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REVIEW

mTOR inhibitors in pediatric kidney transplantation

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Abstract The mammalian target of the rapamycin (mTOR) inhibitors sirolimus and everolimus are increasingly being used in pediatric kidney transplantation in different combinations and doses. Several studies have shown beneficial effects of using mTOR inhibitors in children after pediatric renal transplantation. A switch to a low-dose calcineurin inhibitor (CNI) and mTOR inhibitor has been proven to stabilize the glomerular filtration rate. Additionally, de novo studies using a low-dose CNI and an mTOR inhibitor have shown good graft survival and a low number of rejections. Side effects of mTOR inhibitors, such as hyperlipidemia, wound healing problems, and proteinuria, mainly occur if high doses are given and if treatment is not combined with a CNI. Lower doses of mTOR inhibitors do not result in growth impairment or reduced testosterone levels. Treatment with mTOR inhibitors is also associated with a lower number of viral infections, especially cytomegalovirus. Due to their antiproliferative effect, mTOR inhibitors could theoretically reduce the risk of post-transplant lymphoproliferative disease. mTOR inhibitors, especially in combination with low-dose CNIs, can safely be used in children after kidney transplantation as de novo therapy or for conversion from CNI- and mycophenolate mofetil-based regimens.

Keywords Everolimus · Sirolimus · mTOR inhibitors · Pediatric kidney transplantation · Survival

Introduction

The immunosuppressive regimens currently used in children are often based on those evaluated in adults, but generalizing adult data to children does not take into account unique features of the pediatric patient population. Long-term survival rates of pediatric kidney transplant grafts have not improved in recent years [1]. Approximately 20 % of pediatric patients experience acute rejections within the first year after kidney transplantation (KTX), and reducing this rate remains an important therapeutic objective [2, 3]. However, treatments designed to prevent graft rejection have often resulted in over-immunosuppression and led to complications, including infection and malignancy [2]. Moreover, the nephrotoxicity of calcineurin inhibitors (CNIs) is a significant problem contributing to chronic deterioration of graft function. Recent emphasis has been on steroid-free regimens [4]. The ultimate aim of any chosen therapeutic regimen is to achieve sufficient immunosuppression with a low number of acute and chronic rejections along with a low number of side effects and low nephrotoxicity. Mammalian target of rapamycin (mTOR) inhibitors are a new group of immunosuppressants that, in combination with other drugs, may help to achieve these goals more successfully than standard therapies.

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How do mTOR inhibitors work?

Mammalian target of rapamycin (mTOR) inhibitors have entered the market within the last 10 years as a new group of immunosuppressants. They have been evaluated in several studies and approved by governmental authorities for immunosuppression in adults after KTX. mTOR inhibitors such as sirolimus and everolimus act as immunosuppressants by blocking the signaling pathway of T-cell growth factors and thereby inhibiting the proliferation of antigen-activated T cells (Fig. 1). mTOR is a serine/threonine kinase which belongs to the phosphatidylinositol-3 kinase-related kinases family. It regulates cellular metabolism, growth, and proliferation, making it a suitable drug target. mTOR exists in two distinct multiprotein complexes, denoted mTORC1 and mTORC2, which regulate key enzymes involved in cell cycle regulation and proliferation. mTOR inhibitors bind the cytosolic protein

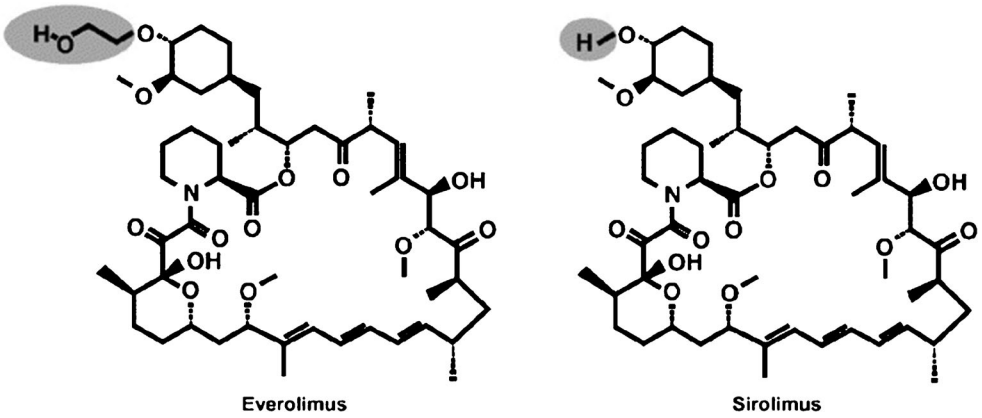

Fig. 1 Chemical Structures of everolimus and sirolimus (reproduced from Pascual et al. [89] with permission)

FK-binding protein 12 (FKBP12) and form the mTOR inhibitor–FKBP12 complex, which directly binds and thereby inhibits mTORC1. Consequently, mTOR inhibitors inhibit the proliferation of antigen-activated T cells by blocking their growth factor signaling pathways and arresting the cells in the G_1 stage of the cell cycle. In this way, mTOR inhibitors also inhibit the proliferation of vascular muscle and cancer cells. The antiproliferative action of mTOR inhibitors in the arterial vessel wall has been shown to inhibit atherogenic remodeling and neointima formation preclinically [5, 6] and to reduce transplant vasculopathy clinically [7]. Since mTOR inhibitors are involved in signaling pathways that block the development of malignant tumors [8], they have antineoplastic potential as antiangiogenic compounds [9] and as promoters of apoptosis [10–13]. This dual immunosuppressive and antiproliferative effect is an important feature for long-term treatment after transplantation and may improve the long-term outcome after KTX. Other advantages of mTOR inhibitors include a lack of nephrotoxicity [14, 15] and a reduced number of gastrointestinal side effects when compared to mycophenolate mofetil [16]. The method of action as compared to other immunosuppressive drugs is shown in Fig. 2.

The two mTOR inhibitors used in transplantation

Two mTOR inhibitors are currently available for use in transplantation: sirolimus, which was the introduced first, and everolimus, a modified molecule of sirolimus that was approved later. The major difference between the two drugs is the shorter half-life of everolimus. The substance now primarily referred to as sirolimus was first found on Easter Island (also known as Rapa Nui), accounting for its original name, rapamycin. Most of the studies of sirolimus in children were carried out between 2000 and 2010, mainly in the USA. After two early studies with everolimus [16, 17] that started in 2002 and 2004, the next monocenter trial with everolimus started in Europe in 2008, and the first large multinational study of pediatric KTX was initiated in 2012 with results expected in 2014.

Other mTOR inhibitors on the market (e.g., temsirolimus) are only used in cancer therapy but not in transplantation.

Approval

Everolimus was approved for the prevention of organ rejection after KTX in 2010 by the U.S. Food and Drug Administration (FDA) [18] and in 2003 by the European Medical Agency (EMEA) for prophylaxis of organ rejection in adult patients following allogeneic renal or cardiac transplant [19]. In 2012 the EMEA also approved everolimus for prophylaxis of organ rejection in adult patients following allogeneic liver transplantation.

Everolimus has also been approved by the FDA and EMA for the treatment of subependymal giant cell astrocytoma associated with tuberous sclerosis in patients who are assessed as not suitable for surgical intervention (2010) and for breast cancer treatment in postmenopausal women with advanced hormone-receptor positive, HER2-negative type cancer, in conjunction with exemestane (2012). The EMEA has approved everolimus additionally for "treatment of patients with advanced renal cell carcinoma whose disease has progressed on or after treatment with VEGF-targeted treatment" (2009) and for adults with tuberous sclerosis and renal angiomylipoma (2012).

