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m-TOR Inhibitors in Kidney Transplantation: A Comprehensive Review

Mekki M[1,2], **Bridson JM**[2], **Sharma A**[2,3] **and Halawa A**[2,4*]

[1]*Renal Department, Royal Shrewsbury Hospital, UK*

[2]*Faculty of Health and Science, Institute of Learning and Teaching, University of Liverpool, UK*

[3]*Royal Liverpool University Hospital, Liverpool, UK*

[4]*Sheffield Teaching Hospitals, Sheffield, UK*

[*]**Corresponding author:** Ahmed Halawa, MD PhD, Consultant Transplant Surgeon, Sheffield Teaching Hospital, University of Sheffield-University of Liverpool, United Kingdom, Tel: +00447787542128; E-mail: ahmed.halawa@sth.nhs.uk

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Abstract

Mammalian target of rapamaycin inhibitors (mTOR-I) has been in use in kidney transplantation for over two decades. Since their introduction, they have been used in different combination immunosuppressant for low risk group renal transplantation. They have been in use in the various (Calcineurin inhibitors) CNI-free protocols, either as *De novo* regiments, or by conversion from CNIs at a later stage. Many of these studies reported comparable graft rejection rate and a better kidney function (eGFR) compared to standard CNI protocols. Also mTOR-I when used in combination with CNIs, facilitated the use of lower doses of CNIs with the resultant reduction of CNI related side effects, without seriously compromising graft outcome. They are of particular interest among certain group of renal transplant recipients, including those with malignancy, post-transplant encapsulating peritoneal sclerosis, CMV and BK virus infections. Moreover, protocols containing mTOR-I showed comparable results to standard protocols among recipients of kidneys from extended-criteria transplantation. However, only few reports studied their use in high risk group renal transplantation, with variable outcomes. There is a noticeable drop in their popularity in the recent years, and their use was associated with multiple adverse events, in addition to a recent concern of their link to increased mortality. Also, a high discontinuation rate was demonstrated across many of the studies available to date. More studies are still needed to clarify the above-mentioned concerns.

Keywords: Mammalian target of rapamycin inhibitors; Renal transplantation; Immunosuppressant

Methods and Data Collection

Search of the literature including pubmed, NCBI and Google search engine using the key words immunosuppression, mammalian target of rapamycin inhibitors, and kidney transplantation. All relevant articles which were provided in English were reviewed.

Introduction and Overview of Immunosuppressant in Kidney Transplantation

Recent years witnessed a substantial improvement in the field of kidney transplantation. The rate of acute allograft rejection (AR) has reduced in both cadaveric and living donors renal transplantation, with an increased graft survival in the first year [1,2]. Graft survival after the first year of transplantation has been improving [2]. Since the first kidney transplantation in the 1960s [3], there has been an observed increase in the development and introduction of newer immunosuppressive agents [1]. In 1960s, kidney transplantation immunosuppressant protocols consisted of total body irradiation, steroids, and azathioprine (Aza) [4]. Cyclosporine A (CyA), a calcineurin inhibitor (CNI), was introduced in 1980s. It provided a dramatic reduction of acute rejection episodes and was shown to improve the graft survival in the first few years of transplantation [5,6]. In the 1990s onwards, various combinations of immunosuppressive medications were introduced into practice in addition to induction therapy [2,7]. Micro-emulsion formulation of CyA (Neoral) was introduced with subsequent improvement in acute graft rejection compared to the conventional formulation (Sandimmune) [8]. Afterwards prograf, mycophenonlate mofetil (MMF), and mammalian target of rapamycin inhibitors (mTOR-I) were introduced. Induction treatments include anti-thymocyte globulin, basliximab, daclizumab [2,8] and alemtuzumab (Campath) [9]. Accompanying this was the increasing challenge of transplanting higher risk groups including those with more HLA mismatches (Tables 1A and 1B) [2], re-transplants [10,11], ABO incompatible [12-15] and patients with preformed donor specific antibodies (DSA) [16,17]. Also there is an observed trend of steroid avoidance/minimization with the adoption of more powerful immunosuppressant [18].

The transplanted kidney got rejected following its recognition by the recipient's immune system. This would be followed by activation of T cells through the three signal pathway. Signal 1 started upon binding of the T cell with donor's antigens presented in the context of major histocompatibility complex (MHC). This involves CD3 complex, which is in close proximity to T-cell receptor. Signal 2 involves co-stimulation and involves binging of CD 28 on the surface of T-cells with CD 80 and CD 86 on the dendritic cells. Signal 3 involves activation of calcium-calcineurin pathway and other pathways leading to increased transcription of IL2, CD154, CD25 and other cytokines, which in turn lead to T cell proliferation.

Immunosuppressive medications target different points in the immune system (Figure 1) [19]. There are 3 stages of immunosuppression: induction, maintenance, and treatment of established rejection. Induction therapy is important in reducing acute rejection and help delay the use of CNI.

