

m-TOR Inhibitors in the Current Practice

Fakhriya Alalawi^{1,2*}, Ajay Sharma^{2,3} and Ahmed Halawa^{2,4}

¹Department of Nephrology, Dubai Health Authority, UAE

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

³Royal Liverpool University Hospital, UK

⁴Sheffield Teaching Hospitals, Sheffield, UK

* **Corresponding author:** Fakhriya J Alalawi, Department of Nephrology, Dubai Hospital, Dubai Health Authority, UAE, Tel: 00971509787597/0097402195000; E-mail: fjalalawi08@yahoo.co.uk

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Abstract

for long time, calcineurin-inhibitors (CNIs) was the best broadly used immunosuppressant in organ transplantation; since it had proven its efficacy in the reduction of acute rejection episodes and early graft loss, though their use in the long-term, are associated with chronic allograft nephropathy (CAN), besides it increases the cardiovascular risks such as diabetes, hypertension, and dyslipidemias. Moreover, CNIs in combination with other immunosuppressant's increases the risk of fatal infections and malignancy. Ultimately the introduction of the mammalian focus of rapamycin (mTOR) inhibitors, such as sirolimus and everolimus in 1990s, had changed the face of transplantation and conveyed a great hope to transplant physicians as an innovative class of effective immunosuppressants with a unique mechanism and less nephrotoxic effects (1-6).

In this review article we will try briefly to elicit the clinical indications, advantage and disadvantage of m-TOR inhibitors over other immunosuppressant's medications and their clinical indications inside and outside the transplant field.

adverse effects of immunosuppressant's, with allograft loss mainly due to chronic allograft nephropathy (CAN) [1,3].

CAN describe the inevitable steady decrease in renal capacity with time; characteristically renal biopsy shows tubular atrophy, interstitial fibrosis, glomerulosclerosis and vascular occlusive changes [1,3].

Other causes of renal allograft loss beside CAN, are death from cardiovascular diseases, infections or malignancy in patients with a functioning graft, which explains the remaining 50% of graft losses.

Unpredictably, with utilization of strong immunosuppressant's medications; the occurrence of de novo malignancies had expanded rapidly, which was attributed to the direct oncogenic impacts of these medications.

The introduction of m-TORI; sirolimus and everolimus in the field of transplantation had brought a great hope to transplant physicians as they have distinctive method of activity and different adverse effects profile (i.e. lower nephrotoxicity, less hypertension and perhaps a protective probability against neoplastic activities) than CNI therapy [1-6].

Mode of Action of m-TOR Inhibitors

Sirolimus (Rapamune®/rapamycin) is a lipophilic microcyclic lactone antibiotic, segregated from a strain of fungus called *Streptomyces hygroscopicus*, found at Rapa Nui in 1969 [7,8]. Though it was created as an antifungal drug against *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*, it was found later to have an immunosuppressive and anti-proliferative activities that led to its launch as a major immunosuppressant against transplant rejection [1,5-9].

Sirolimus is the prototype of the first generation of mTOR inhibitors and resemble in its structure tacrolimus structure, as it fixes to the immunophilin FK binding protein-12 (FKBP-12), to form an immunophilin complex (catalyst). The objective of this SRL-FKBP-12 complex is the serine-threonine kinase of the phosphatidylinositol-3-kinase pathway, which is called mTOR

Keywords: m-TOR Inhibitors; Sirolimus; Everolimus; CNI; Chronic Allograft Nephropathy (CAN).

Introduction

Renal transplantation remains the best treatment approach for most patients with end-stage renal disease. It yields superior life quality compared with dialysis therapy.

With the great advances in the medical field and the introduction of new and potent immunosuppressant medications, the incidence of acute rejection episodes had declined markedly over the last decade to 5-20% in the first post-transplant year; yet the graft half-life remained practically unaffected [1,2]. Reasons behind that were attributed to the

and acts through the co-stimulatory and cytokine-driven pathways, to prevent the signal III pathway (G1 to S transition, interpretation, and cytokine-driven T-cell multiplication) [1,4-10]. The serine/threonine kinase mTOR is a downstream effector of the PI3K/AKT pathway, and forms two distinct multiprotein complexes, mTORC1 and mTORC2 (figure 1). These two complexes have a separate network of protein partners, feedback loops, substrates, and regulators [11].

It appears that growth factors, amino acids, ATP, and oxygen levels regulate mTOR signalling. Several downstream pathways that regulate cell-cycle progression, translation, initiation, transcriptional stress responses, protein stability, and survival of cells are signalling through mTOR [12].

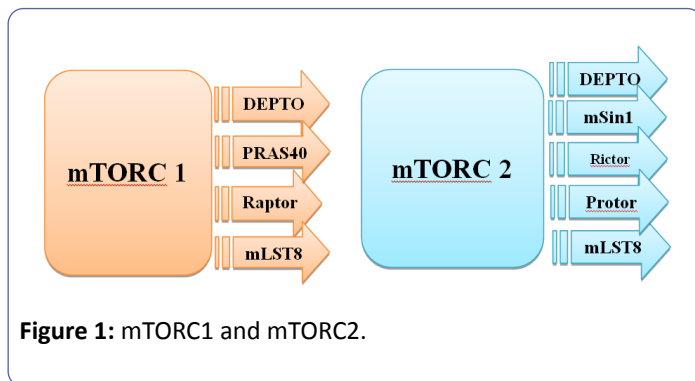


Figure 1: mTORC1 and mTORC2.

mTORC1 consists of mTOR and two positive regulatory subunits, raptor and mammalian LST8 (mLST8), and two negative regulators, proline-rich AKT substrate 40 (PRAS40) and DEPTOR [13], while mTORC2 consists of mTOR, mLST8, mSin1, protor, rictor, and DEPTOR [14]. mTORC1 is sensitive to rapamycin but mTORC2 is considered to be resistant and is generally insensitive to nutrients and energy signals. mTORC2 is activated by growth factors, and it phosphorylates PKC α , AKT and paxillin, and regulates the activity of the small GTPase, Rac, and Rho related to cell survival, migration and regulation of the actin cytoskeleton. The activity of this complex is regulated by rapamycin, insulin, growth factors, phosphatidic acid, certain amino acids and their derivatives (e.g., l-leucine and β -hydroxy β -methylbutyric acid), mechanical stimuli, and oxidative stress [12].