Sirolimus was approved for combined immunosuppression after KTX by the FDA in 2006. In Europe, sirolimus was indicated by the EMEA in 2004 for the prophylaxis of organ rejection in adult patients who are receiving a renal transplant and who are at a low-to-moderate immunological risk. The EMEA recommends "that sirolimus be used initially in combination with a cyclosporine A (CsA) microemulsion and corticosteroids for 2 to 3 months. Sirolimus may be continued as a maintenance therapy with corticosteroids only if cyclosporine can be progressively discontinued" [19].

There is no approval for either of these substances for children in organ transplantation. The EMEA has decided on a mandatory pediatric investigational plan for everolimus. Therefore, a prospective randomized trial with everolimus

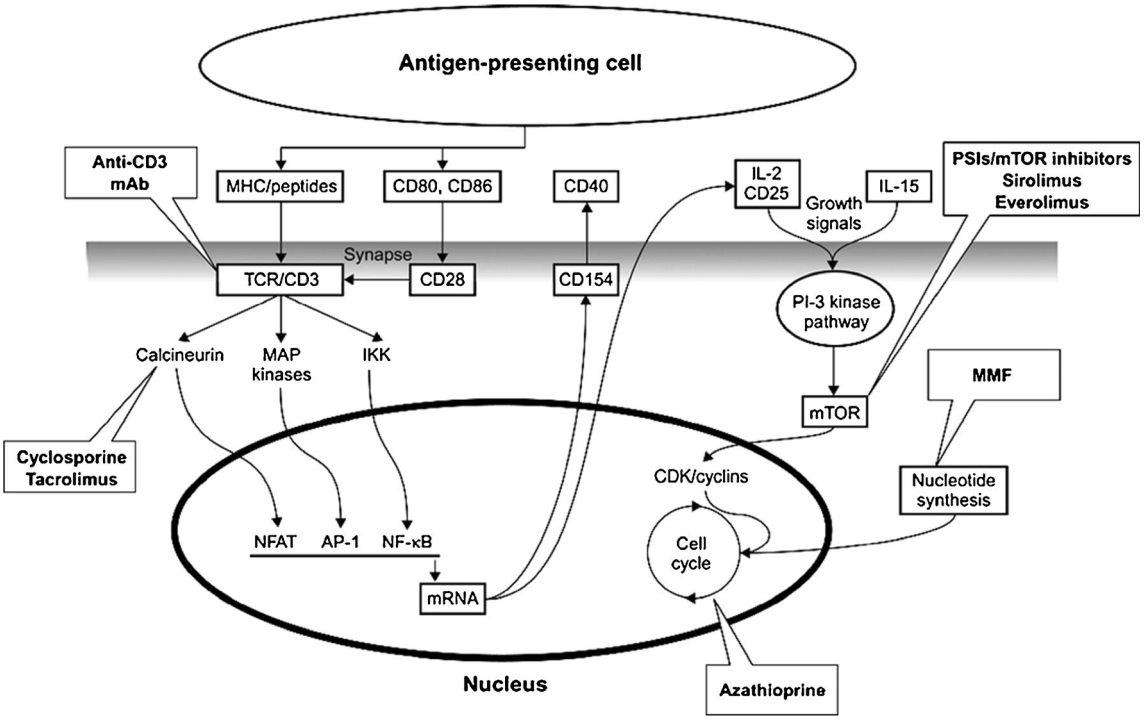

Fig. 2 Mechanism of action of mammalian target of rapamycin (*mTOR*) inhibitors (reproduced from Zuckermann et al. [90] with permission). *PSIs* Proliferation signal inhibitors

was started in pediatric KTX in 2012 and a one-arm prospective trial in pediatric liver transplantation was started in 2013.

Maintenance therapy

Maintenance therapy with everolimus

The use of everolimus was initially proposed for the long-term treatment of patients after KTX instead of CNIs, such as tacrolimus and CsA, to reduce the amount of CNI toxicity. It was speculated that patients with signs of CNI toxicity in kidney biopsy could benefit from a switch from CNIs to everolimus. Our own group reported on maintenance therapy with everolimus and low-dose CsA in 13 renal transplant children and adolescents with a mean age of 13 years. These patients had biopsy-confirmed transplant nephropathy with a mean decrease in glomerular filtration rate (GFR) from 55 to 45 mL/min/1.73 m^2 within 1 year on CsA+ mycophenolate mofetil and prednisolone combination therapy [17]. Mycophenolate mofetil was discontinued, the CsA dose was halved, and the everolimus dose was adjusted to 1.6 mg/m^2/day. After 6 months, the mean trough level was 52 ng/L for CsA and 4 ng/mL for everolimus. After 12 months, the GFR showed a slight mean increase to 47 mL/min/1.73 m^2 which was not statistically significant but led to the conclusion that GFR stabilized under this regimen. Furthermore, the switching of patients with a GFR of approximately <30 mL/min/1.73 m^2 onto everolimus and low-dose CsA did not improve in renal function. No serious adverse drug reactions or acute rejections occurred. Serum cholesterol and the albumin-to-creatinine ratio did not increase significantly.

Maintenance therapy with sirolimus

Several studies have been conducted on the switch to a sirolimus-based therapy in pediatric KTX. The outcomes were mostly associated with an increase in GFR and an acceptable number of side effects. These outcomes were observed when CNI was reduced to 50 % [20]. Also, in cases where interstitial fibrosis and tubular atrophy were detected in kidney biopsies, a conversion from CNI to sirolimus was found to stabilize graft function for at least 1 year [21, 22]. In other studies in which CNI-free immunosuppression was used with a combination of sirolimus and mycophenolate mofetil, only a select group of patients with a low amount of proteinuria, tubular atrophy, good baseline graft function, no acute rejections, and CNI toxicity benefitted from a switch to sirolimus [23–26].

De novo therapy

De novo therapy with everolimus

Ettenger et al. [27] published results of an open-label multicenter study with 19 renal transplant recipients (<16 years

of age) who had received de novo everolimus, CsA, and steroid treatment for at least 1 year. Of the 19 patients, 15 were followed for an additional 2 years. The mean daily dose of everolimus was 1.53 mg/m^2. CsA was adjusted to a trough level of 75–150 ng/mL, and the steroid level was reduced to a low maintenance dose. Three patients had biopsy-confirmed acute rejection episodes, and four had chronic allograft nephropathy. One patient lost his graft after more than 1 year. The survival rate in the extension study was 100 %. Aside from two incidences of cytomegalovirus (CMV) infection, two other viral infections developed. Four patients required the use of HMG-CoA-reductase inhibitors. The mean serum creatinine concentration after 3 years was 1.1 mg/dL. Therefore, this first long-term prospective study examining de novo everolimus+CsA treatment demonstrated good efficacy and safety after pediatric RTX.

Our own group conducted a prospective trial in 20 children after KTX who were initially treated with basiliximab, CsA [with a trough level (C0) of 200–250 ng/mL], and prednisolone [28]. After 2 weeks, the CsA dose was reduced to 50 % (C0 75–100 ng/mL), and after 6 months it was further reduced (C0 50–75 ng/mL). Six months after KTX, the prednisolone dose was set to alternating doses, and it was discontinued 3 months later. At 6 months post-KTX, all 20 protocol biopsies showed no acute rejection or borderline findings. Interstitial fibrosis and tubular atrophy (IF/TA) were only detected in 2/20 patients. Indication biopsies showed no acute rejections and only two borderline findings. At 1 year post-KTX, the mean GFR was 71± 25 mL/min/1.73 m^2. Without CMV prophylaxis, only two primary CMV infections were observed, despite a positive donor/recipient CMV-constellation in 10/20 children.