Year	Number of HLA Mis-matches (% of renal transplantation in the year)							
	0	1	2	3	4	5	6	Unknown
1998	-10.3	-6.2	-9.2	-19.9	-23.2	-20.3	-10.2	-0.5
1999	-11.1	-6.3	-8.3	-18.4	-22.7	-21.3	-11.3	0.5
2000	-10.5	-6.5	-8.5	-17.6	-23.2	-22.3	-11.2	-0.3
2001	-11.5	-5	-8.3	-18.5	-22.9	-21.5	-12.2	-0.2
2002	-11.5	-4.1	-7.7	-16.8	-22.6	-22.7	-14.1	-0.4
2003	-10.6	-3.5	-5.2	-13.3	-23.3	-27.9	-15.4	-0.7
2004	-12	-3	-3.3	-11.8	-24.9	-30.1	-14.3	-0.6
2005	-12	-2.4	-3.3	-11	-24.4	-31.1	-15.1	-0.7
2006	-10.9	-2	-3.3	-10.8	-25.1	-31.1	-16	-0.8
2007	-12	-2.2	-3.7	-10.6	-24.7	-30.6	-15.4	-0.8
2008	-12.2	-2	-3.8	-11.8	-23.2	-30.1	-16.3	-0.6
2009	-6.8	-1.4	-3.9	-12.4	-25.8	-32.1	-17.3	-0.5
2010	-6.8	-1.1	-4	-13	-26.3	-32.1	-16.1	-0.6
2011	-7.2	-0.9	-4	-13.4	-27.1	-31.4	-15.1	-0.7
2012	-7.5	-0.9	-4.1	-13	-26.7	-31.6	-15.4	-0.8

Table 1A: Total HLA mismatches among adult kidney transplant recipients, deceased donors. From the OPTN, SRTR report 2012 [2]. OPTN=Organ Procurement and Transplantation Network, SRTR=Scientific.

Medications used for induction can be divided into depleting and non-depleting agents in Table 2 [4]. The use of induction agents, as per the reports of OPTN/SRTR during the years 1998-2012, is depicted in Figure 2 [2].

Basiliximab and daclizumab bind to CD 25 to inhibit IL2 production, which is necessary for T-cell activation and proliferation. Basiliximab is a non-depleting humanized chimeric monoclonal antibody (75% human and 25% mouse). It has greater affinity to CD25 than daclizumab (which is a humanized monoclonal antibody with 90% human and 10 mouse) [4]. Basiliximab is shown to have similar efficacy and better safety profile compared to Anti thymoglobulin [21-23]. It reduces graft rejection and mortality compared to placebo without increasing adverse effects, which include infection and malignancy [22,23]. The dose for basiliximab is shown in Table 2 [20]. Equine anti-thymocyte globulin (ATGAM) and rabbit anti-thymocyte globulin (Thymoglobulin) are polyclonal depleting agents used for induction of immunosuppression in kidney transplantation [4]. Their doses are shown in Table 2 [20]. Both ATGAM and thymoglobulin contain antibodies active against several T-cell antigens including CD2, CD3, CD4, CD5, CD8, CD11, CD18, CD45, anti β-2- microglobulin and anti HLA DR antibodies [24]. Thymoglobulin was also shown to induce complement-independent apoptosis of naive and activated B cells in addition to plasma cells [25]. They are used for induction especially in high-risk groups and also for treatment of acute rejection [21,26]. The main side effect of thymoglobulin and ATGAM include cytokine release syndrome [27], infections [28] and malignancy especially post-transplant Lymphoproliferative diseases (PTLD) [29,30]. There is a recent concern of graft loss on the long term among patients who had developed serum sickness following induction with thymoglobulin [31].

The newer depleting agent alemtuzumab (Campath®) is a humanized monoclonal antibody directed against CD52 of T-cells [4]. Dose and side effects of Campath are summarized in Table 2 [20]. It is used as an induction agent in high-risk group [32] and causes profound lymphocyte depletion [33]. Risk of rejection is shown to be lower compared to conventional methyl prednisolone induction [34], without significant increase in malignancy [35]. It is also used for treatment of steroid-resistant acute rejection [36]. Other agents to mention in this context are the ones used in the desensitization protocols for high-risk groups. These include intravenous immunoglobulins (IVIG), plasma exchange, rituximab (anti CD 20) and bortezomib (proteasome inhibitor) [37-40]. Maintenance immunosuppressants include steroids, Calcineurin inhibitors (CNI), anti-proliferatives (azathioprine and mycophenolate mofetil), mTOR-I (Sirolimus and Everolimus), and belatacept. Table 3 summarizes the different maintenance immunosuppressive medications, dosing, and common side effects [20].

Steroids have been in use since the early days of transplantation [4]. They work by binding to steroid receptors and inhibit transcription of cytokine genes and cytokine receptors. Their use is associated with multiple side effects (Table 3) [20], which led to emergence of various regiments minimizing their use in kidney transplantation [18]. There is a noticeable drop of the use of steroid both in the early and late stages of transplantation among renal transplant recipients, over the recent decades in Figure 3 [2].

Year	Number of HLA Mis-matches (% of renal transplantation in the year)							
	0	1	2	3	4	5	6	Unknown
1998	-13.6	-7.2	-19.1	-29.4	-11	-10.9	-5.7	-3.1
1999	-12.1	-6.9	-18.8	-29.1	-12.4	-12.4	-5.4	-2.8
2000	-11.7	-6.2	-18.9	-28	-12.7	-14	-6.5	-2
2001	-11.1	-6.8	-18.1	-28.1	-12.9	-14.3	-7.6	-1.1
2002	-10.8	-6.5	-17.3	-29.1	-12.7	-14.9	-7.8	-0.8
2003	-9.6	-5.7	-18.1	-27.9	-13.5	-15.7	-8.9	-0.7
2004	-9.1	-5.5	-16.9	-28.5	-13.5	-16.3	-9.2	-0.9
2005	-8.1	-5.9	-17.2	-28.1	-14.3	-17	-8.8	-0.7
2006	-9.2	-5.3	-16.4	-27.8	-14.7	-16.8	-9	-0.7
2007	-8.1	-4.9	-15.9	-27.6	-15.1	-17.5	-9.8	-1
2008	-8.8	-4.7	-16.8	-25.9	-15.3	-17.5	-10.1	-0.9
2009	-7.8	-4.6	-15.9	-26.7	-15.9	-18.1	-9.9	-1
2010	-7.8	-4.2	-14.7	-25.1	-16.4	-20	-10.8	-1
2011	-7.8	-4.4	-14.1	-24.9	-17	-20.4	-10.5	-0.9
2012	-6.5	-4.3	-13.8	-24.4	-17.1	-19.3	-11.6	-3