The mTORC1 signalling cascade is activated by phosphorylated AKT and results in phosphorylation of S6K1, and 4EBP1, which lead to mRNA translation [13].

In contrast to SRL, everolimus (EVR/RAD/Certican) is considered as a derivative of sirolimus, structurally looks similar to SLR but with additional extra hydroxyethyl chain substitution at position-40 on the sirolimus molecule, which make it more hydrophilic and bioavailable than sirolimus (figure 2) [1,4,5,7-15].

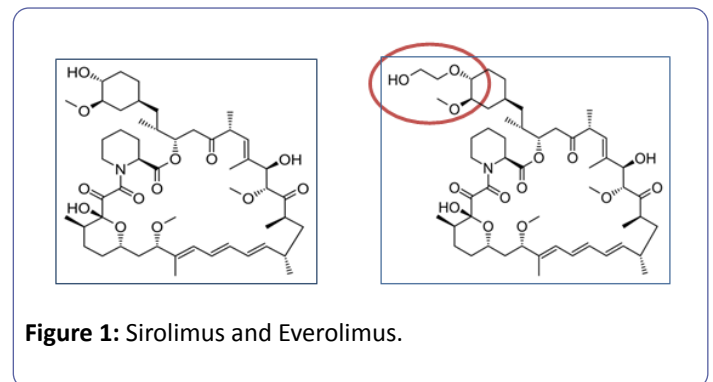


Figure 1: Sirolimus and Everolimus.

Similarly EVR/FKBP-12 multiplexes acts directly to mTOR, leading to inhibition of T-cell progression from the G1 to the S stage of cell cycle, thus results in inhibition of IL-2-induced protein synthesis and cellular proliferation and inhibition of IL-4-dependent multiplications of T- and B-cells [1,4,15,16]. Moreover, B-cell activation, proliferation, differentiation into antibody-producing cells, and antibody release are also inhibited [5,6,17]. Indirectly, mTOR inhibitors (sirolimus and RAD) inhibits effector functions of CD4- helper cells and CD8- cytotoxic cells, and activate monocytes, macrophages and other pro-inflammatory leukocytes [5,6,17].

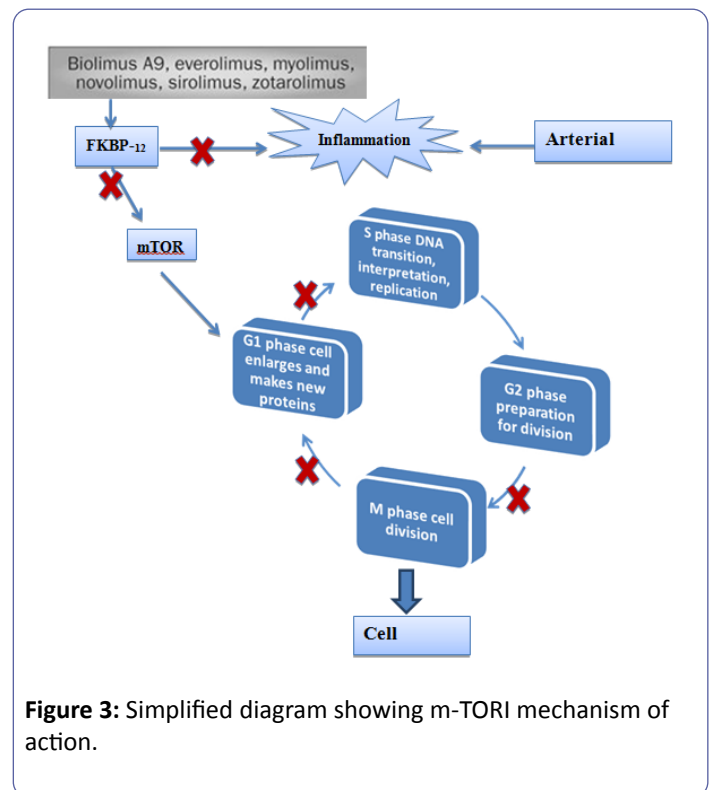


Figure 3: Simplified diagram showing m-TOR1 mechanism of action.

Sirolimus has shown an affinity for FKBP12 that is 2 times greater than tacrolimus and 3.3 times greater than RAD [1,5].

Each of these inhibitors can work synergistically if added to CNIs, although their pharmacokinetic and pharmacodynamic characters can differ between the two drugs owed to the minor differences between their chemical structures.

Pharmacokinetics and Pharmacodynamics m-TOR I in Renal Transplantation

Sirolimus is rapidly absorbed in the intestines, with median T_{max} (time-of-maximal-concentration) of 0.5 to 2 hours. It is metabolized by both cytochrome P450 IIIA4 (CYP3A4) and P-glycoprotein, which explains the interactions with other drugs and high-fat meals and their metabolites are eradicated through gastrointestinal tract (91%) and kidneys [7,18-21].

The maintenance dose for sirolimus is usually 2 to 5 mg/day, aiming at trough levels of 5 to 15 ng/ml. SRL has a very long half-life (nearly 62- 65 hours), so trough level monitoring should be done 5 to 7 days after initiating the medication, which makes once daily dosing probable. In contrast to cyclosporine A, sirolimus trough concentrations are stable over time and strongly correlate with 24-hour exposure (area under the curve [AUC]). There is no significant effect on C_{max} (maximal concentration) or AUC by sex, age, or ethnic origin [19,20,22].

However, cyclosporine A increases sirolimus bioavailability by 240% when administered simultaneously and by only 80% when administered 4 hours apart. On the other hand, tacrolimus does not have the similar pharmacokinetic interactions with sirolimus as cyclosporine, though (as cyclosporine) it may enhance the adverse effect of sirolimus (including hemolytic uremic syndrome/thrombotic thrombocytopenic purpura/thrombotic microangiopathy (HUS/TTP/TMA) if given concurrently. In a crossover study, simultaneous and separate (by four hours) administration of sirolimus and tacrolimus were compared, and no significant interactions were found in pharmacokinetic parameters, including AUC and C_{max}, hence combination of sirolimus and tacrolimus can be taken concurrently while administration of cyclosporine should be separated four hours apart from sirolimus [21,23,24]. Moreover, cyclosporine should be administered at lower doses with lower target serum cyclosporine concentrations when given with sirolimus [24].

