). Enrollment in the stratum “GFR 20 to 40 mL/min” was halted prematurely when the primary

safety endpoint of acute rejection, graft loss or death was reached by 8 patients. Intention-to-treat analyses at 12 and 24 months showed no significant differences in GFR, but on-therapy analysis of the SRL patients of the stratum "baseline GFR > 40 ml/min" showed a significantly higher GFR at 12 and 24 months post conversion (62.6 vs. 59.9 ml/min; P=0.009). Rates of BCAR, graft survival and patient survival were similar between groups but malignancy rates were significantly lower after conversion to SRL (4% vs. 11%; P<0.001). At 12 months, a higher percentage of SRL patients discontinued treatment (15.7% vs. 9,5%; P=0.013), but the rates were similar by 24 months. The most common adverse events registered in the SRL group were hyperlipidemia, diarrhea, anemia, peripheral edema and albuminuria. The median UPr/Cr ratios were similar at baseline but were significantly increased after SRL conversion and there were also some patients who developed *de novo* proteinuria after SRL conversion. Moreover, global graft loss after randomization was associated with significantly higher baseline UPr/Cr and significantly lower baseline GFR. In this regard, post-hoc analyses identified a subgroup with "baseline GFR > 40 mL/min and UPr/Cr ≤ 0.11" whose risk-benefit profile was more favorable after conversion than that of the remaining SRL-conversion cohort (this group had less increment in UPr/Cr after conversion, better renal function, and experienced fewer deaths and graft losses than the remaining SRL-conversion cohort). The researchers therefore concluded that conversion from CNIs to SRL should be made as early as possible, preferably before the allograft has sustained permanent parenchymal injury and that the target population for conversion should be those with baseline GFR > 40 mL/min and with urinary protein excretion within normal limits. (43)

Another randomized-controlled trial was recently made by Han F. *et al*. (2011). In this study, 51 kidney transplant recipients with an estimated GFR between 30 and 60 mL/min/1.73 m^2 were randomized, at least 6 months (mean time 4.2 years) after transplantation, from a triple-therapy of CsA/MMF/Prednisone to either a therapy of SRL/MMF/Prednisone (n=22) or

“low-dose” CsA/ ”high-dose” MMF/Prednisone (n=29). The conversion was abrupt, and after 4 years of follow-up, there was a significant decrease in eGFR in the CsA group, while there was an increase in the eGFR of the SRL group, the latest being higher than that of the CsA group at 48 months ($p < 0.05$). The researchers also observed a significant increase in blood lipid levels in the SRL group. The Kaplan-Meier estimate of 4-year graft survival for the endpoints “graft loss” and “return to dialysis” was 77.3% in de SRL group and 55.2% in the CsA group (P=NS). Two acute rejection episodes were detected in the SRL group, while none was detected in the CsA group (P=NS). (84) A summary of these RCTs can be seen in Table 1.

Table 1: Summary of CNI combination, avoidance and conversion RCTs with SRL usage in renal transplantation.

