Chapter 1

Proliferative Signal Inhibitor in Cardiac Transplantation

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Abstract

Immunosuppression following cardiac transplantation has traditionally comprised a calcineurin inhibitor (CNI) in combination with mycophenolate mofetil or azathioprine and corticosteroids. This combination provides effective immunosuppression but associated with long term adverse events. Proliferation signal inhibitors (PSI) (such as sirolimus and everolimus) have been shown to be effective in attenuating the development of CAV following cardiac transplantation, reduced the incidence of clinically significant cardiac events and improved CNI induced nephropathy. Therefore, PSI have the potential to improve long-term survival following cardiac transplantation.

Abbreviations

HTx–Heart Transplantation; CNI–Calcineurin Inhibitor; PSIs–Proliferation Signal Inhibitors; CAV–Cardiac Allograft Vasculopathy; CMV–Cytomegalovirus; EVR–Everolimus; SRL–Sirolimus; CsA–Cyclosporine; TAC–Tacrolimus; AZA–Azathioprine; ISHLT–International Society of Heart and Lung Transplantation; IVUS–Intravascular Ultrasound; VH-IVUS–Virtual Histology Intravascular Ultrasound; BPAR–Biopsy Proved Acute Rejection; MIT–Maximal Intimal Thickening
Introduction

Heart transplantation (HTx) has become a well-established therapeutic option for patients with end-stage heart disease. In the early 1980s, the introduction of Calcineurin Inhibitors (CNIs) as the mainstay of maintenance immunosuppression was followed by a significant improvement in rates of acute rejection and survival after HTx. CNIs have become a part of standard immunotherapy in conjunction with antimetabolites such as MMF with one-year survival approaching 90%, five-year survival of 70%, and 10-year survival of 50%. These improvements, however, come at the cost of significant adverse effects associated with CNI-based immunosuppression. One major adverse effect is renal failure, which affects 26%, 52%, and 68% of HT recipients during the 1.5 and 10 years, respectively, after HTx and is associated with a 4 4-fold increase in mortality [1]. Moreover, prolonged use of CNIs may contribute to CAV by remodeling of cardiac tissue [2] and stimulating fibrogenic growth factor in genetically predisposed cardiac transplant recipients. The International Society of Heart and Lung Transplantation (ISHLT) registry in 2013 indicates that CAV affects 8% by the first year, 30% by 5 years, and 50% by 10 years after HTx and 5 years after HTX, CAV and graft failure together account for 30% of deaths [1]. Modification of traditional risk factors may attenuate disease progression and improve outcomes [3-5], but in severe CAV, the prognosis is grave and the only treatment option is re-transplantation.

Proliferation Signal Inhibitors (PSI)

The history of proliferation signal inhibitors (PSIs) or mammalian target of rapamycin (mTOR) inhibitors dates from early 1970, when rapamycin was isolated from a strain of Streptomyces hygroscopicus in soil at Easter Island (Rapa Nui) and its antifungal and immunosuppressive properties [6] were confirmed. PSIs form a complex with the intracellular binding protein FKBP-12, but unlike cyclosporine (CsA) and tacrolimus (TAC), which block T cell activation induced by stimuli using Ca2+-dependent pathways, PSIs inhibit the activity of a serine/threonine kinase [7-10] and arrest the cell cycle in the mid-to-late G1 phase [11-14]. In vitro, PSIs inhibit vascular smooth muscle cell migration [15] and proliferation [16,17]. PSIs reduce protein and collagen synthesis by 40% to 60% [40] and inhibit endothelial progenitor cells [18]. Therefore PSIs, in addition to potent immunosuppressive ability, have other beneficial therapeutic effects in heart transplant recipients, including attenuation of CAV as well as regression of cardiac hypertrophy with consequent improvement in cardiac allograft function [19,20].

PSIs include two drugs currently available for clinical use: sirolimus (SRL) previously known as rapamycin) (Rapamune; Wyeth Pharmaceuticals, USA) (Rapamune; Wyeth Pharmaceuticals, Philadelphia, PA, USA), and its derivate everolimus (EVR, Certican; Novartis Pharma AG, Basel, Switzerland).
**SRL** is rapidly absorbed with a peak blood concentration reached after $2.7 \pm 2.1$ hours and is dose dependent [21]. The absolute bioavailability of SRL in humans is approximately 15% and it is highly variable and strongly affected by food [22]. Intestinal CYP3A metabolism and intestinal P-gp counter-transport, intestinal membrane permeability, and hepatic first-pass all affect bioavailability and may influence SRL absorption because the drug is a substrate for these enzymes and transporters [23].