Table 1B: Total HLA mismatches among kidney transplant recipients, living donors. From the OPTN, SRTR report 2012 [2].

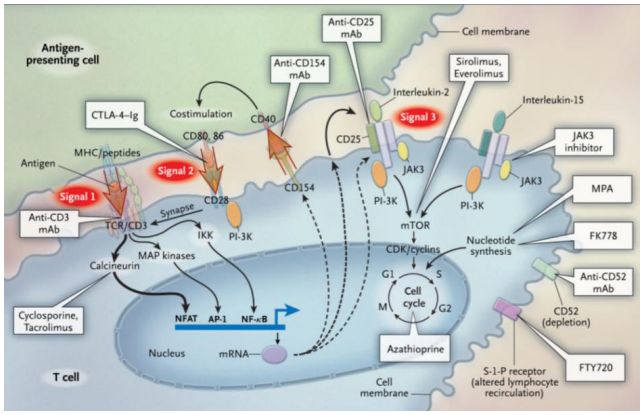

Figure 1: Three signal pathways of T-cell activation [19].

CNIs, including CyA and tacrolimus, work by binding to an intracellular protein (cyclophillin and FK-binding protein, respectively). This action blocks signal 1 of T-cell activation by inhibition of dephosphorylation and translocation of the nuclear factor of activate T-cells (NFAT). CNIs form the cornerstone of immunosuppressants [5,6]. Common side effects and doses are outlined in Table 3 [20]. There are concerns regarding their link to chronic allograft fibrosis and nephropathy [41,42]. CNIs require frequent monitoring of blood levels [4,43] and exhibit interaction with a wide variety of medications, as they are metabolized by liver cytochrome p450 [4]. Tacrolimus-based immunosuppressants were shown to be superior to CyA-based ones in terms of survival and/or acute rejection rate [43-46]. Recent years showed much more use of tacrolimus, while the use of CyA has substantially reduced in Figure 4 [2].

Other maintenance immunosuppressant agents include the anti-proliferative group. This includes azathioprine, MMF and its enteric-coated formulation, myfotic. Azathioprine has been in use since the early days of transplantation [4]. It is an imidazole derivative of 6-mercaptopurine, and is incorporated in DNA to inhibit gene transcription [21]. Table 3 lists doses and side effects. It has been largely superseded by the mycophenolate medications in Figure 5 [2]. Mycophenolate is a reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), which is the rate-limiting enzyme of purine synthesis. IMPDH is found mainly in lymphocytes thus mycophenolate inhibit proliferation of T as well as B-lymphocytes [47].

Generic Name (Brand name)	Depleting/non-depleting	Dosing	Common side effects	Generic Name (Brand name)
Basiliximab (Simulect®)	Non depleting	20 mg IV × 2 doses	None reported compared to placebo	Basiliximab (Simulect®)

Antithymocyte globulin equine (ATGAM®)	Depleting	15 mg/kg/day IV × 3–14 days	Flu-like symptoms, GI distress, rash, back pain, myelosuppression	Antithymocyte globulin equine (ATGAM®)
Antithymocyte globulin rabbit (Thymoglobulin®)	Depleting	30 mg IV × 1–2 doses	Flu-like symptoms, GI distress, rash, back pain, myelosuppression	Antithymocyte globulin rabbit (Thymoglobulin®)
Alemtuzumab (Campath®)	Depleting	30 mg IV × 1–2 doses	Flu-like symptoms, GI distress, dizziness, myelosuppression	Alemtuzumab (Campath®)

Table 2: Common induction agents in transplantation, doses and side effects. Modified from: Gabadi et al. [20].

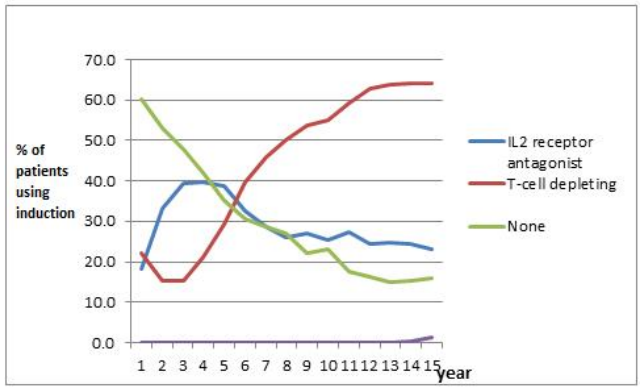

Figure 2: Use of the different induction agents in renal transplantation, data from the OPTN/SRTR 12th annual report. Modified from: OPTN/SRTR, 12th annual report [2].