Study	No of patients	Study design	F-up (months)	BCAR	Renal function	Comments
CNI COMBINATION WITH SRL						
Rapamune US study group	692	CsA/Pred/AZA or CsA/Pred/(SRL 2mg/d) or CSA/Pred/(SRL 5mg/d)	24	32.3% (AZA); 23.6% (SRL 2mg); 17.5% (SRL 5mg)	Lower in both SRL groups	P=NS when comparing SRL 2mg with AZA.
Rapamune Global study group	576	CsA/Pred/Placebo or CsA/Pred/(SRL 2mg/d) or CSA/Pred/(SRL 5mg/d)	24	43.1 % (Placebo); 29.5% (SRL 2mg); 26.0% (SRL 5mg)	Lower in both SRL groups	
RMR study	430	SRL/CsA/ST to either SRL/CsA/ST or SRL/ST	48	Similar at 48 months	Higher GFR in SRL group (58.3 vs 44.5 mL/ min)	
Baboolal et al.	133	SRL/CsA/ST to either SRL/"low-dose"CsA/ST or SRL/ST	6	Similar between the groups	Higher Cr clearance in the SRL/ST group (65 vs 57 mL/min)	
Tedesco-Silva et al.	207	SRL/CsA/ST to either SRL/"low-dose"CsA/ST or SRL/ST	12	Similar between the groups	No differences between the groups	
CNI AVOIDANCE						
SYMPHONY	1645	Low-dose CsA/MMF/ Pred or Standard dose CsA/MMF/Pred or TAC/MMF/Pred or Rapa/MMF/Pred	12	12.3% (TAC) vs 24% (LD CsA) vs 25.8% (SD CsA) vs 37.2% (SRL)	SRL group had the lowest mean calculated GFR.	Graft survival lower with SRL.
EARLY CONVERSION						
CONCEPT	192	CsA/MMF/ST to either CsA/MMF or SRL/MMF	12	17% (SRL) vs. 8% (CsA), p = NS	Higher CrCl in SRL group (68.9 vs 64.4 ml/min)	
POST CONCEPT	162	CsA/MMF/ST to either CsA/MMF or SRL/MMF	48	Similar between groups	Higher CrCl in SRL group (62.6 vs 57.1 ml/min)	Increase of new-onset diabetes in the SRL group
Spare-The-Nephron	299	CNI/MMF to either CNI/MMF or SRL/MMF	24	Late BCAR lower for the SRL/MMF group (2.9% vs 8.0%)	Increase of 8.6%(SRL); 3.4%(CNI); P=NS	
SMART	132	CsA/MMF/ST to either CsA/MMF or SRL/MMF	36	Similar during the 36 months follow-up	Higher eGFR in SRL group (60.88 vs 53.72 mL/ min)	Patients with low immun. risk
LATE CONVERSION						
Stallone et al.	84	CNI to either MMF/CNI(40% dose red.) or SRL	24	No acute rejection after randomization	No differences between the groups	CAN lesions stabilized in SRL group
Watson et al.	38	CNI-based to either CNI maintenance or SRL	12	No acute rejection after randomization	Improvement of 8.5 mL/min (SRL) vs fall of 4.3 mL/min (CNI)	
CONVERT	830	CNI-based to either CNI maintenance or SRL	24	Similar during the 24 months follow-up	Higher GFR in the stratum "baseline GFR > 40 ml/min" after SRL conversion	Increase of proteinuria after SRL conversion
Han et al.	51	CsA/MMF/ST to either "Low-dose" CsA / /"High-dose" MMF / ST or SRL/MMF/ST	48	Similar during the 48 months follow-up	Higher eGFR in SRL group (P<0.05)	Increase of hyperlipidmia after SRL conversion.

Note: Some trials would fit in more than one category but are listed only in one.

VI – Sirolimus induced proteinuria and renal injury?

Although initially SRL was thought to be non-nephrotoxic (40), recent studies support that SRL may have some nephrotoxicity: in kidney-graft transplant patients, some studies suggest that SRL may be responsible for not only an increase in the risk of delayed graft function, but also proteinuria and the development of *de novo* focal segmental glumerulosclerosis. (81, 85)

Sirolimus seems to be responsible for an increase in the risk of delayed graft function (DGF) in the immediate post-transplant period, mainly when using a combination of SRL/MMF and when the allograft is from a deceased donor. (75, 86) However, some studies suggest that the incidence of DGF is reduced when SRL is used in a lower dose. (74) This adverse effect may be due to the interaction of SRL with mTOR, which may promote apoptosis of tubular epithelial cells and impair their regeneration, thereby delaying recovery of renal function in some patients. (87)

Regarding the management of this adverse effect, Knight *et al.* (2004) concluded that the usage of an induction therapy with basiliximab or thymoglobulin in combination with SRL leads to a faster recovery of renal function. (88) Campistol *et al*. (2009) also suggest a reduction of SRL dose (trough levels of 4-8 ng/mL), a temporary SRL withdrawal if DGF is severe and performing transplant biopsies every 1-2 weeks to exclude acute rejection, during the period when DGF is noted to persist. (89)

Proteinuria after SRL administration is a frequent and important side effect, although some studies report that its incidence can be similar between regimens that compare combinations of SRL/CsA with CsA elimination and replacement with SRL. (69, 90) Proteinuria appears to be more frequent in patients who already had some levels of protein excretion before the immunosuppressant exchange, (91, 92) and many studies concluded that higher levels of proteinuria at baseline are correlated with a decrease in renal function after SRL conversion.(43, 79-81) However, proteinuria can also appear in patients without proteinuria at

baseline, (48, 91) as was shown by Stephany *et al.* (2006) who concluded that SRL was an independent predictor factor for proteinuria at 12 months, in *de novo* kidney transplant patients. (93) Some studies have also found that this proteinuria is associated with *de novo* focal and segmental glomerulosclerosis-like lesions. (81, 94)