Differing only by the presence of an extra hydroxyethyl group at position 40, there are important pharmacokinetic and pharmacodynamic differences between **EVR** and SRL. EVR is rapidly absorbed, achieving a peak concentration within 1 to 2 hr, has a higher oral availability and lower plasma protein binding than SRL. Food, in particular high-fat meals, delays absorption and time to steady state; therefore, consistent administration with or without meals is recommended. Twice daily administration of EVR 0.75 mg, with a goal trough level of 3 to 8 ng/dL has become standard with this drug [24-26]. Target concentrations of EVR are verified using a commercially approved immunoassay (Thermo Fisher Scientific, Fremont, CA). Metabolism occurs predominantly by means of the hepatic cytochrome P450 enzyme system, using 3A4, 3A5, and 2C8, and is reduced with moderate hepatic dysfunction. EVR is a substrate for these enzymes as well as P-glycoprotein. EVR has a considerably shorter half-life than SRL (28 hours compared with 62 hours, respectively) and a more rapid time to steady state (4 days versus 6 days for SRL) [27]. The mean elimination half-life is shorter for EVR (estimated 30 +/- 11 hours) and the clearance of EVR is 20% higher in black patients. Noteworthy interactions exist when EVR is administered with grapefruit juice and agents in the macrolide,azole, antiepileptic, and anti-human immunodeficiency virus drugs. A well-documented drug to drug interaction has been identified between EVR and Cyclosporine (CsA; Neoral; Sandoz Pharmaceuticals, Princeton, NJ) [28]. Fewer reports are available evaluating the interaction between EVR and tacrolimus (TAC; Prograf; Astellas Pharmaceuticals, Deerfield, IL); [29], and this combination after HTX although administered in selected patients in some German centers, remains off-label use. An EVR dose-dependent decrease in TAC exposure has been documented when the two were administered together [25]. TAC does not influence EVR blood levels.

**PSIs and CAV**

**Preclinical Studies**

The potent antiproliferative properties of PSIs have been demonstrated in a number of studies using animal models of transplantation. The anti-CAV effectiveness of SRL was demonstrated in rodent model with a minimal myocardial cellular infiltrate and was not explained solely by a reduction in T-cell–mediated immunity.
was thought to inhibit the final common pathway of CAV pathophysiology, smooth muscle cell growth [30]. Although SRL has been shown to act synergistically with CNI to prolong rat cardiac allograft survival [31], it has effectively reduced intimal hyperplasia in rat cardiac allografts as the sole therapy [32]. It has been demonstrated that SRL not only halts progression, but also prevents development of CAV. Even a single preoperative intravenous dose of SRL significantly reduced the incidence of cardiac allograft arteriopathy [33]. In the rat aortic allograft model, SRL effectively prevented both acute rejection and graft arteriopathy and was as effective as high-dose CsA therapy [34]. Similar findings have been reproduced in a non-human primate. De-novo SRL monotherapy prevented the development of CAV as determined by IVUS [35]. Late onset treatment with SRL not only halted the progression of preexisting vasculopathy, but also was associated with partial regression in intimal area up to 20% in four out of the six treated animals [36]. These studies suggest that besides its antiproliferative properties, there are mechanisms by which SRL may also be cytotoxic. It was shown that SRL may induce apoptosis [37,38], prevent degradation of p27KIP1, a cyclin-dependent kinase inhibitor [20,39] and activates autophagy [40-42]. The development of graft vascular disease was also examined following administration of EVR. [43,44].

Clinical Studies

Denovo PSIs immunosuppression

The first clinical evidence that PSIs could limit the development of allograft vasculopathy in heart transplant recipients was provided by an international multicenter randomized, double blind study (RAD B253) comparing EVR as a secondary immunosuppression (1.5 mg/kg/day or 3 mg/day) with azathioprine (AZA, 1-3 mg/kg/day) given with full dose of CsA within the first 72 hours after HTxin 634 de-novo recipients[45]. The occurrence of graft loss and death at 12 months were not statistically different between the groups; however, the incidence of multiple episodes of biopsy-proven acute rejection (BPAR) (at least grade 3A) was higher in the AZA group (P=0.02).

The results of the intravascular sub-study with matched analysis of baseline and 1-year images were available for 211 patients (70 patients treated with 1.5 mg EVR daily, 69 patients treated with 3.0 mg EVR daily and 72 AZA-treated patients). The average increase in intimal thickness was significantly lower with EVR 1.5 mg/day (0.04 mm; P=0.01) or 3.0 mg/day (0.03 mm; P=0.003) than with AZA (0.10 mm) despite worsening of dyslipidemia in the EVR group. This was accompanied by a significantly lower incidence of CAV in the EVR 1.5 mg/day (35.7%; P=0.045) and 3.0 mg/day (30.4%; P=0.01) groups compared with the AZA group (52.8%).
There was a 12 month of open-label period [46] following completion of the original blinded study. In a group of 149 HTx recipients continued treatment with EVR limited the progression of intimal thickening and lowered the incidence of allograft vasculopathy at 24 months when compared with AZA. The difference was significant for patients receiving EVR 1.5 mg, whereas for patients treated with EVR 3 mg decrease in the incidence of CAV did not reach statistical significance. Lipid-lowering therapy with statins was mandatory for all patients.

The four-year follow-up data (211 HTx patients: AZA n=72; EVR 1.5 mg/day n=70; EVR 3.0 mg/day, n=69) showed that the benefits of EVR translate into reduced rates of cardiovascular events with cost savings relative to the use of AZA [47]. Notably, the reduction in cardiovascular events in the EVR groups was largely accounted for by a reduction in PCI (mainly angioplasty), with six incidences of PCI in the AZA group compared with one in the EVR 3.0 mg/day group. There were no incidences of PCI in the EVR 1.5 mg/day group. Therefore, the results from this study were the first to establish the value of EVR 1.5 mg in limiting the development of CAV in HTx recipients.