Analysis of 3 years of cumulative data from this study [29] revealed no loss of follow-up of graft or patient. Indication biopsies showed no acute rejection (Banff classification≥IA). IF/TA was detected in three children. One of the patients presented with transplant glomerulopathy (C4d negative) as a sign of chronic humoral rejection, which was combined with the detection of a donor-specific antibody in the Luminex assay. At 3 years post-KTX, the mean GFR rate was 61±27 mL/min/1.73 m^2. No cases of post-transplant lymphoproliferative disease (PTLD) or polyomavirus nephropathy were diagnosed. After 3 years, 17 of the 20 patients were still on the original immunosuppressive regimen. We therefore concluded that this treatment regimen might be a promising therapy after pediatric KTX. In October 2012, Novartis (Basel, Switzerland) initiated the CRADA2314 trial, a worldwide, multicenter, randomized controlled trial comparing de novo therapy with tacrolimus and everolimus + prednisolone to a standard therapy with tacrolimus, steroids and mycophenolate mofetil, in 200 children after KTX. Originally, induction with basiliximab was mandatory, but with a study amendment, the decision on the use of induction therapy has been left to the participating center. The results of this trial will hopefully show whether initial immunosuppression with everolimus and low-dose CNI is advantageous for children after KTX or not.

De novo therapy with sirolimus

In all drug regimens using sirolimus for initial therapy in children, sirolimus has been administered in combination with basiliximab, daclizumab, or antithymocyte globulin induction in which sirolimus was combined either with a CNI or with mycophenolate mofetil. In all of the studies, the number of side effects was acceptable, and graft survival was good [30–34]. The largest trial with sirolimus in children after KTX was carried out by Benfield and Bartosh [35]. The main goal of this randomized, controlled trial was to eliminate steroids. One group of patients was treated with daclizumab, steroids, tacrolimus, and sirolimus, and the other group was treated with the same regimen but without steroids. This trial, which used full doses of CNI (trough levels 175–400 ng/mL first 2 weeks after transplatation, 175–300 ng/mL week 3 to month 3, 50–250 ng/mL afterwards) and sirolimus (6 mg/m^2; trough levels 10–20 ng/mL), was terminated early after randomization of 131 patients because of an unexpectedly high rate of PTLD in the intervention group (14 %). These findings were associated with the known risk factor of the majority of children with an Epstein–Barr virus (EBV) donor+/recipient− serostatus. The researchers concluded that steroid elimination was possible with this protocol, but that a combination of full-dose sirolimus and full-dose tacrolimus could not be recommended for routine use because of the side effects. No controlled, randomized, prospective studies using low-dose sirolimus and low-dose CNI have as yet been carried out in pediatric renal transplantation patients.

Growth and development

Since mTOR inhibitors are antiproliferative agents, immunosuppression with these drugs might negatively influence growth. Sirolimus has been demonstrated to inhibit longitudinal growth in fast-growing rats [36] and to decrease endochondral bone growth [37]. In one case report, a girl stopped growing after being switched from CsA to sirolimus [38]. In one study involving conversion to sirolimus, no difference in height standard deviation score levels was observed [39]. Two other trials showed impaired growth [40, 41]. Good growth was observed in the largest trial using sirolimus in which there was a greater standardized height velocity in the steroid-free group, which received sirolimus, than in the control group [35]. The longest trial using everolimus showed no evidence of impaired growth [28,

29]. A matched pairs study that compared children treated with everolimus and low-dose CsA also showed no significant difference in growth (our data, not yet published).

Sex hormones

Sex hormone levels were normal in the two everolimus studies conducted to date [28, 29]. Regarding sirolimus, three studies showed a dose-dependent decrease in testosterone levels after the switch, resulting in suppressed levels of testosterone in some adolescents [41–44] that was associated with an increase in luteinizing hormone. This effect has also been shown in heart transplantation [45]. In rats, this negative effect was shown to be reversible after withdrawal of sirolimus medication [46]. Based on these findings, regular assessment of sex hormones is advocated and would seem advisable in cases involving changes, dose reduction, or discontinuation of the mTOR inhibitor.

Potential advantages of the use of mTOR inhibitors

Therapy with mTOR inhibitors has a number of potential advantages, since the possibility of minimizing the dosage of CNIs may lead to less CNI nephrotoxicity. The use of mTOR inhibitors might also enable early steroid withdrawal and thereby possibly improve growth and reduce the side effects of steroid treatment. These results were partially demonstrated in a randomized trial that unfortunately had to be discontinued due to the incidence of PTLD, presumably from overimmunosuppression [35]. Therefore, no final conclusions as to whether a combination of sirolimus and low-dose-CNI is a secure treatment without corticosteroids can be drawn. In trials with early steroid withdrawal in combination with everolimus therapy the patients showed similar growth as those in other trials with non-mTOR immunosuppression [2, 4]. The antiproliferative effect of mTOR inhibitors might reduce the risks of malignancy (mainly the incidence of PTLD) and reduce the incidence of viral infections, such as CMV or BK polyomavirus infections. These effects have been partially shown in adult studies [47, 48]. Studies have also demonstrated that the incidence of CMV infections is lower if patients are treated with an mTOR inhibitor [49]. Treatment with an mTOR inhibitor might decrease the need for nephrotoxic, myelotoxic, and expensive antiviral medications, such as valganciclovir. Additionally, CMV infections have a negative impact on graft survival, so a reduction in CMV infections is expected to lead to better long-term graft function. This positive effect on viral infections occurs only if the overall immunosuppression is low. When full-dose CNI and sirolimus are used, an increased incidence of PTLD has been demonstrated [33]. A conversion to sirolimus [50–53] and everolimus [54] has been shown to improve the course of PTLD in adults, as well as in children with sirolimus after liver transplantation [55]. Long-term results of larger studies are necessary to demonstrate whether mTOR inhibitors have a positive influence on the development of PTLD in children.

Side effects

Hyperlipidemia

Hyperlipidemia has been reported in most of the pediatric trials using mTOR inhibitors. The concentrations of triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol increase in association with the mTOR inhibitor dose [56]. In the everolimus conversion and initial therapy studies, in which a low dose of everolimus was used, cholesterol values increased only gradually [12, 16, 17, 27–29]. In studies using sirolimus, hyperlipidemia was detected in approximately 10–60 % of the children [15, 25, 33, 57, 58], with maximum detection early after transplantation [25]. A substantial number of patients in these trials had to be treated with HMG-CoA-reductase inhibitors. The effect seems to be dose-dependent, and the influence on long-term complications remains unclear.