Similarly, co-administration of diltiazem, verapamil, fluconazole, ketoconazole, anticonvulsants and rifampicin can alter the AUC of sirolimus [5,7,23].

Sirolimus should be taken consistently either with or without food. Grapefruit juice should be avoided since it can unpredictably alter sirolimus pharmacokinetics. Oral tablets of sirolimus are not bioequivalent to the oral solution [23].

Contrary, EVR shows a slightly greater AUC, with half-life of 30 hours compared to sirolimus, hence twice-daily dosing is necessary. Equally to sirolimus; it is metabolized by liver and intestinal cytochrome P-450 enzyme CYP-3A, therefore it is affected by drugs and dietary changes.

Pharmacokinetics of EVR are less affected by cyclosporine than sirolimus, therefore it's possible to be administered with cyclosporine or tacrolimus simultaneously [5]. Beyond that, the efficacy of both drugs appears to be similar, as well as their toxicity profiles [7,25-28].

Liver disease significantly increases sirolimus/everolimus bioavailability, reducing its clearance and prolonging its elimination half-life [23].

There are numerous clinical trials of m-TOR-I since the time it was approved for practice in the renal transplant field; various regimens had appeared aimed at minimizing CNI or eliminating them. Accordingly three CNI-sparing strategies: CNI withdrawal, CNI minimization, and CNI avoidance have been studied [29]; here we summarize the most famous clinical trials used with mTOR inhibitors:

Table 1: Trials of CNI elimination using sirolimus in kidney transplantation.

Trial Name & method	Time of conversion	Results
SMART: is an observational study, included 132 patients followed for 36 months, the aim was to compare early conversion to SRL while watching such effect on eGFR	Early conversion of CNI to SRL at 10-24 days	<ul style="list-style-type: none"> After 36 months, renal function was better in SRL-group (ITT-eGFR at 36 months: 60.88 vs 53.72 [CsA] ml/min/1.73 m², P=0.031), however, many patients had to stop the treatment in the SRL group 59.4% vs 42.3% (CsA). Survival at 36 months was excellent for both groups (99% [SRL] vs 97% [CsA] and graft: 96% [SRL] vs 94% [CsA]).
Rapamune Maintenance Regimen [30] is a prospective, open-labeled, randomized, multicenter trial, included 525 patients with aim to compare early conversion to SRL while watching the effect on cGFR over 24 months	Early conversion of CsA to SRL at 3 months	<ul style="list-style-type: none"> At 24 months, the differences in patient survival (94.0% vs 95.3%), graft survival (91.2% vs 93.5%), BPAR after randomization (5.1% vs 9.8%) or discontinuations (34% vs 33%) for SRL-CsA-ST vs SRL-ST, respectively, all were not statistically significant SRL therapy resulted in significant long term improvement in graft survival and BP without increased risk of graft loss or late acute rejection, Calculated GFR (43.8 vs 58.3 mL/min, P<.001).
Spare-the Nephron [31] is a prospective, open-label, randomized, multicenter trial, includes 305 patients, examined early withdrawal from CNI-based therapy post transplantation while maintaining patients on MMF together with SRL. Patients were followed for a median time of 519 days.	Early withdrawal of CNI at 4-12 weeks	<ul style="list-style-type: none"> Patients who were maintained on MMF/CNI for ≤6 months and then converted to maintenance immunosuppression with MMF/SRL had greater improvement in mGFR vs patients who remained on MMF/CNI. The incidence of BPAR was significantly greater with MMF/SRL (12.2%) vs MMF/CNI (4.1%, P=0.02). Graft loss occurred in 3.4% of the MMF/SRL-treated patients and in 8.3% of the MMF/CNI-treated patients (P=0.04). Malignancies were seen less frequent with MMF/SRL. Withdrawal for adverse events was 34.2% of the MMF/SRL-treated patients and for 24.1% for MMF/CNI-treated patients (P=0.06).

<p>CONCEPT [32] is a prospective, open-labeled multicenter trial, included 237 patients, evaluated for early conversion from a CsA-regimen to a SRL-regimen, measuring eCrCl (Cockcroft and Gault) at 52 weeks</p>	<p>Early conversion at 3 months from CNi to SRL</p>	<ul style="list-style-type: none"> CrCl at 52 weeks was superior in SRL group (68.9 vs 64.4 mL/min, P=0.017), though patients and grafts survival were statistically insignificant. The frequency of acute rejection, had occurred mostly following steroids withdrawal, however, although it was numerically higher but statistically insignificant in the SRL group (17% vs 8%, P=0.071). 	<p>ASCERTAIN [35] is an open-label, multicenter RCT aimed at CNi elimination or minimization while introducing EVL, patients included (N=394 ITT; 127 CNi elimination vs 144 CNi minimization), all followed for 24 months.</p>	<p>Late conversion (>6 months, mean follow-up 5.6 years)</p>	<ul style="list-style-type: none"> No overall renal benefit of EVL was shown, adverse events and discontinuations were more frequently noted in the EVL group, however, Post hoc analyses showed that patients with CrCl >50 mL/min at baseline had considerably greater increase in mGFR after CNi eradication vs controls (variance of 11.4 ml/min/1.73 m² vs 20.8 ml/min/1.73 m², P=0.17).
<p>CONVERT [33] 830 patients were randomly assigned to continue CNi (n:275) or to be converted from CNi to SRL (n:555), Endpoint were GFR (Nankivell), rates of BPAR, allograft loss, or death at 12-24 months.</p>	<p>Late conversion from CNi to SRL (6-12 months).</p>	<ul style="list-style-type: none"> ITT analysis of the primary endpoint failed to demonstrate any significant benefit of SRL conversion group over CNi-group, however, among SRL converted-group; patients with GFR >40 ml/min/1.73 m² at baseline were accompanied with superior patient and graft survival, while there was no difference in acute rejection episodes and had lower incidence of neoplasia compared with CNi group, as demonstrated by retrospective analysis of binary outcome for the overall stratum. Superior renal function was observed in SRL-group that persisted through 12-24 months, particularly those with baseline GFR >40 ml/min/1.73 m² and UPr/Cr ≤0.11. An unexpected finding of new onset proteinuria had appeared with an increase in the preexisting proteinuria following conversion to SRL. 	<p>CENTRAL [36] is an open-label, multicenter RCT, included 202 patients, followed-up for 36 months.</p>	<p>Early conversion of CsA to EVL at 7 weeks</p>	<ul style="list-style-type: none"> Conversion from CsA to EVL at 7th weeks was accompanied with significant enhancement in mGFR at 36 months vs CsA-treated patients, although drug withdrawals and BPAR episodes were more common There was no overall advantage in an intent-to-treat population.