Similar benefit was also seen in patients treated with SRL. In an open-labeled study [48], 136 de-novo HTx patients were randomized to SRL or AZA. The IVUS data showed that the use of SRL significantly reduced the progression in intimal plus media proliferation by 6 months, whereas AZA therapy resulted in increased plaque volume and plaque burden over time. This effect was sustained at 2 years.

While the evidence that PSIs used in combination with full CNIs dose may reduce the incidence of CAV, questions were raised regarding the increased renal toxicity which is thought to be due to potentiation of the nephrotoxic effect of CNIs [49,50]. Moreover, regimens incorporating PSIs with reduced CNIs doses were associated with a lower incidence of hypertension, hypercholesterolemia and hypertriglyceridemia [51]. These trials were the basis for subsequent studies involving early dose reduction of CsA with PSIs and steroids.

A previous IVUS study in which patients treated with MMF had significantly less progression of first-year intimal thickening compared to patients treated with AZA suggested that MMF may also have antiproliferative effects and limit the occurrence of CAV [52]. Therefore, an open-label, multicenter study was conducted from January 2006 to July 2011 and included 63 transplant centers in Europe, North and South America, Asia and Australasia (A2310). De novo HTx recipients were randomized within 72 h of HTxin a 1:1:1 ratio to receive: 1) EVR 1.5 mg (target trough concentration 3 to 8 ng/ml) with reduced-dose CsA; 2) EVR 3.0 mg (target trough concentration 6 to 12 ng/ml) with reduced-dose CsA; or 3) MMF 3 g (1.5 mg b.i.d) with full dose CsA [52,53]. Enrollment into the
EVR 3.0 mg/day group was stopped early, in March 2008, given an increased rate of death within the first 90 days post-randomization in this treatment group. Of the 721 patients originally enrolled in the trial, 553 remained in the 1.5 mg/day of EVR or 3 g/day of MMF groups.

EVR with reduced-dose CsA achieved similar efficacy to MMF with standard-dose CsA following HTx, although adverse events and study drug discontinuations were more frequent with EVR 1.5 mg than MMF. EVR at higher levels of exposure (6–12 ng/mL) is not advised due to an increased mortality rate. Moreover, in patients receiving rATG induction, the mortality rate for the EVR 1.5 mg group was higher at 12 month (7.8% [22/282] vs. 4.8% [13/271] for MMF) due to a high rate of early infection, but similar at 24 months (10.6% [30/282] vs. 9.2% [25/271]).

IVUS data at baseline and 12 months was available for 35% of patients, which included 88 patients assigned to the EVR 1.5 mg group and 101 to the MMF group. The incidence of CAV defined as first-year change in MIT >0.5 mm, was significantly lower in the EVR 1.5 mg group compared with the MMF group (12.5% vs. 26.7%, p = 0.018). The EVR 1.5 mg group compared to the MMF group demonstrated a significant reduction in first-year intimal thickening regardless of donor age and lipid level. The percentage of patients with de novo CAV was also significantly lower in the EVR 1.5 mg group compared with the MMF group (9.1% vs.19.8%, p = 0.042).

In contrast to findings from a retrospective study of SRL with reduced-exposure CsA [54], sternal and other wound healing was not impaired in the EVR group [55,56].

Despite several retrospective studies demonstrating successful use of SRL as a primary immunosuppres-sant after HTx [57,58,59], the only randomized trial of de novo SRL treatment with complete CNI withdrawal was terminated prematurely due to increased rate of acute rejection [60] and reported to the US Food and Drug Administration (http://www.fda.gov/medwatch/safety/2007/cellcept DHCP letter 02-01-2007.pdf). This study proposed an abrupt switch from CNI-based immuno-suppression at 3 months after HTx, and SRL levels in patients who experienced rejection were low.

Due to negative experience with SRL in de-novo primary immunosuppression, additional randomized studies of PSI treatment with complete CNI withdrawal in de novo HTx recipients were conducted in Europe. De novo EVR was compared to CsA in a randomized, open-label trial (SCHEDULE) [61]. 115 heart transplant recipients treated with lymphocyte-depleting inductionimmunosuppression were assigned within 5 days post-transplant to low-exposure EVR (target level 3–6 ng/mL) with reduced-exposure CsA (n = 56), or standard-exposure CsA (n = 59), with both MMF and corticosteroids. In the EVR group, CsA was withdrawn after 7–11 weeks and EVR exposure increased (target level 6–10 ng/mL). At the end of the 12-month trial, the mean increase in MIT by IVUS
was smaller (0.03 mm vs. 0.08 mm, p = 0.03), and the incidence of CAV was lower (50.0% vs. 64.6%, p = 0.003) with EVR versus CsA [62], but mild to moderate acute rejection was more frequent after CNI therapy was withdrawn (p = 0.03). Left ventricular function was not inferior with EVR versus CsA.