Proteinuria

Whereas no significant albuminuria was detected in any of the pediatric everolimus studies (that used a combination of low-dose everolimus and low-dose CNI) [12, 16, 17, 26, 27], studies with sirolimus did show an increase in albuminuria [59]. This side effect was mainly observed after CNI elimination, but not in patients receiving a combination of CNI and sirolimus. Thus, it may not be sirolimus itself but CNI elimination that causes the increase in proteinuria. Interestingly, this possibility is supported by the observation that when the same dose of sirolimus was used and CNI was re-introduced into the therapeutic regimen, proteinuria was once again lowered [60]. However, pre-existing proteinuria could be worsened after the introduction of mTOR inhibitors and therefore be a limitation for its use, even in combination with a CNI. It has been shown that mTOR inhibitors decrease the production of vascular endothelial growth factor (VEGF) [61] and block VEGF signaling pathways [62] in the podocyte and thereby may lead to albuminuria. Additionally, it is hypothesized that mTOR inhibitors can induce proximal tubular epithelial cell dysfunction and reduce receptor-mediated albumin uptake [63].

Wound healing

Wound healing problems have been reported with both compounds. With respect to everolimus, this problem was primarily described when everolimus therapy was initiated directly after transplantation [28]. Consequently, several trials examining the use of everolimus delayed the introduction of the mTOR inhibitor for 2–4 weeks after KTX. The same is true for the use of sirolimus [51, 64, 65]. The antiproliferative effect of mTOR inhibitors is believed to be responsible for these findings. Since there are also other studies in adults in which no influence of mTOR inhibitors on wound healing was observed [66], it remains unclear whether mTOR inhibitors affect wound healing or not. The problem of wound healing may be dosage related as the first studies that reported these effects administered mTOR inhibitors at very high doses.

Other side effects

Aphtous ulcers and herpetic lesions are seen quite often, but these are transient in most children and do not require any dose reduction of mTOR inhibitors. Interstitial pneumonia caused by *Pneumocystis carinii* pneumonia (PCP) or other microorganisms has been diagnosed in some patients, mainly under high doses. Therefore PCP-prophylaxis is recommended when mTOR inhibitors are used. In the case of a diagnosis of interstitial pneumonia, the dose of the mTOR inhibitor should be significantly reduced or therapy with the agent should be discontinued.

Hematologic abnormalities, such as anemia and thrombocytopenia, occur due to the antiproliferative effects of mTOR-inhibitors. In some children erythropoetin stimulating agents have to be administered. Platelet count has to be taken into consideration before surgery.

Several other less common side effects of mTOR inhibitors have been described, including lymphedema, nail disorders (e.g.,paronychia), acne, constipation, bleeding, glucose intolerance, and thrombotic microangiopathy.

Combination with other immunosuppressive drugs

Everolimus is only approved in combination with a low-dose CNI. Also, all pediatric studies involving conversion to mTOR inhibitors or initial use of these drugs have been conducted in combination with low-dose CsA or low-dose tacrolimus. Studies using everolimus in combination with mycophenolate mofetil have only been carried out in adults [67].

In contrast, most of the sirolimus studies in children were carried out with the aim to eliminate CNI [15, 22–24, 33, 51–58]. This aim was associated with a need for a higher sirolimus dosing; consequently, there were a higher number of side effects. The historic focus on the use of sirolimus without CNI might be explained by the negative effects of an interaction between this drug and CsA [14, 21, 30], as well as early reports of increased nephrotoxicity when full-dose sirolimus was combined with a CNI in adults.

The differences between the immunosuppressive combinations of everolimus and sirolimus make it quite difficult to compare the two drugs. Two factors may explain these differences. First, most studies on sirolimus were carried out several years before the studies on everolimus started, so the design of the everolimus trials benefitted from the experiences of the sirolimus trials. Secondly, most studies on sirolimus were carried out in North America, whereas the everolimus studies were mainly conducted in Europe. Traditionally, there are some differences in the use of immunosuppressants between North America and Europe.

Dosing

Sirolimus and everolimus have significant pharmacokinetic differences; however, both substances are chemically quite similar. Sirolimus was isolated from *Streptomyces hygroscopicus*. Interestingly, its structure is related to that of tacrolimus, an isolate of a Japanese *Streptomyces* subgroup, but it has a completely different mode of immunosuppressive action. Everolimus is the synthetic 40-*O*-(2-hydroxyethyl) derivative of sirolimus. The drugs are mainly hepatically eliminated by the cytochrome P 450 isoenzyme 3A4 and then by the multidrug transporter p-glycoprotein via the gut [68, 69]. Both mTOR inhibitors have known interactions with other drugs, including antibiotics, antiepilieptics, and antifungals (Table 1). The therapeutic windows of both substances are narrow, yet there is significant intra- and interindividual variability in their pharmacokinetics. Therefore, therapeutic drug monitoring by liquid chromatography with tandem mass spectrometry (LC-MS/MS) is highly recommended. Sirolimus has a more age-dependent half-life than everolimus; however, in children, twice-daily dosing of both substances corrected to body surface area is recommended [16, 21, 30], whereas in adolescents, sirolimus can also be administered once daily, as in adults [69]. The exact age of a switch to once-daily dosing is different in the publications cited. It is recommended that the treating physician delay the switch until adolescence. Because of the long half-life of sirolimus, in fact no real “trough levels” but steady state levels are measured in older children. This makes sirolimus therapy more forgiving when doses are not administered according to the time schedule and might make it a more appropriate drug for adolescents.

If sirolimus is used in combination with a CNI, several aspects of the treatment must be taken into

Table 1 Medications with important drug interactions with mammalian target of rapamycin inhibitors and therapeutic consequences[a]

ACE inhibitors: mTOR inhibitors may enhance the adverse/toxic effect of ACE Inhibitors. Specifically, the risk of angioedema may be increased. Risk C: Monitor therapy
Clozapine: Myelosuppressive agents may enhance the adverse/toxic effect of clozapine. Specifically, the risk for agranulocytosis may be increased. Risk X: Avoid combination
CYP3A4 inducers (i.e., phenobarbital, carbamezepine, phenytoine, oxcarbazepine, rifampicin, rifabutin): May decrease the serum concentration of mTOR inhibitors. Management: Avoid concurrent use of strong CYP3A4 inducers, but if strong CYP3A4 inducers cannot be avoided, consider gradually increasing the mTOR inhibitor dose. Risk X: Avoid combination
CYP3A4 inhibitors (i.e., protease inhibitors, erythromycin, clarithromycin, ketoconazole, itraconazole, fluconazole, verapamil, diltiazem): May increase the serum concentration of mTOR inhibitors. Risk X: Avoid combination
Dasatinib: May increase the serum concentration of CYP3A4 Substrates. Risk C: Monitor therapy
Deferasirox: May decrease the serum concentration of CYP3A4 substrates. Risk C: Monitor therapy
Denosumab: May enhance the adverse/toxic effect of immunosuppressants. Specifically, the risk for serious infections may be increased. Risk C: Monitor therapy
Echinacea: May diminish the therapeutic effect of immunosuppressants. Risk D: Consider therapy modification
Efavirenz: May decrease the serum concentration of mTOR-inhibitors. Management: Closely monitor mTOR inhibitor serum concentrations when starting, stopping, or changing doses of efavirenz, particularly during the first 2 weeks after any change. Dose adjustment of mTOR inhibitors may be required. Risk D: Consider therapy modification
Grapefruit juice: May increase the serum concentration of mTOR-inhibitors. Risk X: Avoid combination
Ivacaftor: May increase the serum concentration of CYP3A4 substrates. Risk C: Monitor therapy
Leflunomide: Immunosuppressants may enhance the adverse/toxic effect of leflunomide. Specifically, the risk for hematologic toxicity such as pancytopenia, agranulocytosis, and/or thrombocytopenia may be increased. Management: Consider not using a leflunomide loading dose in patients receiving other immunosuppressants. Patients receiving both leflunomide and another immunosuppressant should be monitored for bone marrow suppression at least monthly. Risk D: Consider therapy modification
Mifepristone: May increase the serum concentration of CYP3A4 substrates. Management: Minimize doses of CYP3A4 substrates, and monitor for increased concentrations/toxicity, during and 2 weeks following treatment with mifepristone. Avoid cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, and tacrolimus. Risk D: Consider therapy modification
Natalizumab: Immunosuppressants may enhance the adverse/toxic effect of natalizumab. Specifically, the risk of concurrent infection may be increased. Risk X: Avoid combination
Pimecrolimus: May enhance the adverse/toxic effect of immunosuppressants. Risk X: Avoid combination
Roflumilast: May enhance the immunosuppressive effect of immunosuppressants. Risk D: Consider therapy modification
St Johns wort: May decrease the serum concentration of mTOR inhibitors. Management: Concurrent use of mTOR-inhibitors with St Johns wort is not recommended. Risk X: Avoid combination
Tacrolimus (topical): May enhance the adverse/toxic effect of immunosuppressants. Risk X: Avoid combination
Tocilizumab: May decrease the serum concentration of CYP3A4 substrates. Risk C: Monitor therapy
Tofacitinib: Immunosuppressants may enhance the immunosuppressive effect of tofacitinib. Management: Concurrent use with antirheumatic doses of methotrexate or other non-disease modifying antirheumatic drugs is permitted, and this warning seems to particularly focused on more potent immunosuppressants. Risk X: Avoid combination
Trastuzumab: May enhance the neutropenic effect of Immunosuppressants. Risk C: Monitor therapy