Table 2: Trials of CNi elimination using everolimus in kidney transplantation. Till date there is no head to head trial comparing everolimus to sirolimus in renal transplant recipients, and therefore there is no evidence for superiority of either drug on the other.

Trial Name & method	Time of conversion	Results
<p>ZEUS [34] is an open-label, multicenter RCT, includes 300 patients, followed up for 12 months.</p>	<p>Early conversion of CsA to EVL at 4.5 months</p>	<ul style="list-style-type: none"> Everolimus therapy was accompanied with an improvement in GFR compared to cyclosporin (71.8 ml/min per 1.73 m² vs 61.9 ml/min per 1.73 m², respectively). BPAR Rates were greater in the everolimus group than cyclosporin after randomisation (15 [10%] of 146; p=0.036), Early replacement of CNi with everolimus-based therapy had improved renal function at 12 months and preserved efficacy and safety.

Recommendations for Conversion to m-Tor Inhibitors in Renal Transplantation

There is no special protocol for conversion to m-TOR I in renal transplant, however, proteinuria, frequency of rejection episodes that precedes conversion, higher chronic Banff score and high score of vascular intimal thickening were connected with non-responder in a univariate investigation [19]. Diekmann had proposed conversion for CAN at creatinine less than 2.5mg/dl and proteinuria beneath 800 mg/day [22].

Current literature had suggested the likely beneficial evidences for conversion to SRL-based regimen if there are: [1,4,29]

- CNi- related symptoms, for example, nephrotoxicity, arterial hypertension and diabetes mellitus.
- Early chronic transplant dysfunction, however, GFR should be more than 40 mL/min with normal urinary protein excretion.
- Possibility of tumor recurrence/occurrence with the administration of CNi.

On the other hands; the possible contraindications for conversion to SRL-based regimen are:

- Hyperlipidemia with serum cholesterol >300 mg/dl and/or serum triglycerides >400 mg/dl, despite using lipid-lowering agents.
- Advanced renal failure with elevated serum creatinine that exceeds 4 mg/dl.
- Glomerular damage with proteinuria >1 g/day at baseline.

The optimum time for early conversion is about 3 months post-transplant.

Notably; The administration of SRL in high-risk group, like those with diabetes mellitus, body mass index >30 kg/m², major re-operations or delayed graft function, should not occur until four to six weeks post transplantation, after the wound had

healed and serum creatinine level stabilized at 2.5 mg/dl or below.

The dosage of MMF/AZA must be decreased by 50% at the beginning of SRL therapy while steroid treatment might be continued [1].

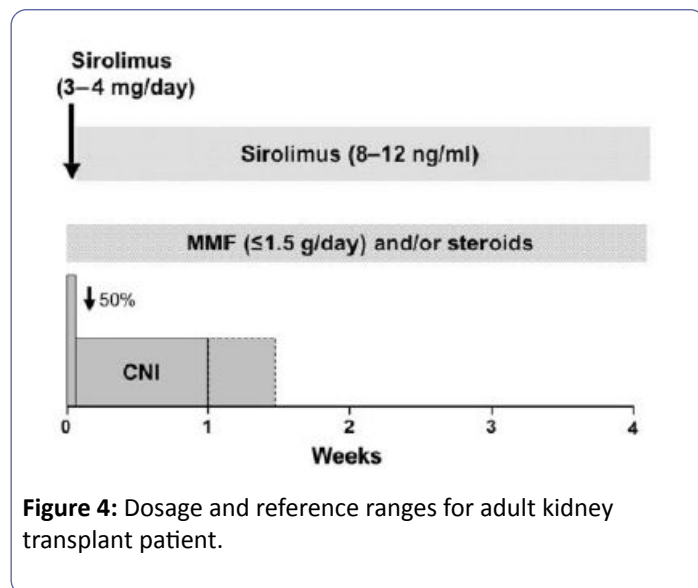
The CNI dosage must simultaneously decreased by 50% and it can be ceased totally when the SRL trough level reach 5–10 ng/mL. Further trough level measurements are suggested weekly after each extra dose adjustment [1].

Additional trough level estimations are suggested for patients with liver impairment or when using particular substances that might hinder CYP3A4 and/or P-gp metabolism.

Two SRL conversions protocols can be used;

- Abrupt stop of CNI therapy with a single loading dose of sirolimus of 10mg followed by 5 mg/day in African-Americans or a loading dose of 8 mg followed by 4 mg/day in Caucasians [37].
- To introduce sirolimus at a dose of 3-4 mg/day with a synchronous decrease of CNI dosage to 50%. Once target level had achieved (8-12ng/ml), then CNI can be withdrawn totally, commonly in 7-10 days [38,39].

Note: Mycophenolate dose should be ≤ 1.5 g/day [40].



The initial oral sirolimus maintenance dose is 2 to 5 mg daily. Subsequent doses are based on the attainment of therapeutic trough concentrations [1,23].

Table 3: Sirolimus Trough Concentration (ng/mL).

	≤ 3 months	>3 months
(When sirolimus is used with tacrolimus or cyclosporine +/- MMF/mycophenolic acid and steroids):	8-12	5-10

(When sirolimus is used as a single agent +/- steroids)	10-12	8-10
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For everolimus, an initial dose regimen of 0.75 mg twice daily concurrently with ciclosporin is recommended for the general kidney and heart transplant population, while a dose of 1.0 mg twice daily in co-administration with tacrolimus is recommended for the hepatic transplant population. Subsequent doses are tailored based on blood concentrations level, initial adjustments can be made at 4-5 day intervals after initiating therapy. Recommended everolimus range to be 3 to 8 ng/mL for renal and liver transplant patients [8,23,26].

Table 4: suggested target CNI trough concentration windows with m-TOR I therapy in renal transplant recipients. CO levels monitoring is reliable measure with mTORI therapy [41].