SCHEDULE trial also suggested that EVR initiation and CNI withdrawal early after HTx may have some impact of systemic inflammation as reflected by a significant decline in sTNFR-1 in the EVR group.

SCHEDULE sub-study also investigated invasive hemodynamics during rest and exercise in EVE (n=28) and CsA (n=34) patients, early (7 weeks) and later (52 weeks) after HTx and found no difference in cardiac reserve between the groups [64].

The three year follow-up data, which included 102 patients [65,66] revealed significantly reduced progression of CAV assessed by IVUS in the EVR group compared to the CNI group. During months 12-36, BPAR grade ≥2R occurred in 10.2% and 5.9% (NS) and serious adverse events occurred in 37.3% and 19.6% of EVR versus CNI-treated patients (p = 0.08). These results suggested that in cardiac transplant recipients, early CNI withdrawal with replacement by EVR as a primary immunosuppression combined with MMF and steroids is safe at intermediate follow-up.

Maintenance PSI immunosuppression

There is a growing body of evidence indicating that the underlying pathophysiologic mechanisms is markedly different in the early versus late stages of CAV development [67-69]. This suggests that the results of de novo PSIs treatment may not be applicable to maintenance recipients with established CAV. Although intimal thickening traditionally has been the focus of research, several observations indicate that impaired positive remodeling also contributes to a net lumen loss [70]. The same signals perpetuating intimal proliferation initiate a cascade of pathways adversely affect the ability of the coronary vessel to positively remodel [71].

The first evidence suggesting that PSIs may be effective in preventing worsening of CAV came from a randomized study by Mancini [72] which assigned 46 patients approximately 3 years after HTx to treatment with SRL (n=22) as a secondary immunosuppressive agent or continued conventional immunosuppressive agents (n=24). The primary endpoint, a composite of death, myocardial infarction, revascularization, or worsening of a coronary angiographic score, was significantly reduced in the SRL group showing that SRL reduced the incidence of clinically significant cardiac events. However, MIT was measured with IVUS only in a minority of patients and no difference was found.

Several IVUS-based retrospective single center obser-
vational studies suggested that the benefit from PSI therapy appears to be time dependent and most pronounced if it introduced early post-transplant. A retrospective study from Mayo Clinic compared 3D IVUS findings in 45 HTx recipients treated with SRL based immunosuppression after complete CNI withdrawal to 58 patients treated with standard CNI protocols. SRL as a primary immunosuppressive was found to attenuate the progression of CAV by reducing intimal hyperplasia and plaque burden when initiated early (< 2 years) after HTx. In patients converted to SRL late (≥ 2 years) after HTx SRL treatment did not affect intimal thickness, but improved positive vascular remodeling (P=0.01). Treatment with AZA or MMF did not affect the results; there was no difference in late rejection episodes [71]. Importantly, five-year survival (97.4±1.8% versus 81.8±4.9%; P=0.006), and freedom from cardiac-related events (93.6±3.2% versus 76.9±5.5%; P=0.002) was improved with SRL [73].

Similar results were found by Masetti et al. in analysis of early and late CAV development of 143 consecutive HTx patients treated with EVR or MMF as a secondary immunosuppression in combination with CNI. EVR treatment was associated with a reduced progression of coronary intimal hyperplasia during the first post-transplant year, but did not influence changes in IVUS parameters assessed between years 1 and 5 after transplant [74]. In contrast, in a study from Japan the conversion to EVR instead MMF in combination with a low-dose CNIs resulted in a suppressive effect on the development of CAV in both the early (< 2 years after HTx) and late (≥ 2 years) period after HTx; EVR treatment in this study was effective not only by reducing plaque progression but also by suppressing negative vessel remodeling [75].

In the 12-month, open-label, multicenter, randomized, controlled trial of 282 heart and lung transplant patients in Scandinavia (NOCTET, Nordic Certican Trial in Heart and lung Transplantation), patients were randomized 5.8±4.3 years following transplantation to continue their CNI based immunosuppressive regimen or start EVR therapy with a predefined reduction in CNI exposure. While introduction of EVR with CNI reduction achieved a significant improvement in renal function [76,77], there was no difference in CAV progression between the treatment groups (P = 0.30) in the IVUS sub-study of 111 patients. Interestingly, CAV progression was attenuated with EVR versus standard CNI in patients receiving concomitant AZA therapy (change in MIT 0.00 ± 0.04 and 0.04 ± 0.04 mm, change in percent atheroma volume 0.2% ± 3.0% and 2.6% ± 2.5%, mm3, [P < 0.05]). However, in patients receiving MMF (MMF), accelerated CAV progression occurred with EVR versus standard CNI (change in MIT 0.06 ± 0.12 vs. 0.02 ± 0.06 mm and change in percent atheroma volume 4.0% ± 6.3% vs. 1.4% ± 3.1% mm3, P < 0.05). Moreover, the levels of C-reactive protein and vascular cell adhesion molecule-1 declined significantly with EVR+ AZA, whereas EVR + MMF pa-
patients demonstrated a significant increase in levels of C-reactive protein, vascular cell adhesion molecule-1, and von Willebrand factor. The different effect of EVR when combined with AZA versus MMF could potentially reflect an unknown interaction.