ACE, Angiotensin-converting enzyme; mTOR, mammalian target of rapamycin; CYP, cytochrome P450

[a] Reproduced from Lexicomp, Inc. [91, 92] with permission

account. If both medications are used in standard doses, overimmunosuppression might occur and lead to a higher number of PTLDs and viral infections [15, 34, 35]. Therefore, the aim in long-term use should be lower trough levels, such as 2–4 ng/mL tacrolimus, 30–50 ng/mL CsA, and 4–5 ng/mL sirolimus. It is important to recognize that sirolimus can increase tacrolimus clearance in children leading to lower trough levels [70]. CsA exposure is not altered by sirolimus [71, 72]. Tacrolimus only slightly decreases sirolimus clearance, whereas CsA is responsible for a significantly higher sirolimus metabolism in children [30]. This is in contrast to findings in adults, where the half-life of sirolimus is prolonged by CsA [73]. Interestingly, sirolimus levels significantly decrease in children if administered at the same time as CsA [16, 72], but not when sirolimus is administered 4 hours after CsA. However, separate dosing is very impractical in routine use [16]. In contrast to CsA, tacrolimus alters sirolimus exposure only marginally [74]. Since these interactions with CNI change when CNI is reduced or eliminated, individualized dosing and therapeutic drug monitoring are important when sirolimus is used. In combination with mycophenolate mofetil, no significant drug interactions have been reported.

Everolimus also inhibits clearance of CsA and vice versa, since the same pathway is used for elimination. Therefore,

lower doses of everolimus and CsA should be administered when these substances are used in combination [74]. If everolimus is used in combination with mycophenolate mofetil or tacrolimus, these interactions do not occur. Therefore, the doses of everolimus administered in combination with mycophenolate mofetil or tacrolimus are approximately double those used in combination with CsA [75]. One study reported that tacrolimus exposure is reduced by everolimus [76].

The gold standard for drug monitoring of both mTOR inhibitors is liquid chromatography/mass spectrometry (LC/MS). This method is used routinely in most laboratories. However, a chemiluminescent microparticle immunoassay (CMIA) on the Architect® analyzer for measuring sirolimus levels is also available. This assay has a bias of +15–20 % [77] and can also be used for the measurement of everolimus levels due to the similarity of both substances, but also with a significant bias as compared to LC/MS [78].

Table 1 lists the reported drug interactions that have to be taken into account when prescribing sirolimus and everolimus.

Comparison between everolimus and sirolimus

As described above, a comparison between sirolimus and everolimus is complicated by the different context in which these drugs have been administered in their respective studies. Unfortunately, no head-to-head trials that directly compare both substances in a similar setting have been carried out in adults or children. However, some aspects may be considered from the results of studies in adults. Practical experience with everolimus and sirolimus has revealed that there are a number of special benefits to using everolimus in terms of its side-effect profile that appears to be less severe, since it is associated with a lower incidence of hyperlipidemia [17], fewer instances of de novo proteinuria, a lower rate of wound healing disorders [64], less pneumonitis [16], better controllability because of its shorter serum half-life, and simultaneous use with CNI without a time interval between doses.

mTOR inhibitors and adaptive immunity / tolerance

Mammalian target of rapamycin is involved in adaptive immunity, including T-cell differentiation into effector or regulatory T cells [79–83]. T-cell/dendritic cell interaction activates mTOR in a dose- and time-dependent manner [84]. In CD4 T cells, mTOR integrates signals from immune activation, metabolic cues, and environmental stimuli. mTOR mediates response to cytokines [83], and recent experiments with genetically modified animals have shown that mTORC1 is essential for Th1 and Th17 differentiation, whereas mTORC2 is involved in Th2 differentiation [82, 83]. Finally, PIK3/AKT seems to negatively regulate FoxP3 expression via mTORC1, and inhibition of mTOR thereby leads to the generation or expansion of regulatory T cells [80, 85, 86]. It is speculated that this increase of regulatory T cells might lead to operational tolerance. Proliferation of T cells is also affected. The TSC1 complex, which negatively controls mTORC1, maintains quiescence of T cells or NK cells [82]. The mTOR pathway is a major regulator of memory CD8 cells. Inhibition of mTOR by sirolimus alters the process of short-lived and memory effector cells [83] and provides the signal for CD8 T cell expansion [87, 88]. This might be the explanation for a better replication of virus-specific CD8 cells and thereby for the lower incidence of viral infections under mTOR inhibitor therapy.

Conclusion

Mammalian target of rapamycin (mTOR) inhibitors can safely be used after pediatric KTX as an initial immunosuppressive therapy or for a switch in immunosuppressive therapy in cases of clinical need. However, the question of impaired wound healing is not yet finally answered. The use of mTOR inhibitors with reduced doses of CNIs may be advisable in patients with slowly decreasing renal function as a means of reducing CNI toxicity. Its use in well-advanced renal insufficiency (GFR<30 ml/min/1.73m^2) does not provide any benefit for the patients. In some patients with long-term CNI toxicity, CNI-free immunosuppression using mTOR inhibitors with mycophenolate mofetil may be advisable. The risk of CMV and EBV infections is lower than in standard protocols, which may benefit patients. mTOR inhibitors may reduce CNI toxicity in all pediatric solid organ transplants, and by means of steroid reduction/withdrawal they may also improve long-term growth and reduce steroid side effects. The number of side effects of mTOR inhibitors is acceptable in our experience if the right mode of dosing and continuous drug monitoring are used. Everolimus may have some advantages over sirolimus. The first small de novo trials in KTX with both mTOR inhibitors have had encouraging results if care is taken to avoid overimmunosuppression. Long-term results and additional prospective, randomized trials are needed, and one is presently being conducted. The results of these trials must be awaited before final conclusions on the use of mTOR inhibitors in children after KTX can be drawn.