Maintenance immunosupressive treatment also includes mTOR-I, comprised of sirolimus and evarolimus, binds to FK protein but would not inhibit calcineurin. Their complex with calcineurin binds to rapamycin target and inhibit signal 3 of T-cell activation, through inhibition of cytokines activating T-cell cycle [19].Again they have a lot of drug interactions as they are metabolized by p450, and interact with CNIs [4]. They have many side effects, including infection [48-55], GI symptoms [20,49,52,53,56,57], poor wound healing [20,48,51,52,58-60], hyperlipidaemia [20,50,52-55,57,58,61-63], deceased sperm count in males on sirolimus [64] and worsening proteinuria [54,55,57,59,61,62,65,66]. Data from the OPTN/SRTR showed that their use in kidney transplantation has largely reduced in the recent years see Figure 6 [2].

The use of combination of immunosuppressive agents helps controlling immune response to the graft [20] however, this beneficial effect occurred at the expense of reducing immunity to infection [20,21]. Also their use is associated with more liability to malignancy including Kaposi's sarcoma, post-transplant lymphoproliferative disease (PTLD), lung, kidney and prostate cancers [63]. The mTOR-I is associated with reduction of malignancy, mainly non-melanoma skin cancer [64,67,68]. Moreover, they are associated with lesser CMV viremia [69], and everolimus was shown to be superior to MMF in that aspect in a pooled analysis of three clinical trials [70]. Additionally, mTOR-I showed favourable effect in BK viremia/BK associated nephropathy [71,72] and post-transplant encapsulating peritoneal sclerosis [73-75].

Immunosuppressive Protocols Using mTOR-I Among Low Risk Renal Transplantation

Given the side effects of CNIs outlined earlier [20,41,42], and the reduced malignancy risk observed with mTOR-I [65,67,68], several studies looked into their potential use in the context of kidney transplantation. Some studies looked into CNI avoidance with *De novo* m TOR-I introduction [45,56,76-82], while others looked into conversion from CNI into mTOR-I at a later stage [54,63,66,83,84] Table 4. Several studies showed that mTOR-I have good effect on preserving kidney function (as noted by changes in eGFR). Moreover, mTOR-I was used in combination with CNI as CNI sparing regiments and their use in combination with low dose CNI showed good graft survival and low rejection rate in Table 4. [48,50-53,57,84-90]. Larson et al. studied mTOR-I while completely avoiding CNI in a prospective randomized trial, comparing sirolimus, MMF and prednisolone (81 patients), to tacrolimus, MMF and prednisolone [84 patients], for an average of 33 months. At one year, patients' survival, graft survival, eGFR and acute rejection were similar in the two groups [58]. Additionally, Oh et al. studied 148 renal transplant recipients for 1 year. Patients were randomized one month after transplantation to receive everolimus, low dose CyA or to have MMF plus standard CyA dose. AR rate were similar in the two groups, but there was significantly higher eGFR among the everolimus group [91]. In the CONCEPT trial (2009), 192 patients who were on dacluzimab induction, and baseline CyA, MMF and prednisolone (which was withdrawn at 8 months), were studied prospectively. At 3 months post transplantation 95 were randomized to convert to sirolimus and 97 remained on CyA. Patient survival was similar and there was improved eGFR on the sirolimus group. However, the sirolimus group showed non-significantly more acute rejection (17% *vs.* 8%), and significantly more hyperlipidemia, lower HB, and more proteinuria [60]. Similarly, in the Spare-the-nephron trial (2011), the feasibility of mTOR-I use in a CNI-free regiment was studied [62]. In this study, 299 patients who were on initial CNI/MMF protocols were randomized after 30 to 180 days into MMF/ sirolimus (148 patients) and MMF/CNI (151 patients). Patients received variable induction therapy in both arms of the trial, including thymoglobulin, basiliximab, dacluzimab and Muromonab-CD3. At 24 months, patients who were on sirolimus/MMF had significantly higher eGFR compared to those on CyA/MMF. Also the eGFR was non-significantly higher among patients who were on sirolimus/MMF compared to tacrolimus/MMF. Patients on the sirolimus/MMF has similar opportunistic infection to the CNI/MMF group, however, they had significantly more dyslipidaemia, oedema, proteinuria and mouth ulcers. There was significantly lower BP among the sirolimus/MMF group compared to the Tacrolimus/MMF one, while hyperglycaemia was non-significantly worse in CNI/MMF group [62]. On the other hand, some studies showed less favourable effects of mTOR-I.