Further, the virtual histology (VH) IVUS sub-study from the NOCTET revealed a significant increase in calcified (2.4 ± 4.0 vs. 0.3 ± 3.1%; p = 0.02) and necrotic component (6.5 ± 8.5 vs. 1.1 ± 8.6%; p = 0.01) among EVR with reduced CNI patients (n = 30) compared to standard CNI-therapy (n = 48) HTx recipients. The increase in necrotic and calcified components was most prominent in EVR patients with time since HTx >5 years and was accompanied by a significant increase in levels of von Willebrand factor (p = 0.04) and vascular cell adhesion molecule (p = 0.03) [78].

A similar VH IVUS finding was reported with SRL treatment: plaque progression was attenuated owing to a decrease in the progression of fibrous plaque component during the first 5 years after HTx. However, late (>5 years post HTx), SRL conversion was associated with a significant increase in the inflammatory plaque (necrotic core and dense calcium) component, while the overall plaque volume was not affected at all [79]. As recognized by the authors, early and late CAV lesions are pathologically heterogeneous and can also be affected differently by SRL.

These findings raise the concern that initiation of PSIs in some patients with established CAV may not slow progression and may potentiate inflammation and the development of necrosis and calcification in the CAV tissue with uncertain consequences [80].

A Meta-Analysis of five Randomized Clinical Trials showed no significant difference in MIT changes (Mean difference: -0.03mm [95%CI: -0.06, 0.01]; I2=78%) between standard and PSI based immunosuppression. The progression of CAV (mean difference: -0.04mm. [95%CI: -0.06, -0.02mm]; I2=0%) and the incidence of CAV (Risk ratio: 0.6 [95%CI: 0.45, 0.81]; I2=0%) was attenuated in the subgroup of patients receiving de-novo PSI therapy. This benefit, however, did not confer a lower risk of 1-year mortality (RR: 1.12 [95%CI: 0.61, 2.07]; I2=35%), and was associated with 52% increased risk of adverse event (RR: 1.52 [95%CI: 1.19, 7.93]; I2=0%), NNH:13 [95%CI: 8, 33]) [81].

In summary, the data demonstrated that immunosuppressive potency can be maintained in HTx patients receiving PSI, despite marked CNI reduction or even complete withdrawal. The optimal time for conversion and the adequate reduction in CNI exposure remains to be defined. The early initiation of PSI offers greater benefits in the treatment of CAV; however, a very early CNI withdrawal may be inadvisable. In randomized studies, EVR has been combined with reduced CsA dose and compared to standard CsA immunosuppression. TAC-based immunosuppression offers superior prevention of rejection compared with CsA [82], appears to promote less intima
proliferation in the first post HTx year [83] and the combination of TAC and MMF is the base immunosuppression regimen currently used in the majority transplant centers [1]. The safety and efficacy of EVR in combination with TAC needs further clarification.

The ongoing 12-month multicenter, randomized, open-label, parallel-group study (MANDELA) will enroll approximately 200 HTx patients. Patients will receive CNI therapy, steroids and EVR or MMF during months 3 to 6 post-transplant, and will be then be randomized at month 6 post-transplant to 1) conversion to CNI-free immunosuppression with EVR and MMF or 2) continue reduced-exposure CNI, with concomitant EVR. Patients will be followed up to 18 post-HTx and efficacy and safety of the treatments will be compared [84].

**PSIs and renal function**

There have been a number of studies evaluating the use of PSI inhibitors in HTx recipients and their role in reducing kidney damage.

In the landmark EVR trial, RAD B253, full dose CsA in combination with EVR 1.5 and 3mg/day versus AZA resulted in significant worsening of renal function [45]. PSI with full-dose CNIs showed worse renal outcomes in several observational studies and questions were raised regarding the increased renal toxicity due to potentiation of the nephrotoxic effect of CNIs in combination with by PSIs [48-50]. These data suggested that lower doses of CsA for therapy are needed to decrease the risks of nephrotoxicity.

Several studies evaluated de novo PSIs based immunosuppression with complete CNI withdrawal. González-Vilchez and colleagues found a significant improvement in eGFR from 28 to 67 mL/min/1.73 m2 at one month with the initiation of a PSI, which remained significant at 3 and 6-months post-HTx; however, this immunosuppression regime was associated with very high rates of rejection [85].

In the SCHEDULE trial [86] there was a significant improvement in at 12 months GFR (79.8 mL/min/1.73 m2 vs 61.5 mL/min/1.73 m2, p<0.001) in patients treated with EVR as a primary immunosuppression compared to the CsA group. The improvement in renal function was significant at every time point from week 4 onward, with the benefits becoming more pronounced after discontinuation of CsA. This study demonstrated significantly higher BPAR (76.9% vs. 66.1%, p=0.03) which included significantly higher rejection graded ≥ 2R in the EVR group (40% vs. 18%, p=0.012).