Period	Month 1	Months 2-3	Months 4-5	Months 6-12
Target cyclosporin Co (ng/ml)	100-200	75-150	50-100	25-50
Target tacrolimus (ng/ml)	3-7	3-7	3-7	3-7

Clinical Advantages of mTOR-inhibitors Therapy over Other Immunosuppressants

Antitumoral activity

Immunosuppressants in conjunction with other oncogenic stimuli, such as viruses and radiation can be a major risk for development of certain cancers like Kaposi sarcoma, lymphoma and skin cancers, which is considered the leading reasons for morbidity and death after solid organ transplantation. There is, however, persuading evidence that avoiding CNIs and uses of mTOR-inhibitors can convey advantages with respect to the progression of malignancy [2,19,25,42,43], this was proven on several studies, e.g. patients in CONVERT study who were converted to sirolimus; had a diminishment in the rate of malignancies compared to the individuals who stayed on CNI at 12 and 24 months (3% vs 10%) [1,4,32,33], similarly Campistol JM et al, had reported successful recession of Kaposi's sarcoma and PTLD with no alteration in renal function after substitution of CNI with mTORIs [44].

mTOR-I has cytostatic effect without cytotoxic activities, so the clinical outcome is stabilization of the tumor cells rather than progression.

The mechanism by which m-TOR-I can incite this anti-tumor activity is related to up-regulation of adhesion molecules and to a change to less aggressive phenotype of tumoral cells, as appeared with Luan et-al. who found that SRL could modify tumor cells from aggressive phenotypes like spindle- or dome-shaped cells to a less aggressive cuboidal form in renal cell carcinoma [45].

Moreover, m-TOR1 can block growth-factor-stimulating cell proliferation of haematopoietic and non-haematopoietic cells (CNS, hepatocytes, renal, melanocytic, osteoblastic, myogenic, fibroblasts and endothelial cells) by creating a complex with the intracellular immunophilin FKBP-12. Furthermore it inhibits proliferation of T and B cells altered by HTLV-1 and EBV [1,15].

In the pre-clinical models; m-TOR1 had exhibited growth inhibition of tumoral cells by increasing expression of E-cadherin and p27-kip1, also it decrease cyclin-d1 expression, causing arrest in cellular progression from G1 to S-phase [1,4,6,7,15]. Recent reports demonstrated that sirolimus inhibits UV-B activation of several metalloproteinases that can enhance tumor development and premature skin aging [34].

Lower rate of viral disease after transplantation

It was observed that the rate of viral infections (particularly cytomegalovirus and BK virus infections) are much lower in patients taking mTOR-inhibitor-based regimens than other immunosuppressant, which is true for both sirolimus and everolimus and in all recipients of kidney and heart grafts whether anti-viral prophylaxis were given or not [2].

Everolimus showed a profound inhibitory effect on human Epstein-Barr virus and lymphoblastoid B-cell lines in vitro [5].

Effect of mTOR inhibitors on cardiovascular disease

Cardiovascular disease is known to have a great influences on morbidity and mortality for kidney transplant recipients, risk factors (such as hypertension, left ventricular hyperplasia, hyperlipidemia and Post-transplant diabetes mellitus (PTDM)) has been linked greatly with CNI use [2,4], and by replacing/minimizing them with mTOR-I we can reduce such risk factors.

Additionally, conversion from CNI-therapy to mTORi-based regimen had improved blood pressure significantly as shown in the RMR study and may achieve regression of left ventricular hyperplasia [39].

Although hyperlipidemia is considered to be a major concern in patients converted to mTOR-inhibitors, with highly elevated cholesterol and triglycerides levels, with subsequent increased use of lipid-lowering agents [2]; yet the long-term follow-up in most studies does not suggest an increased association between mTOR-I's and cardiac events. Indeed, pre-clinical studies had suggested a beneficial effect of mTORIs on atherosclerosis with favorable effects were found in human cardiac transplantation, in which SRL has been shown to reduce the progression of allograft vasculopathy [4].

Experimental studies have shown that SRL could be effective in reducing aortic atherosclerosis in apo-E-deficient mice independent of lipid levels [2,4].

Clinical Indications of m-TOR I in Non-Transplant Patients

Anticancer therapies

Tumors may occur due to dysregulation of mTOR signals and can confer higher susceptibility to mTOR inhibitions.

Deregulations of multiple elements of the mTOR pathway, like PI3K amplification/mutation, PTEN loss of function/mutation, AKT mutations/amplifications, and S6K1, 4EBP1, and eIF4E overexpression have been related to many types of cancers. Both eIF4E and S6K1 are included in cellular transformation and their overexpression has been linked to poor cancer prognosis [46,47]. Therefore, mTOR is an interesting therapeutic target for treating multiple cancers alone or in combination with inhibitors of other pathways.

Currently m-TOR-I (rapamycin and several rapalogs, including temsirolimus, everolimus, and ridaforolimus which are the first generation mTOR inhibitors) are being widely investigated outside the transplant field for their anti-proliferative properties on certain kinds of tumors, including glioma, non-small cell lung cancer, breast cancer, rhabdomyosarcoma, B- and T-cell lymphoma, multiple myeloma, colonic carcinoma, pancreatic cancer, hepatocellular carcinoma, ovarian cancer, endometrial carcinoma, renal cell cancer, prostatic cancer, bladder cell carcinoma and melanoma, however, their use remained limited within experimental field [1,48]. Probably this is related to the fact that rapalogs are primarily cytostatic, and therefore effective as disease stabilizers rather than for regression, hence the response rate in solid tumors where rapalogs have been used as a single-agent therapy have been modest [49-52]. Another reason for the limited success is the presence of feedback loop between mTORC1 and AKT in certain tumor cells. It seems that mTORC1 inhibition by rapalogs fails to suppress a negative feedback loop that results in phosphorylation and activation of AKT [48,53-55]. These limitations have led to the development of the second generation of mTOR inhibitors known as ATP-competitive mTOR kinase inhibitors [55].

Coronary stent coating

The antiproliferative effect of m-TOR I has also been used in conjunction with coronary stents to prevent restenosis in coronary arteries following balloon angioplasty. The sirolimus or everolimus is formulated in a polymer coating that affords controlled release through the healing period following coronary intervention.

Several large clinical studies have demonstrated lower restenosis rates in patients treated with sirolimus-eluting stents when compared to bare-metal stents, resulting in fewer repeated procedures [56].