Generic name (brand name)	Common oral dosage	Common adverse effects
CyA (Sandimmune®, Neoral®, Gengraf®)	4-5 mg/kg by mouth twice a day	Neurotoxicity, gingival hyperplasia, hirsutism, hypertension, hyperlipidemia, glucose intolerance, nephrotoxicity, electrolyte abnormalities
TAC (Prograf®)	0.05-0.075 mg/kg by mouth twice a day	Neurotoxicity, alopecia, hypertension, hyperlipidemia, glucose intolerance, nephrotoxicity, electrolyte abnormalities
AZA (Imuran®)	1-2.5 mg/kg by mouth once a day	Myelosuppression, gastrointestinal disturbances, pancreatitis
MMF (CellCept®)	0.5-1.5 g by mouth twice a day	Myelosuppression, gastrointestinal disturbances
EC-MPA (Myfortic®)	720 mg by mouth twice a day	Myelosuppression, gastrointestinal disturbances
Sirolimus (Rapamune®)	1-10 mg by mouth once a day	Hypertriglyceridemia, myelosuppression, mouth sores, hypercholesterolemia, gastrointestinal disturbances, impaired wound healing, lymphocele, pneumonitis
Everolimus (Zortress®)	0.75 mg by mouth twice a day	Hypertriglyceridemia, myelosup-pression, mouth sores, hypercholesterolemia, gastrointestinal disturbances, impaired wound healing, lymphocele, pneumonitis
Belatacept (Nulojix®)	10 mg/kg/dose IV on post-op days 1 and 5 and at the end of post-op weeks 2, 4, 8 and 12.5 mg/kg/dose given every 4 weeks starting at the end of post-op week 16.	Anaemia, leukopenia, peripheral oedema, gastrointestinal disturbances, headache
Prednisone (Deltasone®)	Maintenance: 2.5-20 mg by mouth once a day	Mood disturbances, psychosis, cataracts, hypertension, fluid retention, peptic ulcers, osteoporosis, muscle weakness, impaired wound healing, glucose intolerance, weight gain, hyperlipidaemia

Table 3: Common maintenance immunosuppressant, doses and side effects.

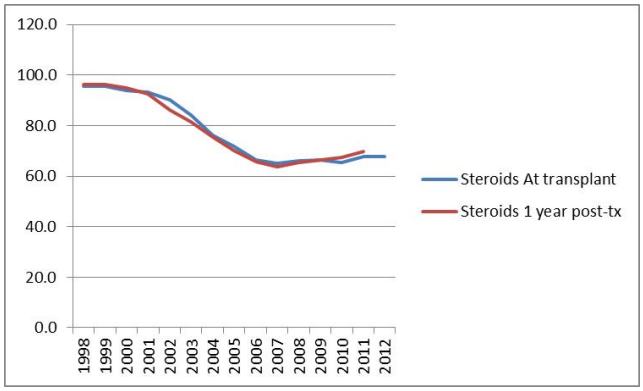

Figure 3: Use of steroid, data from the OPTN/SRTR 12th annual report. Modified from: OPTN/SRTR, 12th annual report [2].

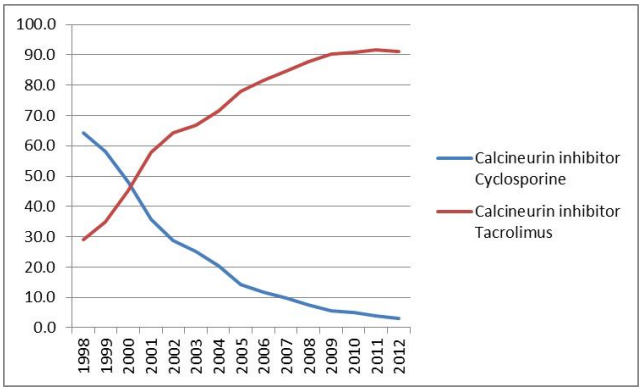

Figure 4: Use of CNI, data from the OPTN/SRTR 12th annual report. Modified from: OPTN/SRTR, 12th annual report [2].

In the ORION study (2011), Flechner et al. studied 3 treatment groups: sirolimus plus tacrolimus (tacrolimus withdrawal after 13 months, n=152 patients), sirolimus plus MMF (n=152 patients) and tacrolimus+MMF (139 patients). There was more biopsy-proven rejection among the sirolimus/MMF group leading to discontinuation of that arm. There was more rejection in the sirolimus group; however, graft survival was similar in the remaining two groups [59]. Less favourable results were also shown in a 36 months prospective randomized controlled study which was conducted in 11 centers from Australia, New Zealand, Taiwan, Malaysia, and Korea: The SCORATES study (2014). In this study, 126 patients on CyA, MMF, steroids and basiliximab for induction were randomized 14 days after transplantation into three groups to eliminate MMF plus either CNI or steroids. Group 1 (n=45): The CNI withdrawal group (CNI-WD), CNI plus MMF were withdrawn and continued on everolimus plus steroids; group 2 (n=40): The steroid withdrawal group (steroid-WD) in which MMF and steroids were withdrawn, and they continued on everolimus plus CNI; and group 3 (n=22): The control group with CNI, MMF and steroids.

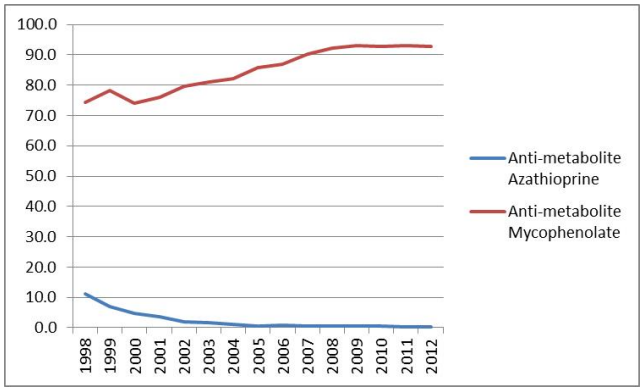

Figure 5: Use of antimetabolites, data from the OPTN/SRTR 12th annual report. Modified from: OPTN/SRTR, 12th annual report [2].