Due to the concerns of complete CNI withdrawal in de novo immunosuppression, additional investigations were conducted to assess CNI-minimization with PSI use. Observational studies by Lehmkuhl and colleagues have shown that reduced CsA concentrations correlate with improved renal function when EVR is used in place...
of MMF. However, randomized studies investigating de novo PSI in combination with low dose CNI have revealed conflicting results.

In A2411, the prospective, multi-center trial, Lehmkuhl and colleagues randomized patients with a CrCl of at least 50mL/min to de novo EVR 0.75mg BID (target levels of 3-8 ng/mL) with reduced dose CsA or standard dose CsA and MMF 1500mg BID [87]. There was no significant difference in renal function at 6 or 12 months between the EVR and MMF groups (65.4 mL/min vs. 72.2 mL/min, and 68.7 mL/min vs. 71.8 mL/min, respectively) and in both groups, there was a reduction in eGFR from baseline to month 12. However, the diabetes incidence and the baseline creatinine clearance (72.5 ± 27.9 mL/min in the EVR group and 76.8 ± 32.1 mL/min in the MMF group) was lower in EVR group at randomization. Moreover, CsA level in EVR group was higher than targeted.

In the A2310 study, Eisen and colleagues investigated three different regimens effects on renal function [53]. In the EVR 1.5mg arm, CsA levels were at the upper limit, to above normal from month 3 onward; however, they were within goal in the MMF arm. Baseline eGFR was similar between the groups. At 12 months, non-inferiority was not established with EVR compared to MMF with a mean eGFR of 59.4 mL/min/1.73 m2 vs. 64.7 mL/min/1.73 m2. There was a lower reduction in eGFR with EVR compared to mycophenolate from month 1 to month 12 (-8.6 vs. -14.6 mL/min/1.73 m2, p = 0.009) which may be due to the fact that CsA concentrations were the same during the first month post-transplant but subsequently decreased over the 12 months. There was no difference in the rates of rejection in both A2411 and A2310.

Overall, de novo use of PSIs in combination with reduced-dose CNIs demonstrate a neutral effect on renal function compared to full dose CNIs in combination with MMF; however, the evidence is concerning for higher rates of rejection in this population, especially when used as the primary immunosuppressant.

In maintenance immunosuppression, there were several single center reports of PSIs as a primary immunosuppressant with complete withdrawal of CNIs in patients with CNI-induced nephrotoxicity [59,88]. In the Mayo clinic study patients with renal dysfunction (CrCl <50 mL/min by iohalamate clearance) at a mean of 4.6 years after HTx were converted to SRL in place of CNIs. Renal function significantly improved from 47.7 mL/min at baseline to 56.9 mL/min at 12 months and 61.3 mL/min at 24 months in 70% of patients in the SRL group. Tis study reported of increase in proteinuria in SRL treated patients at 12 months from 299mg/day to 517mg/day (p=0.002) and them plateau up to 24 months of follow up. Patients with ischemic cardiomyopathy, diabetes mellitus and proteinuria prior to SRL conversion were less likely to respond to SRL.

Due to the positive results seen in single-center studies, Gude and colleagues investigated the ideal timing of
conversion from CNIs to PSIs [57]. They randomized patients to early (within first post HTx year) or late (> 1 year) conversion to CNI-free regimens with an overnight switch from CsA to EVR. In the early group, estimated glomerular filtration rate (eGFR) improved from 29 to 62 mL/min/1.73 m² (p<0.001); however, in the long-term CNI group there was only a modest increase in eGFR from 26 to 28 mL/min/1.73 m² (p=0.225). This study suggests that early conversion is necessary for patients to receive the renal benefits of CNI withdrawal.

PSIs as secondary immunosuppression in maintenance therapy have been investigated in several studies. In the single-center SHIRAKISS study, 34 patients with moderate renal dysfunction (creatinine clearance 20-60 mL/min) were randomized to EVR (target 3-8 ng/mL) with CsA (40-90 ng/mL) or MMF with CsA (100-150 ng/mL) [89]. Baseline CrCl went from 43.5 mL/min to 49.8 mL/min with EVR vs. 44.5 mL/min with MMF at 12 months (p=0.5). The beneficial effects of EVR were only seen in patients with no proteinuria at baseline (urine protein <150 mg/day). The same observation was made in their previous study where baseline proteinuria of less than 150 mg/24 hours was the only independent predictor of improvement in creatinine clearance (odds ratio 5.5 [1.05-29.4]; p=0.04) [90]. In patients with proteinuria of greater than 150 mg/24 hours, EVR treatment was associated with significantly worse renal function.

In a large, randomized, multi-center study of both heart and lung transplant recipients, NOCTET, patients renal function was compared after initiating EVR with reduced CNI exposure compared to standard-CNI exposure with MMF [76]. In the EVR group, the levels of CNI were significantly different. At 12-months, the mean eGFR increased in the EVR group (+4.6 mL/min) and decreased in the MMF (-0.5 mL/min, p<0.0001). There was a greater benefit observed in HTx patients compared to lung transplants. Similar to the Gude study, patients with a shorter time post-HTx had the largest improvement in eGFR after conversion to EVR. Additionally, patients who were on CsA showed a significant improvement in renal function (+4.6 vs -0.5 mL/min, p<0.001) while patients on TSC did not (6.3 vs. -0.5, p=0.06). Patients receiving CsA were within goal levels (p<0.001); whereas patients on TAC were consistently above goal (p=0.77).