Moreover, sirolimus-eluting stents (SES) were found to have superior results compared to everolimus-eluting stents (EES), which were associated with greater angiographic in-segment late loss and higher rates of in-segment restenosis compared

with SES implantation, though clinical outcomes were both excellent and not statistically significant [57].

Lymphangiomyomatosis

(LAM) is a rare, progressive and systemic disease, predominantly affects women of childbearing age and typically resulting from proliferation in the lung, kidney, and axial lymphatics of abnormal smooth muscle-like cells (LAM cells) that exhibit features of neoplasia and neural crest origin. Cystic destruction of the lung with progressive pulmonary dysfunction and presence of abdominal tumors (eg, angiomyolipomas [AML], lymphangiomyomas) characterizes the disease. It usually occurs as a feature of an inherited tuberous sclerosis complex (TSC-LAM), with mutations of the tuberous sclerosis complex gene (*TSC2*), though sporadic forms of the disease (S-LAM) are present. Loss of *TSC2* gene function activates the mTOR signaling pathway, resulting in the release of lymphangiogenic growth factors and this can be blocked with m-TOR I.

Sirolimus was approved by the Food and Drug Administration for their use in LAM in May 2015, based on the results of Multicenter International LAM Efficacy and Safety of Sirolimus (MILES) Trial. The MILES data supports the use of sirolimus in patients who have abnormal and rapidly declining lung function (i.e. FEV1<70% predicted). Sirolimus also appears to be effective for the treatment of chylous effusions. The benefits of sirolimus only persist while treatment continues, so the safety of long term therapy must be addressed in further studies [58-60].

Sirolimus is the only and first drug therapy approved by the FDA in the management of lymphangiomyomatosis (LAM).

Neurological Indications

Tuberous sclerosis complex and epilepsy

Sirolimus/Everolimus shows a promising role in treating tuberous sclerosis complex (TSC), elicited on several studies that conclusively linked mTOR inhibitors to remission in TSC tumors, specifically subependymal giant-cell astrocytomas in children and renal angiomyolipomas in adults, and pulmonary lymphangiomyomatosis [61-64]. Further trials are ongoing, till then their uses will remain off-label [46], moreover, tumors were often re-grow when the treatment stopped.

Topical sirolimus therapy (applied in different formulations such as ointment, gel, solutions, and creams, ranging from 0.003 to 1% concentrations) was used for treatment of facial angiofibromas with positive outcomes results reported in sixteen separate studies involved a total of 84 patients and improvement was observed in 94% of subjects, especially if treatment began during the early stages of the disease [47,65].

In addition to the known role of the mTOR in tumorigenesis and the associated utility of mTOR inhibitors for treating tumors in TSC, the importance of mTOR in the common, disabling neurological symptoms of TSC, in particular epilepsy, autism, and cognitive deficits, is not well established and appear to be unrelated to tumor growth per se, but probably linked to other cellular and molecular abnormalities, such as aberrant circuit

formation and dysregulated neurotransmitter receptors or ion channels. However, whether mTOR inhibitors represent a rational treatment for seizures and neuropsychiatric symptoms in TSC patients, this has not been prompted yet in the clinical practice [63]. Furthermore, there is increasing interest as to whether the mTOR pathway may be involved in other types of epilepsy (other than genetic epilepsies), such as epilepsy following acquired brain injury. There is some evidence that mTOR inhibitors can inhibit existing seizures or can prevent epilepsy in some animal models of acquired epilepsy, though other studies have found negative results [65-69].

Non-TSCrelated brain tumors

In addition to SEGAs in TSC, the mTOR pathway has been implicated in the pathophysiology of other brain tumors unrelated to TSC, particularly other types of gliomas [65]. The mechanism of the antitumor effects of rapamycin against gliomas is still being investigated, but may include direct cytotoxic and antiproliferative effects, inhibition of vascular endothelial growth factor and angiogenesis, decreased invasive propensity, and increased sensitivity to radiation [70].

Neurodegenerative diseases

M-TOR pathway has been implicated in a number of neurological conditions including structural brain disorders, inherited neurocognitive disorders such as fragile X syndrome, autism spectrum disorder, and classical progressive neurodegenerative diseases such as Alzheimer disease, Parkinson's and Huntington disease which characterized by accumulation of abnormal toxic proteins and associated neuronal death.

Since mTOR pathway is involved in regulation of cell death and survival mechanisms, it makes sense that mTOR has also been implicated in the pathophysiology of these neurodegenerative disorders. In particular, mTOR signaling may modulate the mechanisms of apoptotic cell death. Furthermore, mTOR pathway normally inhibits autophagic mechanisms, which help degrade and clear aggregated or accumulated proteins. Thus, mTOR inhibitors may represent a rational therapy and protective agents for this group of diseases by inducing autophagy or by directly regulating neuronal death mechanisms. However their use is limited currently to animal's models. Moreover, significant side effects that may occur with mTOR inhibitors, including chronic immunosuppression and associated opportunistic infections, make this prospect of longterm treatment less attractive [65].

Muscular dystrophy in mice

Duchenne muscular dystrophy progresses rapidly in males to severe impairment of muscle function and death in the second or third decade of life. Bibee et al had demonstrate an alternative approaches that rescue defective autophagy in mdx mice, a model of Duchenne muscular dystrophy, with the use of rapamycin-loaded nanoparticles induce a reproducible increase in both skeletal muscle strength and cardiac contractile performance that is not achievable with conventional oral

rapamycin, even in pharmacological doses. This increase in physical performance occurs in both young and adult mice, and, surprisingly, even in aged wild-type mice. Although the exact mechanism responsible for rapamycin's entry into muscle tissue remains to be clarified, the effect on autophagy is clear and seems dependent on NP-linked depot delivery, because oral therapy is ineffective at the doses that were administered [71,72].

Progeria (Hutchinson–Gilford Progeria Syndrome (HGPS))

Progeria or aging disease is an extremely rare genetic disorder in which symptoms resembling aspects of aging and manifested at a very early age leading to an extremely compromised cell-damage repair capacity and typically resulting in death in the early teenage years due to causes which are generally associated with old age such as heart disease or stroke.

A recent discovery that rapamycin suppresses a pro-senescent phenotype in progeric cells in addition to clearance of progerin. Rapamycin in theory would suppress geroconversion downstream of progerin. Furthermore, rapamycin prevents atherosclerosis in animal models of accelerated atherosclerosis and accelerated atherosclerosis is one of the main symptoms of progeria leading to death [73].