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References

1. Joosten SA, Sijpkens YW, van Kooten C, Paul LC (2005) Chronic renal allograft rejection: Pathophysiologic considerations. Kidney Int 68:1–13
2. Offner G, Toenshoff B, Hocker B, Krauss M, Bulla M, Cochat P, Fehrenbach H, Fischer W, Foulard M, Hoppe B, Hoyer PF, Jungraithmayr TC, Klaus G, Latta K, Leichter H, Mihatsch MJ, Misselwitz J, Montoya C, Muller-Wiefel DE, Neuhaus TJ, Pape L, Querfeld U, Plank C, Schwarke D, Wygoda S, Zimmerhackl LB (2008) Efficacy and safety of basiliximab in pediatric renal transplant patients receiving cyclosporine, mycophenolate mofetil, and steroids. Transplantation 86:1241–1248
3. North American Pediatric Renal Trials and Collaborative Studies (2011) Annual dialysis report. Available at: https://Web.emmes.com/study/ped/annlrept/annualrept2011.pdf
4. Grenda R, Watson A, Trompeter R, Tonshoff B, Jaray J, Fitzpatrick M, Murer L, Vondrak K, Maxwell H, van Damme-Lombaerts R, Loirat C, Mor E, Cochat P, Milford DV, Brown M, Webb NJ (2010) A randomized trial to assess the impact of early steroid withdrawal on growth in pediatric renal transplantation: The TWIST study. Am J Transplant 10:828–836
5. Cole OJ, Shehata M, Rigg KM (1998) Effect of SDZ RAD on transplant arteriosclerosis in the rat aortic model. Transplant Proc 30:2200–2203
6. Schuurman HJ, Pally C, Weckbecker G, Schuler W, Bruns C (1999) SDZ RAD inhibits cold ischemia-induced vascular remodeling. Transplant Proc 31:1024–1025
7. Matsumoto Y, Hof A, Baumlin Y, Muller M, Hof RP (2004) Differential effects of everolimus and cyclosporine A on intimal alpha-actin-positive cell dynamics of carotid allografts in mice. Transplantation 78:345–351
8. Faivre S, Kroemer G, Raymond E (2006) Current development of mTOR inhibitors as anticancer agents. Nat Rev Drug Discov 5:671–688
9. Rubinsztein DC, Gestwicki JE, Murphy LO, Klionsky DJ (2007) Potential therapeutic applications of autophagy. Nat Rev Drug Discov 6:304–312
10. Majumder PK, Febbo PG, Bikoff R, Berger R, Xue Q, McMahon LM, Manola J, Brugarolas J, McDonnell TJ, Golub TR, Loda M, Lane HA, Sellers WR (2004) mTOR inhibition reverses akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. Nat Med 10:594–601
11. Hartford CM, Ratain MJ (2007) Rapamycin: Something old, something new, sometimes borrowed and now renewed. Clin Pharmacol Ther 82:381–388
12. Chapman JR, Valantine H, Albanell J, Arns WA, Campistol JM, Eisen H, Frigerio M, Lehmkuhl H, Marcen R, Morris R, Nashan B, Pascual J, Pohanka E, Segovia J, Zuckermann A (2007) Proliferation signal inhibitors in transplantation: Questions at the cutting edge of everolimus therapy. Transplant Proc 39:2937–2950
13. Gaumann A, Schlitt HJ, Geissler EK (2008) Immunosuppression and tumor development in organ transplant recipients: The emerging dualistic role of rapamycin. Transpl Int 21:207–217
14. Gupta P, Kaufman S, Fishbein TM (2005) Sirolimus for solid organ transplantation in children. Pediatr Transplant 9:269–276
15. Hymes LC, Warshaw BL (2005) Sirolimus in pediatric patients: Results in the first 6 months post-renal transplant. Pediatr Transplant 9:520–522
16. Ettenger RB, Grimm EM (2001) Safety and efficacy of TOR inhibitors in pediatric renal transplant recipients. Am J Kidney Dis 38:S22–S28
17. Pape L, Ahlenstiel T, Ehrich JH, Offner G (2007) Reversal of loss of glomerular filtration rate in children with transplant nephropathy after switch to everolimus and low-dose cyclosporine A. Pediatr Transplant 11:291–295
18. Federal Drug Administration. Available at: http://www.acessdata.fda.gov/scripts/cder/drugsatfda/index.cfm. Accessed 25 Apr 2013
19. European Medicines Agency. Available at: http://www.emea.europa.eu/ema/index.jsp?curl=pages/medicines/landing/epar_search.jsp&mid=WC0b01ac058001d123. Accessed 25 Apr 2013
20. Garcia CD, Bittencourt VB, Alves AB, Garcia VD, Tumelero A, Antonello JS, Malheiros D (2006) Conversion to sirolimus in pediatric renal transplantation recipients. Transplant Proc 38:1901–1903
21. Vilalta R, Vila A, Nieto J, Callis L (2003) Rapamycin use and rapid withdrawal of calcineurin inhibitors in pediatric renal transplantation. Transplant Proc 35:703–704
22. MacDonald AS (2003) Rapamycin in combination with cyclosporine or tacrolimus in liver, pancreas, and kidney transplantation. Transplant Proc 35:201S–208S
23. Falger JC, Mueller T, Arbeiter K, Boehm M, Regele H, Balzar E, Aufricht C (2006) Conversion from calcineurin inhibitor to sirolimus in pediatric chronic allograft nephropathy. Pediatr Transplant 10:565–569
24. Ibanez JP, Monteverde ML, Diaz MA, Goldberg J, Turconi AF (2007) Sirolimus in chronic allograft nephropathy in pediatric recipients. Pediatr Transplant 11:777–780
25. Hymes LC, Warshaw BL, Amaral SG, Greenbaum LA (2008) Tacrolimus withdrawal and conversion to sirolimus at three months post-pediatric renal transplantation. Pediatr Transplant 12:773–777
26. Weintraub L, Li L, Kambham N, Alexander S, Concepcion W, Miller K, Wong C, Salvatierra O, Sarwal M (2008) Patient selection critical for calcineurin inhibitor withdrawal in pediatric kidney transplantation. Pediatr Transplant 12:541–549
27. Ettenger R, Hoyer PF, Grimm P, Webb N, Loirat C, Mahan JD, Mentser M, Niaudet P, Offner G, Vandamme-Lombaerts R, Hexham JM, Everolimus Pediatric Study Group (2008) Multicenter trial of everolimus in pediatric renal transplant recipients: Results at three year. Pediatr Transplant 12:456–463
28. Pape L, Offner G, Kreuzer M, Froede K, Drube J, Kanzelmeyer N, Ehrich JH, Ahlenstiel T (2010) De novo therapy with everolimus, low-dose ciclosporine A, basiliximab and steroid elimination in pediatric kidney transplantation. Am J Transplant 10:2349–2354
29. Pape L, Lehner F, Blume C, Ahlenstiel T (2011) Pediatric kidney transplantation followed by de novo therapy with everolimus, low-dose cyclosporine A, and steroid elimination: 3-year data. Transplantation 92:658–662
30. Schachter AD, Benfield MR, Wyatt RJ, Grimm PC, Fennell RS, Herrin JT, Lirenman DS, McDonald RA, Munoz-Arizpe R, Harmon WE (2006) Sirolimus pharmacokinetics in pediatric renal transplant recipients receiving calcineurin inhibitor co-therapy. Pediatr Transplant 10:914–919
31. El-Sabrout R, Delaney V, Qadir M, Butt F, Hanson P, Butt KM (2003) Sirolimus in combination with tacrolimus or mycophenolate mofetil for minimizing acute rejection risk in renal transplant recipients—a single center experience. Transplant Proc 35:89S–94S
32. Ibanez JP, Monteverde ML, Goldberg J, Diaz MA, Turconi A (2005) Sirolimus in pediatric renal transplantation. Transplant Proc 37:682–684
33. Iorember FM, Patel HP, Ohana A, Hayes JR, Mahan JD, Baker PB, Rajab A (2010) Steroid avoidance using sirolimus and cyclosporine in pediatric renal transplantation: One year analysis. Pediatr Transplant 14:93–99
34. Sindhi R (2003) Sirolimus in pediatric transplant recipients. Transplant Proc 35:113S–114S
35. Benfield MR, Bartosh S, Ikle D, Warshaw B, Bridges N, Morrison Y, Harmon W (2010) A randomized double-blind, placebo controlled trial of steroid withdrawal after pediatric renal transplantation. Am J Transplant 10:81–88