The changes in creatinine clearance with the use of PSIs are more extensively reported; however, the development of proteinuria with PSI-based regimens is also of relevance. Both Potena and Raichlins studies demonstrated worsening renal function in patients with pre-existing proteinuria prior to PSI use. The mechanisms underlying this phenomenon have not been elucidated. Raichlin et. al. found attenuation of PSI induced proteinuria by angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin-receptor blockers (ARB) [88].
In summary, based on existing evidence, de novo use of PSI as a primary immunosuppression is not recommended due to increased rejection rate. Additionally, de novo use of PSI in combination with reduced-exposure CNI may have beneficial effects in patients with pre-existing CNI nephrotoxicity; however, there might be also a higher incidence of rejection in this population. The group that appears to have the largest benefit from PSI use is patients with mild-moderate renal dysfunction and transitioning to a PSI in combination with low-exposure CNI within the first year post-transplant.

PSIs and Cytomegalovirus (CMV) infection

Cytomegalovirus (CMV) infection is the most common viral infection in solid organ transplant patients especially in heart transplant patients. The symptomatology for clinical infectious disease ranges from self-limiting asymptomatic viremia through CMV syndrome to life threatening multiorgan invasive disease [91]. In addition to direct infectious syndromes, CMV infection can induce indirect effects. Among those are increased risk of allograft rejection, accelerated CAV progression and higher mortality [92]. The highest risk for symptomatic disease exists in the CMV-seronegative recipients receiving hearts from CMV-seropositive donor (D+/R-) [93]. Reactivation infection also develops in the patients who are CMV-seropositive before HTx and these patients are also at risk for super infection by CMV from a CMV-seropositive donor, especially in the setting of intense immunosuppression.

There is an increasing amount of evidences to support that PSI inhibitors decrease rate of CMV infection in HTx patients.

In the RAD B253 study, CMV infections occurred less frequently in the EVR 1.5 mg (7.7%) or EVR 3.0 mg (7.6%) than in the AZA group (21.5%, p<0.001 for both comparisons) and were even less common among patients who did not receive induction therapy (3.8% and 3.7 % in the EVR 1.5 mg and 3.0 mg respectively, p=0.01; and 14.3% in the AZA group) [45]. The percentage of patients who received prophylaxis against CMV was similar among the three groups (73.8 % in the AZA group, 79.4% in the EVR 1.5 mg group and 76.8% in the EVR 3.0 mg group) as was the percentage of CMV negative recipients who received a transplant from a CMV positive donor (17.3%, 17.2% and 22.7% respectively).

Lower rates of CMV infection was also shown in the A2411 study [87]. CMV prophylaxis was administered to 49 of 91 EVR patients (54.4%, mean duration 116 days) and 56 of 83 MMF patients (67.5%, mean duration 132 days). The rate of CMV infection was low and there were significantly fewer cases of CMV infection in the EVR group ( 4.4%) versus MMF ( 16.9%, P=0.011).

Lower rates of CMV infection was also shown in the A2310 trial [53]. Lower rate of CMV infection was reported in patients who were treated with EVR 1.5 mg as compared to pa-
patients treated with MMF (7.2% versus 19.4% at month 12 \( p<0.001 \), 7.2% versus 21.6% at month 24, \( \bar{p}<0.001 \)). Since CMV infection is associated with increased risk for CAV, this may have contributed to the lower rate of protocol-defined CAV at month 12 in the EVR 1.5 mg cohort.

To note, the mentioned studies support that lower incidence of CMV infection is not related to differences in CMV prophylaxis between treatment groups and to donor/recipient sero-status [45,53,87]. Since similar rate of CMV infection was observed with EVR and standard CsA versus EVR with reduced CsA, the lower incidence of CMV infection in de novo heart transplant patient treated by EVR cannot be attributed solely to lower CsA exposure [94].

**Meta-analyses** of controlled clinical studies included 10 trials of PSI vs. CNI and 15 trials of PSI +CNI vs. CNI. The risk for CMV events was significantly higher in patients treated with CNI compared to PSI immunosuppression (RR 2.27; 95% CI 1.72-3.01; \( p<0.0001 \)). Moreover, CNI immunosuppression had a 2.45-fold risk for a CMV event compared to a CNI and PSI combination (95% CI 1.76-3.41 \( p<0.0001 \)) [91].

Another big meta-analysis of 1009 cardiac transplant recipients in 3 de novo trials supported those findings [95] and showed that EVR is associated with a lower incidence of CMV infection compared with AZA and MMF. Moreover, the benefit of EVR is retained in CMV-naïve recipients and is independent of anti-CMV prophylaxis or preemptive approaches.