Others genetic diseases

Tuberous sclerosis, Peutz-Jeghers syndrome, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, Lhermitte-Duclos disease, Proteus syndrome, von Hippel-Lindau disease, Neurofibromatosis type1 are all caused by mutations in the mTOR pathway component genes *TSC1*, *TSC2*, *LKB1*, *PTEN*, *VHL*, and *NF1* respectively, hence addition of m-TOR I can play a promising role in such diseases [74].

Systemic lupus erythematosus in murine lupus models and human

Sirolimus decreases the disease activity and prednisone requirement in systemic lupus erythematosus (SLE) patients who are intolerant or resistant to immunosuppressant medications.

Sirolimus acts through blocking the activation of its molecular target, the mechanistic target of rapamycin complex 1 (mTORC1). The mTOR pathway is involved in many aspects of T cell differentiation and function. The activation of mTORC1 is associated with suppression of mTORC2, results in the expansion of pro-inflammatory CD4-CD8- double-negative (DN) T lymphocytes. These DN-T cells produce inflammatory cytokines, interleukin-4 (IL-4) and interleukin-17, and they exhibit predisposition to pro-inflammatory cell death through necrosis. Moreover, Increased IL-4 production is responsible for activation of autoantibody-producing B lymphocytes in SLE. Rapamycin treatment in vivo blocked the IL-4 production and necrosis of DN T cells, increases the expression of FOXP3 in CD25(+)/CD4(+) T cells, and expanded CD25(+)/CD19(+) B cells. These results

identify mTOR activation to be a trigger of IL-4 production and necrotic death of DN T cells in patients with SLE.

It is possible that rapamycin treatment resulted in recovery of IL-2 production along with normalization of calcium signaling, creating a cytokine milieu more favorable to regulatory T cell function. Rapamycin also has been demonstrated to promote an anti-inflammatory environment by affecting the behavior of dendritic cells. M-TOR inhibition leads to complementary changes in myeloid dendritic cells and T cells leading to the production of regulatory T cells. This regulatory T cells together with CD4+CD25-cells increased STAT3 and STAT5 phosphorylation and enhance the differentiation of CD8+ T cells into memory cells in mice inoculated with lymphocytic choriomeningitis virus. Prospective clinical trial in SLE patients with sirolimus is ongoing [74-76].

Scleroderma

m-TOR can play a role in fibrotic diseases and autoimmunity, and blockade of the mTORC pathway through blocking the pro-fibrotic effects of TGF- β can play a part in the treatment of scleroderma (or systemic sclerosis), however, this approach is currently under investigation [77].

Glycogen storage disease (GSD)

In a trial done in a canine model of glycogen storage disease (GSD) IIIa, had suggested that rapamycin can inhibit mTORC1, so that the phosphorylation of GS (glycogen synthase) can be increased in skeletal muscle and glycogen content in primary muscle cells can be reduced significantly in patients with GSD IIIa through suppressing the expression of glycogen synthase and glucose transporter 1, therefore rapamycin had prevented effectively progression of liver fibrosis in GSD IIIa dogs, consistent with markedly inhibiting the transition of hepatic stellate cells into myofibroblasts, the central event in the process of liver fibrosis. This discovery represents a potential novel therapeutic approach for glycogen storage diseases (for GSD III) [78-79].

ADPKD

Sirolimus has been assessed as a therapeutic option for autosomal-dominant polycystic kidney disease (ADPKD). Different case reports demonstrated sirolimus as a key role in reducing kidney volume in patients with early-stage ADPKD and delaying decline in kidney function compared with those receiving standard care. However, the current clinical trials are limited with confounding results [79-81].

Familial cardiac hypertrophy and Wolff-Parkinson-White syndrome

An essential mediator of cardiac myocyte enlargement is protein synthesis, which is controlled at the levels of both translation-initiation and elongation. Eukaryotic elongation factor-2 (eEF2) mediates the translocation step of peptide-chain elongation and is inhibited through phosphorylation by eEF2 kinase. In addition, p70S6 kinase can regulate protein synthesis

by phosphorylating eEF2 kinase or via phosphorylation of ribosomal protein S6. Phosphorylation of eEF2 kinase is also controlled by AMP-activated protein kinase (AMPK), a key regulator of cellular energy homeostasis. Dependent on AMPK-priming phosphorylation of tuberin, GSK3 β phosphorylates tuberin and triggers activation of its potential to inhibit mTOR. AMPK γ 2, an important regulatory subunit of AMPK, is encoded by the gene *PRKAG2*. Mutations in *PRKAG2* are responsible for familial cardiac hypertrophy and Wolff-Parkinson-White syndrome. The knowledge that an important hallmark of cardiac hypertrophy (a major risk for cardiac morbidity and mortality) is hyperactivation of the PI3K-mTOR cascade, initiated a discussion whether inhibition of the mTOR pathway could be beneficial in treating such disorders [79,82,83].

The mTOR pathway in obesity and type 2 diabetes

Recent studies had linked insulin resistance in type 2 diabetes mellitus and obesity to mTOR Complex1 pathway since it is controlled through nutrient-hormonal signaling network.

Insulin resistance can be regulated by mTORC1 activation of p70S6K through the negative feedback loop. p70S6K has been demonstrated to phosphorylate IRS1 on multiple inhibitory sites promoting its degradation. Inhibition of IRS protein function will desensitize cells to insulin. Phosphorylation of IRS1, which is known to antagonize IRS signaling, is elevated in animal models of obesity and in muscle from type 2 diabetic patients. Insulin resistance is the hallmark for both, obesity and type 2 diabetes [79,84,85].

In HIV

In vitro and in vivo studies suggest sirolimus to possess anti-HIV properties that may qualify it as a potential new anti-HIV drug. Sirolimus inhibits HIV replication through different mechanisms, including down-regulation of the co-receptor CCR5 and induction of autophagy. In addition sirolimus synergistically enhances the anti-HIV activity of entry inhibitors such as vicriviroc, aplaviroc and enfuvirtide in vitro.

Additionally, a prospective nonrandomized trial of HIV patient series receiving RAPA monotherapy after liver transplantation indicated significantly better control of HIV and hepatitis C virus (HCV) replication among patients taking RAPA monotherapy [86].