36. Alvarez-Garcia O, Carbajo-Perez E, Garcia E, Gil H, Molinos I, Rodriguez J, Ordonez FA, Santos F (2007) Rapamycin retards growth and causes marked alterations in the growth plate of young rats. Pediatr Nephrol 22:954–961
37. Sanchez CP, He YZ (2009) Bone growth during rapamycin therapy in young rats. BMC Pediatr 9:3
38. Rangel GA, Ariceta G (2009) Growth failure associated with sirolimus: Case report. Pediatr Nephrol 24:2047–2050
39. Hymes LC, Warshaw BL (2011) Linear growth in pediatric renal transplant recipients receiving sirolimus. Pediatr Transplant 15:570–572
40. Gonzalez D, Garcia CD, Azocar M, Waller S, Alonso A, Ariceta G, Mejia N, Santos F (2011) Growth of kidney-transplanted pediatric patients treated with sirolimus. Pediatr Nephrol 26:961–966
41. Cavanaugh TM, Schoenemen H, Goebel J (2012) The impact of sirolimus on sex hormones in male adolescent kidney recipients. Pediatr Transplant 16:280–285
42. Tondolo V, Citterio F, Panocchia N, Nanni G, Favi E, Brescia A, Castagneto M (2005) Gonadal function and immunosuppressive therapy after renal transplantation. Transplant Proc 37:1915–1917
43. Lee S, Coco M, Greenstein SM, Schechner RS, Tellis VA, Glicklich DG (2005) The effect of sirolimus on sex hormone levels of male renal transplant recipients. Clin Transplant 19:162–167
44. Huyghe E, Zairi A, Nohra J, Kamar N, Plante P, Rostaing L (2007) Gonadal impact of target of rapamycin inhibitors (sirolimus and everolimus) in male patients: An overview. Transpl Int 20:305–311
45. Kaczmarek I, Groetzner J, Adamidis I, Landwehr P, Mueller M, Vogeser M, Gerstorfer M, Uberfuhr P, Meiser B, Reichart B (2004) Sirolimus impairs gonadal function in heart transplant recipients. Am J Transplant 4:1084–1088
46. Rovira J, Diekmann F, Ramirez-Bajo MJ, Banon-Maneus E, Moya-Rull D, Campistol JM (2012) Sirolimus-associated testicular toxicity: Detrimental but reversible. Transplantation 93:874–879
47. Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A, Margreiter R, Hugo C, Grinyo JM, Frei U, Vanrenterghem Y, Daloze P, Halloran PF, ELITE-Symphony Study (2007) Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med 357:2562–2575
48. Ekberg H, Bernasconi C, Tedesco-Silva H, Vitko S, Hugo C, Demirbas A, Acevedo RR, Grinyo J, Frei U, Vanrenterghem Y, Daloze P, Halloran P (2009) Calcineurin inhibitor minimization in the symphony study: Observational results 3 years after transplantation. Am J Transplant 9:1876–1885
49. Nashan B, Gaston R, Emery V, Saemann MD, Mueller NJ, Couzi L, Dantal J, Shihab F, Mulgaonkar S, Seun Kim Y, Brennan DC (2012) Review of cytomegalovirus infection findings with mammalian target of rapamycin inhibitor-based immunosuppressive therapy in de novo renal transplant recipients. Transplantation 93:1075–1085
50. Brennan DC, Legendre C, Patel D, Mange K, Wiland A, McCague K, Shihab FS (2011) Cytomegalovirus incidence between everolimus versus mycophenolate in de novo renal transplants: Pooled analysis of three clinical trials. Am J Transplant 11:2453–2462
51. Cullis B, D'Souza R, McCullagh P, Harries S, Nicholls A, Lee R, Bingham C (2006) Sirolimus-induced remission of posttransplantation lymphoproliferative disorder. Am J Kidney Dis 47:e67–e72
52. Pascual J (2007) Post-transplant lymphoproliferative disorder–the potential of proliferation signal inhibitors. Nephrol Dial Transplant 22[Suppl 1]:i27–i35
53. Zaltzman JS, Prasad R, Chun K, Jothy S (2005) Resolution of renal allograft-associated post-transplant lymphoproliferative disorder with the introduction of sirolimus. Nephrol Dial Transplant 20:1748–1751
54. Chiurchiu C, Carreno CA, Schiavelli R, Petrone H, Balaguer C, Trimarchi H, Pujol GS, Novoa P, Acosta F, Gonzalez C, Arriola M, Massari PU, Argentinian Registry of Everolimus Treated Renal Transplant Recipients (2010) Results of the conversion to everolimus in renal transplant recipients with posttransplantation malignancies. Transplant Proc 42:277–279
55. Jimenez-Rivera C, Avitzur Y, Fecteau AH, Jones N, Grant D, Ng VL (2004) Sirolimus for pediatric liver transplant recipients with post-transplant lymphoproliferative disease and hepatoblastoma. Pediatr Transplant 8:243–248
56. Augustine JJ, Bodziak KA, Hricik DE (2007) Use of sirolimus in solid organ transplantation. Drugs 67:369–391
57. Schachter AD, Meyers KE, Spaneas LD, Palmer JA, Salmanullah M, Baluarte J, Brayman KL, Harmon WE (2004) Short sirolimus half-life in pediatric renal transplant recipients on a calcineurin inhibitor-free protocol. Pediatr Transplant 8:171–177
58. Powell HR, Kara T, Jones CL (2007) Early experience with conversion to sirolimus in a pediatric renal transplant population. Pediatr Nephrol 22:1773–1777
59. Bumbea V, Kamar N, Ribes D, Esposito L, Modesto A, Guitard J, Nasou G, Durand D, Rostaing L (2005) Long-term results in renal transplant patients with allograft dysfunction after switching from calcineurin inhibitors to sirolimus. Nephrol Dial Transplant 20:2517–2523
60. Butani L (2004) Investigation of pediatric renal transplant recipients with heavy proteinuria after sirolimus rescue. Transplantation 78:1362–1366
61. Letavernier E, Bruneval P, Vandermeersch S, Perez J, Mandet C, Belair MF, Haymann JP, Legendre C, Baud L (2009) Sirolimus interacts with pathways essential for podocyte integrity. Nephrol Dial Transplant 24:630–638
62. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, Jauch KW, Geissler EK (2002) Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: Involvement of vascular endothelial growth factor. Nat Med 8:128–135
63. Oroszlan M, Bieri M, Ligeti N, Farkas A, Meier B, Marti HP, Mohacsi P (2010) Sirolimus and everolimus reduce albumin endocytosis in proximal tubule cells via an angiotensin II-dependent pathway. Transpl Immunol 23:125–132
64. Valente JF, Hricik D, Weigel K, Seaman D, Knauss T, Siegel CT, Bodziak K, Schulak JA (2003) Comparison of sirolimus vs. mycophenolate mofetil on surgical complications and wound healing in adult kidney transplantation. Am J Transplant 3:1128–1134
65. Dean PG, Lund WJ, Larson TS, Prieto M, Nyberg SL, Ishitani MB, Kremers WK, Stegall MD (2004) Wound-healing complications after kidney transplantation: A prospective, randomized comparison of sirolimus and tacrolimus. Transplantation 77:1555–1561
66. Nashan B, Citterio F (2012) Wound healing complications and the use of mammalian target of rapamycin inhibitors in kidney transplantation: A critical review of the literature. Transplantation 94:547–561
67. Budde K, Lehner F, Sommerer C, Arns W, Reinke P, Eisenberger U, Wuthrich RP, Scheidl S, May C, Paulus EM, Muhlfeld A, Wolters HH, Pressmar K, Stahl R, Witzke O, ZEUS Study Investigators (2012) Conversion from cyclosporine to everolimus at 4.5 months posttransplant: 3-year results from the randomized ZEUS study. Am J Transplant 12:1528–1540
68. Van Damme-Lombaerts R, Webb NA, Hoyer PF, Mahan J, Lemire J, Ettenger R, McMahon L, Cambon N, Boger R, Kovarik JM (2002) Single-dose pharmacokinetics and tolerability of everolimus in stable pediatric renal transplant patients. Pediatr Transplant 6:147–152
69. Zimmerman JJ, Kahan BD (1997) Pharmacokinetics of sirolimus in stable renal transplant patients after multiple oral dose administration. J Clin Pharmacol 37:405–415