Other viral infections such as herpes simplex virus, Epstein-Barr virus, polyoma virus, and herpes zoster virus may be lowered by EVR, but studies have not been designed with these infections as predefined endpoints. Of note, although viral infections are reduced, bacterial or fungal infections may be more frequent with EVR, and avoiding over immunosuppression is critical to reduce this risk [96].

Several biological mechanisms has been proposed in order to explain the anti CMV effect of PSIs [91]; which include: 1) mTOR is essential for CMV replication during the late phases of the viral cycle and PSIs cause inhibition of virus mRNA translation; 2) PSIs affects acquired immunity leading to immune modulatory effects and an increase in the number and quality of antigen-specific CD8+ memory cells [97]; 3)PSIs regulate innate immunity by sustained mTOR activation in human macrophages which is mandatory for efficient viral protein synthesis. Treatment of these cells with an mTOR-I interferes with CMV replication [98].

In conclusion, the strategy of de novo use of PSI in high-risk patients for CMV infection or conversion to PSI in patients with recurrent or intractable CMV disease may be of benefit in order to reduce morbidity associated with CMV infection in heart transplant patients. A de novo PSI immunosuppressive protocol might bean option as a
preemptive strategy for CMV infection avoidance.

**PSIs and Malignancy**

HTx is the gold standard treatment for end stage heart failure; yet, its success is limited by severe cancer occurrence and recurrence. According to the ISHLT report (1) post-transplant malignancy affects 2.6% by the first year, 14.2% by 5 years, and 27.7% by 10 years after HTx and skin cancer is the most common malignancy affecting 1.3%, 9.4% and 19.6% at 1, 5 and 10 years after HTx respectively [1].

PSI inhibitors in addition to their immunosuppressant effects have anti proliferative and antiangiogenic properties suggesting an oncologic therapeutic benefit for these drugs. PSIs influence numerous signaling pathways of cell proliferation including the phosphatidylinositol-3-kinase (PI3K) pathway and signal transducer and transcription activator 3 (STAT3), and have an inhibitory effect on the angiogenic vascular endothelial growth factor [VEGF] [99,100]. Preclinical evidence suggests that PSI inhibitors might also be involved in the replication of human herpes virus 8 associated with Kaposi sarcoma [101,102].

SRL anti-angiogenic activity has been suggested in primary and metastatic tumors [100], and its clinical activity has been shown in some cancers (among them are Kaposi’s sarcoma, malignant glioma, hepatocellular and cholangiocellular cancer) when combined with other tar-

tgeted therapies [102,103].

EVR has been approved for the treatment of advanced RCC, specific types of breast and pancreatic cancer, renal angiomyolipoma and tuberous sclerosis complex [102]. Advanced clinical trials support its efficiency in various other solid tumors (including non-small cell lung cancer, endometrial cancer, gastric cancer and transitional cell carcinoma of the urothelial tract), and various lymphomas [104-107].

These data suggest an antitumor potential of PSI inhibitors treatment after solid organ transplantation. Evidence regarding the influence of PSI inhibitors on malignancies in heart transplant patients is limited, but the data coming from kidney transplant trials suggest PSIs may reduce the rate of new malignancies and non-skin solid cancers in kidney transplant patients.

The Rapamune Maintenance Regimen trial showed that CsA withdrawal at 3 month after renal transplantation followed by concentration-controlled SRL maintenance therapy resulted in a decreased incidence of malignancy (4.2% vs. 9.8%; p = 0.0.36) when compared with a combined SRL and CsA regime [108]. Furthermore, SRL-steroid (ST) therapy was associated with a reduced incidence of both skin (RR: 0.346; 95% CI: 0.23 – 0.53; p < .001) and non-skin malignancies (9.6% vs. 4.0%, SRL-CsA-ST versus SRL-ST, respectively; p = .032) at 5 years after renal transplantation, and delay in the median time to a first skin carcinoma (491 vs. 1126 days; p = .007)
[109]. Similar results were also found in the CONVERT trial following late conversion from CNI to PSI [110,111].

These results, however, were not corroborated in other kidney transplant RCTs. Malignancy incidence was similar between PSII-treated patients and CNI controls in the SYMPHONY [112], ORION [113], and the ZEUS [114] trials.

A recent meta-analysis tried to address these conflicting results and showed that SRL treatment was associated with a 40% reduction in malignancy risk (5876 patients from 21 RCTs, RR: 0.60; 95% CI: 0.39 – 0.93) [115]. In an U.S. registry data analysis, a similar tendency was found [116], (HR: 0.88; 95% CI: 0.70–1.11). Although, it failed to achieve statistical significance, after excluding prostate cancer (which presented a 70% higher incidence during SRL-exposure time) the incidence of all other cancers was 26% lower in the SRL-exposure time (HR: 0.74; 95% CI: 0.57–0.96).

Randomized, controlled trials in kidney transplant recipients focusing on skin cancer have shown that switching from a CNI to SRL therapy significantly lowers the rate of skin malignancies relative to continued therapy in most studies [117,118].

**In summary**, these data suggest that immunosuppression therapy with PSI inhibitors in solid organ transplants might present a safer malignancy profile, yet reduction of malignancy risk is not generally a reason to select de novo therapy with such drugs.

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