The mechanisms behind the origin of this proteinuria are probably multiple: Initially, because proteinuria appeared in patients who were converted from CNIs to SRL because of CAN, it was thought that proteinuria was a manifestation of preexisting glomerular lesions. (95) Other studies suggested that hemodynamic mechanisms are partially responsible for the increase in proteinuria: since CNIs reduce renal blood flow, their disruption can increase glomerular pressure. This was observed by Saurina *et al.* (2006) who concluded that after conversion to SRL, afferent arteriolar resistance and renal functional reserve decreased, while effective renal plasma flow and intraglomerular pressure increased and that, therefore, CNI withdrawal lead to hyperfiltration which, by its turn, was responsible for an increase in urinary protein excretion. (6) However, since proteinuria can occur also in patients who have never received CNI therapy (93) and it is associated with *de novo* renal injuries, (81) suggests that SRL is also directly responsible for an increase in proteinuria. In fact, a study by Letavernier *et al*. (2008) has associated SRL with podocyte injury, which then may be responsible for the focal-segmental glomerulosclerosis-like lesions observed in some patients. Sirolimus may mediate this process by a decrease in vascular endothelial growth factor or through its effect on mTOR, which is known to cause apoptosis. (95) Other studies have reinforced the association between SRL and podocyte dysregulation, but also provided evidence that SRL-induced proteinuria may be a dose-dependent effect. (96) Studies in animal models of kidney diseases also have shown that SRL prevents podocyte regeneration and aggravates renal injury, although it only produces slight alterations in healthy podocytes. (97)

However, the exact mechanism behind SRL interaction with renal cells is not yet fully understood, and since a significant number of patients don't show an increase in proteinuria after SRL conversion, other unknown factors may also contribute to its increase. (95)

VII – Conclusion

Answering the initial question of this review: "Does conversion of CsA to SRL grants renoprotection in kidney transplantation?", the answer is "yes, but with particular caution and close monitoring in some patients".

SRL conversion when the kidneys are already damaged is associated with an increase in proteinuria, a decrease in hemoglobin, the appearance of hyperlipidemia and the biggest randomized controlled trial (43) found no increase in graft and patient survival after 2 years. However, it found that in patients with baseline GFR > 40mL/min and UPr/Cr ≤ 0.11, conversion could be beneficial and the same opinion was obtained from other studies, who have recommended that conversion should be made before a prolonged exposure to CNIs, when baseline proteinuria is low and GFR is within normal range. (79-81) One study has also suggested that conversion can be even more beneficial in patients that were converted from CsA (instead of tacrolimus) or that received an allograft from a living donor. (48) Moreover, late conversion to SRL is associated with similar acute rejection rates than the ones obtained with CNI therapies and in many late-conversion studies reported in this review, a significant portion of the studied patients obtained an improvement or stabilization in renal function and/or stabilization in kidney-allograft lesions after conversion to SRL. (81, 82) This was the case observed in the *RMR* trial, where biopsies made 3 years after conversion showed a decrease in tubular atrophy in the SRL/ST group. (65)

Although some trials have shown that patients needed to be reconverted to CNIs because of adverse events, a meaningful number of those patients have experienced a remission of said

adverse events after reconversion. Therefore, late conversion to SRL appears to be beneficial in some selected patients, but may not grant benefits if advanced loss of renal function and high proteinuria are already present before conversion.

The prevention of the onset of CNI-related lesions is one of the motivations behind early conversion from CsA to SRL. In the analyzed early-conversion trials, some conclusions are evident: there is a significant increase in renal function resulting from conversion to SRL, there were no significant differences in graft and patient survival and there were no statistically significant differences in acute rejection rates. However, these studies have also shown that early conversion is associated with an increase in the incidence of hyperlipidemia and proteinuria, and some works have also reported a decrease in hemoglobin and the appearance of new-onset diabetes. Some studies have also reported increased dropout rates in the SRL groups, although in the *CONCEPT* trial these rates were associated with an increased SRL loading dose and in the *SMART* trial were associated with the conversion being made too early after transplantation (2-3 weeks after transplantation). From these studies, it seems that conversion from CNI-based to SRL-based immunosuppression, when made in an ideal post-transplant time (i.e. 1-6 months after transplantation) is associated with an increase in renal function, a similar risk of acute rejection and patient and graft survival rates similar to those of the CNI-based immunosuppressive regimens. However, both early and late conversion should be avoided in the high metabolic risk patients (with a personal or familial history of either lipid or glucose metabolism disorders) and reconversion should be considered in those cases where proteinuria increases rapidly or reaches high values and whenever significant changes in renal function are observed. Moreover, since the follow-up times are limited to 4 years, the benefits in graft and patient survival for longer periods of time are still unknown. Therefore, longer studies are needed to reinforce the current knowledge that indicates the

favorable use of protocols of conversion to sirolimus as effective alternatives to minimize cyclosporine-induced nephrotoxicity in renal transplant patients.

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