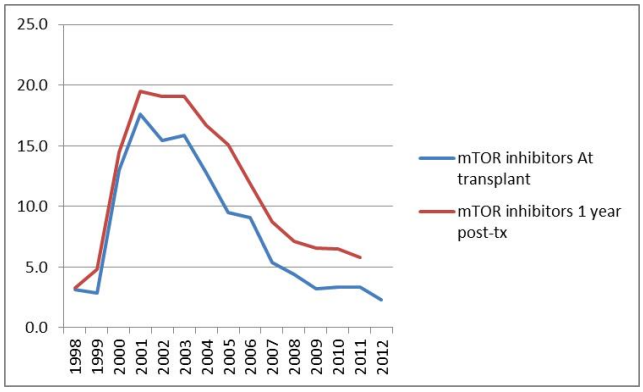

Figure 6: Use of mTOR, data from the OPTN/SRTR 12th annual report. Modified from: OPTN/SRTR, 12th annual report [2].

The steroid-WD was discontinued prematurely as there was high rate of discontinuation. At 12 months, in the everolimus group, the eGFR was non-inferior; however there was significantly more rejection and a trend towards more graft and patient loss [56]. Also, in the ELITE Symphony study (2007), 1645 patients were treated with standard CyA, MMF and steroids; low dose tacrolimus with dacluzimab in the first 2 months; low dose CyA and MMF; and low dose sirolimus with dacluzimab for two months. The patients were followed for one year: the low dose tacrolimus showed significantly higher eGFR, less rejection and better graft survival compared to all other groups [49].

A systematic review and meta-analysis included 2067, studied patients who were on everolimus with CNI minimization or elimination, compared to standard CNI protocols. Patients on everolimus plus CNI elimination have significantly more rejection, without increasing patient mortality or graft loss, and a better eGFR compared to the standard CNI protocols. However patients on everolimus with CNI minimization had similar graft rejection compared to the control CNI protocols [92]. An earlier systematic review and meta-analysis (2006) showed that when mTOR-I replaced CNI (750 patients), there would be same rejection rate, lower creatinine and more marrow suppression; when replaced antimetabolites (3966 patients) there was less rejection and lower CMV infection but higher hyperlipidaemia. However when mTOR-I used in combination with CNI (3175 patients) graft and patient survival were similar in all comparison groups (low dose mTOR-I *vs.* High dose mTOR inhibitors in combination with standard CNI doses, and variable mTOR-I inhibitors doses with variable CNI doses). Patients on low dose mTOR-I had more rejection and better eGFR compared to the high dose with similar CNI doses. Similar results with the low dose mTOR-I plus standard dose CNI compared to the high dose mTOR-I plus low CNI dose [93].

Other studies from Table 4, looked into various mTOR-I based protocols with results comparable to the above-discussed studies. Overall, it appears that there is good evidence of the efficacy of mTOR-I in preserving the transplanted kidney function, with a graft survival comparable to other conventional immunosuppressant protocols, among standard risk renal transplant recipients.

High-Risk Renal Transplantation & Extended-Criteria Renal Transplants and mTOR-I Protocols

Compared to low risk renal transplantation, mTOR-I are less studied among other risk groups. Few studies showed that mTOR-I have good and comparable results to other standard protocols among recipients of extended-criteria donors [92-98]. Even fewer studies looked in their use among high-risk renal transplant recipients. Sirolimus containing regiments, with CyA and prednisolone, were shown to have equal efficacy and graft survival to CyA, azathioprine and prednisolone regiments, among renal transplant recipients at high risk of delayed graft function [99,100]. Uchida et al. studied 16 stable ABO incompatible renal transplant recipients who were on MMF and standard CNI. MMF was substituted by everolimus with low dose CNI, and there was no increased rejection at three months [101]. Other earlier studies showed also good results on mTOR-I among high immunologic risk renal transplants [101,102]. However, Lee et al. reported an unfavourable outcome in a pilot study of 28 sirolimus-based high-risk renal transplant recipient compared to 69 control patients on MMF, CNI and prednisolone. There was non-significantly higher rejection rate, lower survival and lower eGFR among the sirolimus-treated group. Also there were increased side effects on the sirolimus group [103].

Other Aspects of mTOR-I Protocols in Kidney Transplantation

Looking into other aspects related to the use of mTOR-I, sirolimus was shown to be cost effective. The cost of treatment and graft loss were compared among 4 groups: early sirolimus+withdrawal of steroids/CNI, early transition by sirolimus, early transition by everolimus, and the standard prednisolone, MMF and CNI. The lowest cost was for early sirolimus transition group [104].

Safety of mTOR-I Protocols in Kidney Transplantation

Taking into consideration all what is discussed above, safety might be an issue. A recent systematic review and meta-analysis of 21 RCT studies included 5876 patients, was conducted by Knoll et al. It was demonstrated that mTOR-I use, although associated with a 40% reduction of malignancy and 56% reduction of non-melanoma skin cancer, was associated with an increased risk of mortality [105].