70. Schubert M, Venkataramanan R, Holt DW, Shaw LM, McGhee W, Reyes J, Webber S, Sindhi R (2004) Pharmacokinetics of sirolimus and tacrolimus in pediatric transplant patients. Am J Transplant 4:767–773
71. Venkataramanan R, Sindhi R (2006) Sirolimus pharmacokinetic differences between children and adults. Pediatr Transplant 10:872–874
72. Grimm EM, Kelley PA, Seinford RD, Gitomer JJ, Kahan BD (2000) Sirolimus pharmacokinetics in pediatric renal transplants. Pediatr Transplant 4:S86a
73. Zimmerman JJ, Harper D, Getsy J, Jusko WJ (2003) Pharmacokinetic interactions between sirolimus and microemulsion cyclosporine when orally administered jointly and 4 hours apart in healthy volunteers. J Clin Pharmacol 43:1168–1176
74. Vu MD, Qi S, Xu D, Wu J, Fitzsimmons WE, Sehgal SN, Dumont L, Busque S, Daloze P, Chen H (1997) Tacrolimus (FK506) and sirolimus (rapamycin) in combination are not antagonistic but produce extended graft survival in cardiac transplantation in the rat. Transplantation 64:1853–1856
75. Brandhorst G, Tenderich G, Zittermann A, Oezpeker C, Koerfer R, Oellerich M, Armstrong VW (2008) Everolimus exposure in cardiac transplant recipients is influenced by concomitant calcineurin inhibitor. Ther Drug Monit 30:113–116
76. Pascual J, del Castillo D, Cabello M, Pallardo L, Grinyo JM, Fernandez AM, Brunet M (2010) Interaction between everolimus and tacrolimus in renal transplant recipients: A pharmacokinetic controlled trial. Transplantation 89:994–1000
77. Marin-Casino M, Crespo M, Mateu-de Antonio J, Pascual J (2011) Monitoring sirolimus levels: How does it affect the immunoassay used? Nefrologia 31:359–361
78. Coentrao L, Carvalho C, Sampaio S, Oliveira JG, Pestana MI (2010) Relationship between everolimus blood concentration assessed using the innofluor certican fluorescence polarization immunoassay and the architect i system sirolimus chemiluminescent microparticle immunoassay. Transplant Proc 42:1867–1869
79. Katzman SD, O'Gorman WE, Villarino AV, Gallo E, Friedman RS, Krummel MF, Nolan GP, Abbas AK (2010) Duration of antigen receptor signaling determines T-cell tolerance or activation. Proc Natl Acad Sci USA 107:18085–18090
80. Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR, Xiao B, Worley PF, Powell JD (2011) The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. Nat Immunol 12:295–303
81. Thomson AW, Turnquist HR, Raimondi G (2009) Immunoregulatory functions of mTOR inhibition. Nat Rev Immunol 9:324–337
82. Yang K, Neale G, Green DR, He W, Chi H (2011) The tumor suppressor Tsc1 enforces quiescence of naive T cells to promote immune homeostasis and function. Nat Immunol 12:888–897
83. Gulen MF, Kang Z, Bulek K, Youzhong W, Kim TW, Chen Y, Altuntas CZ, Sass Bak-Jensen K, McGeachy MJ, Do JS, Xiao H, Delgoffe GM, Min B, Powell JD, Tuohy VK, Cua DJ, Li X (2010) The receptor SIGIRR suppresses Th17 cell proliferation via inhibition of the interleukin-1 receptor pathway and mTOR kinase activation. Immunity 32:54–66
84. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, Worley PF, Kozma SC, Powell JD (2009) The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity 30:832–844
85. Battaglia M, Stabilini A, Tresoldi E (2012) Expanding human T regulatory cells with the mTOR-inhibitor rapamycin. Methods Mol Biol 821:279–293
86. Brouard S, Puig-Pey I, Lozano JJ, Pallier A, Braud C, Giral M, Guillet M, Londono MC, Oppenheimer F, Campistol JM, Soulillou JP, Sanchez-Fueyo A (2010) Comparative transcriptional and phenotypic peripheral blood analysis of kidney recipients under cyclosporin A or sirolimus monotherapy. Am J Transplant 10:2604–2614
87. Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, Larsen CP, Ahmed R (2009) mTOR regulates memory CD8 T-cell differentiation. Nature 460:108–112
88. Araki K, Youngblood B, Ahmed R (2010) The role of mTOR in memory CD8 T-cell differentiation. Immunol Rev 235:234–243
89. Pascual J, Boletis IN, Campistol JM (2006) Everolimus (certican) in renal transplantation: A review of clinical trial data, current usage, and future directions. Transplant Rev (Orlando) 20:1–18
90. Zuckermann A, Manito N, Epailly E, Fiane A, Bara C, Delgado JF, Lehmkuhl H, Ross H, Eisen H, Chapman J, Valantine H (2008) Multidisciplinary insights on clinical guidance for the use of proliferation signal inhibitors in heart transplantation. J Heart Lung Transplant 27:141–149
91. Lexicomp (2013) Lexicomp Online™. Available at: http://www.uptodate.com/contents/everolimus-drug-information?source=search_result&search=everolimus&selectedTitle=1%7E80#F7710406. Lexi-Drugs Online™, Lexicomp Inc., Hudson. Accessed 25 Apr 2013
92. Lexicomp (2013) Lexicomp Online™. Available at: http://www.uptodate.com/contents/sirolimus-drug-information?source=search_result&search=sirolimus&selectedTitle=1%7E150#F221195. Lexi-Drugs Online™, Lexicomp, Inc., Hudson. Accessed 25 Apr 2013