Author, year/Study design/ (reference)	Number of patients	mTOR-I use *De novo*/ conversion/CNI sparing	Comparison group	Acute rejection	eGFR
Gatault et al. SPIESSER study; Open label comparative randomized study, 8 years follow-up [77]	99	*De novo*	SRL+MMF *vs.* CyA+MMF Thymoglobulin (Ind), steroid (withd) at 5/12	--	↑ in SRL
Uchida et al, 2016 Retrospective pilot [63]	26	Conversion	Antimetabolite+CNI *vs.* EVR+CNI (min.)	--	↑in EVR
Yoshimura et al. Prospective study. [87]	29	29	EVR+(LD) Tac+MZR+ pred *vs.* no EVR. basiliximab (Ind)	Similar	Similar
Yamanaka et al. Retrospective study [86]	12	CNI sparing	EVR+(LD) Tac+MMF+pred *vs.* no EVR	↑ in EVR (Sub-clinical)	↑ in EVR
Carmellini et al. Post-hoc analysis of 2309 study [88]	833	*De novo*	EVR+(LD) CyA *vs.* MMF+CyA. Basiliximab (Ind)	Similar	--
Mühlbacher et al. Open label, multi-centre [50]	420	CNI sparing	SRL+pred+(LD) CyA *vs.* SRL+pred+(Sd) CyA	Similar	↑ in (LD) CyA
Huang et al. Prospective open label [89]	112	112	SRL+MMF+Pred *vs.* CNI +MMF+Pred	Similar	↑ in SRL
Bechstein et al. Prospective open label multi-centre randomized [51]	128	CNI sparing	SRL+(LD) Tac+Pred *vs.* SRL+(Sd)Tac +pred	Similar	↑ in (LD) Tac
Lebranchu et al. Retrospective follow-up of SPIESSER study [55]	131	*De novo*	SRL+MMF *vs.* CyA+MMF	Similar	↑ in SRL
Langer et al. ASSETT study. A phase 111 Open label prospective randomized multicenter [59]	228	CNI sparing	EVR+(LD) Tac+pred *vs.* EVR+(Sd) Tac +Pred. Basiliximab+steroids (Ind)	Similar	Similar
Budde et al. ZEUS study, Open Label multi-centre study [66].	300	Conversion	CyA+Pred+Myfortic *vs.* +EVR+Pred +Myfortic. Basliximab (Ind)	↑ in EVR	↑ in EVR
Paoletti et al. Case control study [83].	39	Basliximab (Ind)	SRL+MMF *vs.* CyA+MMF	Similar	↑ in SRL
Han et al. Prospective randomized open label study [84].	51	Conversion	CyA+MMF+Pred *vs.* SRL+MMF+Pred	--	↑ in SRL
Bertoni et al. Randomized single-centre open-labelled prospective [76]	106	CNI sparing	EVR+(LD) CyA *vs.* CyA+MMF	--	↑ in EVR
Holdaas et al. ASCERTAIN investigator, open labelled randomized multicenter study [53]	404	CNI sparing	EVR+CNI (Withd) *vs.* EVR+CNI (Min.) *vs.* CNI based control	↑ in CNI (Withd)	↑ in CNI (Withd)
Schena et al. CONVERT Trial, Open label prospective comparative [54]	830	Conversion	CNI+Pred+either MMF or Aza *vs.* SRL +Pred+either MMF or Aza	--	↑ in SRL
Tsai et al. Retrospective cohort [85]	206	CNI sparing	(LD) CNI+SRL *vs.* (Sd) CNI+MMF	↓ in SRL	--

Salvadori et al. A multi-centre randomized study, EVEREST trial [91].	285	CNI sparing	(LD) EVR+(HD) CyA *vs.* (HD) EVR+ (LD) CyA	Similar	Similar
Albano et al. 2009. Prospective multi-centre open label randomized trial. [48]	139	CNI sparing	EVR+CyA+Pred *vs.* EVR (MMF conversion)+CyA+Pred. IL2 blocker (Ind)	Similar	Similar
Chan et al. Randomized open label prospective multi-centre [52]	92	CNI sparing	EVR+pred+(LD) Tac *vs.* EVR+(Sd) Tac +Pred. Basiliximab+steroids (Ind)	Similar	Similar
Kumar et al. Prospective randomized pilot study [45]	200	*De novo*	CyA+MMF *vs.* CyA+SRL *vs.* Tac+MMF *vs.* Tac+SRL. Basiliximab+steroid (Ind). Early steroid (Withd)	↓in Tac+MMF, Tac +SRL, CsA+ SRL	Similar
Carmellini et al. Single-centre retrospective. [77]	286	CNI sparing	mTOR+(LD) CNI+pred *vs.* (HD) CNI +MMF+Pred. Basiliximab (Ind)	Similar	--
Flechner et al. Randomized prospective [80].	61	*De novo*	SRL+MMF+Pred *vs.* CNI+MMF+Pred. Basiliximab (Ind)	--	↑ in SRL
Diekmann et al. [79].	108	*De novo*	SRL+MMF+pred+anti CD 25 (Ind) *vs.* pred+MMF +/- anti CD 25	↑ in MMF	--
Kandaswamy et al. Prospective randomized [90].	239	CNI sparing	CsA+MMF *vs.* (HD) Tac+(LD) SRL *vs.* (LD) Tac+(HD) SRL. ATG+Steroids (Ind)	Similar	Similar
Flechner et al. Prospective randomized [80]	58	*De novo*	SRL+MMF+Pred *vs.* CyA+MMF+Pred. Basiliximab (Ind)	--	↑ in SRL
Kreis et al. Open label multi-centre study [81]	78	*De novo*	SRL+MMF+Pred *vs.* CyA+MMF+Pred	Similar	↑ in SRL

Table 4: mTOR-I based protocols[46,48,51-53,55,57,63,65,75,77-79,81-89,91,92]

Author (reference)	Year	No of patients on mTOR/overall No of patients	Discontinuation (%)	Reported side effects
Gatault et al. [78]	2016	50/99	48	--
Uchida et al. [64]	2016	26/26	42.3	Hypercholesterolemia, oedema, aphthous ulcers, fatigue, anaemia, menoxemia, interstitial pneumonitis, acne
Lee et al. [94]	2015	28/97	28.6	Higher hyperlipidemia, BK virus and lymphocele.
Chadban et al. [56]	2014	15/54	31	Diarrhoea
Mühlbacher et al. [50]	2014	101/420	24	Infections (UTIs/pyelonephritis, pneumonia, CMV, wound), lymphocele, oedema, hyperlipidaemia, anaemia, raised creatinine, HTN
Bechstein et al. [51]	2013	128/128	25.8	Infections (wound, UTI, candida, sepsis, CMV, Herpes simplex, Herpes zoster, pneumonia), lymphocele, wound dehiscence
Langer et al. [57]	2012	228/228	13.6	Hyperlipidaemia, hyperglycaemia, hypo and hyperkalaemia, GI disturbance, oedema, HTN, lymphocele, anaemia, insomnia, acne, proteinuria
Budde et al. (66)	2012	138/269	28.4	Aphthous ulcers, proteinuria, anaemia
Euvrard et al. [67]	2012	64/120	23	Aphtous ulcers, oedema, pneumonitis, rash

Campbell et al. [65]	2012	39/87	42.6	Pneumonitis, diarrhoea, decreased tolerance, rash, mouth ulcers, proteinuria, epistaxis
Burkhalter et al. [95]	2012	65/65	25	Leukopenia, anaemia, arthritis, and pneumonitis
Lebranchu et al. [55]	2012	63/131	33	Proteinuria, pneumonia, hyperlipidaemia, hyperglycaemia, oedema, mouth ulcers, pyelonephritis
Holdaas et al. [53]	2011	281/404	28.3 in CNI elimination and 16.7 in CNI minimization	Rash, mouth ulcers, oedema, hyperlipidaemia, anaemia, diarrhoea infection, acne
Weir et al. [62]	2011	148/299	19	Hyperlipidaemia, peripheral oedema, mouth ulcers, proteinuria
Flechner et al. [59]	2011	304/443	33.6- 34.2	Pneumonia, Thrombocytopenia, ATN, UTI, oedema, anaemia, delayed wound healing, lymphoedema
Tedesco Silva et al. [49]	2010	277/833 and 279/833	30-34.1	Wound healing, mouth ulcers
Schena et al. (54)	2009	555/830	25.8	Pneumonitis, infection, acne, aphthous ulcers, hyperlipidaemia, oedema, proteinuria, fever, thrombocytopenia
Albano et al. [48]	2009	139/139	20 & 23	Delayed wound healing, infections
Lebranchu et al. [55]	2009	95/192	16	Aphthous ulcers, diarrhoea, acne, hypertriglyceridemia
Chan et al. [52]	2008	92/92	9.8	Hyperlipidaemia, oedema, ATN/AKI, renal vein thrombosis, effusions, wound drainage, anaemia, leucopoenia, thrombocytopenia, infections (UTI, wound, pneumonia), GI disturbance
Ekberg et al. [49]	2007	401/ 1645	53.2	GI disturbance, Infections, DM, hyperglycemia, hyperlipidemia, hypercholestrolemia, hypertriglyceridemia, Hypophosphatemia
Larson et al. [58]	2006	81/165	36	Poor wound healing, pulmonary complications, sever hypertriglyceridemia, thrombocytopenia, acute rejection

Table 5: Discontinuation rate of mTOR inhibitors and the reported side effects [49,50-64,66-68,70,81,86].

Coupled with it are the frequent adverse reactions necessitating discontinuation of their use. Many studies have showed their high discontinuation/withdrawal rate among patients on mTOR-I reaching up to 53.2%. Proteinuria, primary glomulonephritis, and poor graft function were associated risk factors for withdrawal of mTOR-I, as shown in a recent report on 77 renal transplant recipients in Taiwan [106]. Also, GI symptoms were frequently described among mTOR-I users (Table 5). The presence and severity of GI side effects, among transplant recipients, appear to be associated with lower quality of life [107]. These had often been underestimated by the treating clinicians as shown in a survey conducted by Ekberg et al. [108,109]. Table 5 summarizes the discontinuation rate among patients treated with mTOR-I, together with the commonly encountered side effects. Various studies were examined for discontinuation of mTOR-I secondary to side effect. Included are studies in which all patients received mTOR-I, and other studies involving additional/comparative treatment agent (as expressed as number of patients on mTOR-I/ overall number of patients). High discontinuation rate was shown throughout most of the different studies conducted in the last decade.

Limitation of the Study

An important limitation of this study is that the majority of the studies had a short follow-up of 12 months or less. Few numbers of studies examined the effect of mTOR-I at 2-3 years and only two studies looked at a longer term follow-up at 5 and 8 years respectively. Longer term efficacy and safety of mTOR-I in kidney transplantation is still poorly understood.

Conclusion

Immunosuppressive protocols containing mTOR-I are generally effective in low risk renal transplantation, with a comparable graft survival and a better eGFR, compared to conventional protocols. However, we have limited reports on their efficacy in high-risk groups. They are of particular interest in specific groups of patients including those with malignancies, encapsulating peritoneal sclerosis, BK and CMV virus infections. They were shown to have unacceptable side effect profile leading to high discontinuation rate. These might have contributed to their reduced popularity (both as early and maintenance) in the recent years. Moreover there is a new concern linking their use to increased patients' mortality. More studies, looking into the safety of mTOR-I, are needed.

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