Anti-Aging properties

Rapamycin was first shown to extend lifespan in eukaryotes in 2006 by Powers et al. who showed a dose-responsive effect of rapamycin on lifespan extension in yeast cells. Based on this and other researchers, in 2009, Harrison De et al had demonstrated a significant increase in the lifespans of genetically heterogeneous mice fed with rapamycin with increase in median survival by 14% for females and 9% for males, and he attributed this to retarding ageing mechanisms or probably to delaying death from cancer. Life span lengthening was confirmed on subsequent studies, and is now being tested in the marmoset monkey and dogs [87-91].

It is hypothesized that dietary regimes such as caloric restriction and methionine restriction, causes lifespan extension by decreasing mTOR activity and it is believed that this is achieved by limiting the essential amino acid leucine and methionine, which are potent activators of mTOR. The administration of leucine into the rat brain has been shown to decrease food intake and body weight via activation of the mTOR pathway [61,62,87,92].

Moreover, and based on the free radical theory of aging, reactive oxygen species cause damage of mitochondrial proteins and decreases ATP production. Subsequently, via ATP sensitive AMPK, the mTOR pathway is inhibited and ATP consuming protein synthesis is down-regulated, since mTORC1 initiates a phosphorylation cascade activating the ribosome. Hence, the proportion of damaged proteins is enhanced. Additionally, disruption of mTORC1 directly inhibits mitochondrial respiration. These positive feedbacks on the aging process are counteracted by protective mechanisms: decreased mTOR activity (among other factors) upregulates glycolysis and removal of dysfunctional cellular components via autophagy [64,93,94].

However, this remains within experimental field and it is not known whether rapamycin will have similar lifespan-lengthening effects in humans.

Side Effects of m-TOR Inhibitors

Majority of m-TORI clinical trials have been done in renal transplant patients using SRL. However, it seems reasonable that EVR can induce similar adverse effects. Apparently that time, drug dosage and high drug trough levels might have a primary role in the development of drug-related adverse effects and clinical complications.

M-TOR-inhibitors have considerable adverse effects that can limit its use in some patients. Nearly 30-50% of patients on SRL therapy have to discontinue the therapy due to these related adverse effects [1,17].

Reported Side Effects of m-TORI Includes

- Hypertension in 8 -58% of cases [3,15].
- Fever (23% to 34%), chest pain, headache, insomnia, fatigue, Arthralgia, alterations in taste, and asthenia are common side effects and can be managed by reducing the drug dose [1,3,15].

Hyperlipidemia

Hyperlipidemia was reported in 8% to 57%,

M-TORI increases high-density lipoproteins (HDL), low density lipoproteins (LDL), cholesterol, and triglycerides in approximately 40 to 75% of patients receiving this therapy, it affects the up-regulation of adipocyte fatty acid-binding protein (aP2) expressed in monocytes and macrophages which plays a key role in the increased accumulation of triglycerides [3,15].

Hyperlipidemia is the principle risk factor for post-transplant cardiovascular-related morbidity and mortality with a yearly

hazard appraisal of 50-fold more prominent for renal transplant recipients than for the general population, that is responsible for over one-third of all deaths, however, some authors (as mentioned earlier) claims that the long-term follow-up in patients getting m-TOR1 does not propose an expanded risk for cardiac events. Indeed few had suggested further beneficial benefits of mTORIs on atherosclerosis.

Joannides et al. have demonstrated that SRL-based regimen reduces aortic stiffness, plasma endothelin-1, and oxidative stress in renal recipients proposing a defensive impact on the arterial wall with reduction in the total cardiovascular risk, However, extra studies are necessary to evaluate long-term cardiovascular effects of mTOR-I therapy in renal transplant patients [4,15, 42].

Hyperlipidemia with m-TOR1 is reversible and dose dependent, as shown in Morrisett et al. study; where he observed that cholesterol and triglyceride levels increases 2–4 weeks after initiation of therapy, and reverted to near-baseline levels within 8 weeks after discontinuation of treatment [3,15]. Hyperlipidemia in m-TOR1-based regimen can be treated with statins therapy alone or in combination with a second line agent.

Gastrointestinal Effects

Reported in approximately 15–20%, and include stomatitis, abdominal pain, anorexia, nausea, vomiting, diarrhea, constipation and abnormal LFT [42].

Mucositis and oral ulcerations are the commonest side effects reported with mTOR-I therapy, occurs probably secondary to the direct toxic effect of these drugs on oral and nasal mucous membranes.

Mouth ulcers typically are transient and emerge soon after administration of SRL. It could be treated with topical steroid, iodine, or topical analgesic. If symptoms persisted, mTOR-I ought to be ended and possibly restarted at a lower dosage after resolution of symptoms.

Gastrointestinal leukocytoclastic vasculitis is a rare complication reported in a few cases with use of SRL. It is characterized by diffuse mucosal thickening of the antrum, duodenum, and jejunum. Drug discontinuation is necessary if occurs [1,3,15,29].

Increased Infection Risk

Reported in 2-60% in various literatures, occurs early in the post-conversion period and could be attributed to the high loading dosages [1,3,4,15]. Severe bacterial infections can occur in m-TOR-I group, secondary to inhibition of interleukin-12-induced proliferation of activated T lymphocytes and IFN creation of the lymphocytes. Both cytokines are known to be critical in the defensive insusceptibility to intracellular bacteria (e.g., mycobacterium).

Though bacterial infection is common with m-TOR1, studies had suggested a protective effects against viral infections particularly CMV infection and possibly BK infection, which

could be related to drug inhibitory effects on viral replication [15].

Hematological Effects

Bone marrow toxicity often occurs in patients treated with mTOR-inhibitors, such patients develop anemia, leukopenia, and thrombocytopenia.

Anemia (13-58%), can occurs as early of schedule as 1-month in the SRL-post-conversion group, thereafter, hemoglobin levels typically settled, but at the detriment of incessant utilization of Erythropoietin in around 50% of patients [3,4]. Similarly EVR therapy has been associated with anemia as well [15].

Various mechanisms have been anticipated for mTOR1 induced anemia, one mechanism proposed the anti-proliferative effect of the drug on bone marrow progenitor cells and possible direct impact on iron homeostasis, other mechanism suggested that mTOR inhibition is responsible for blocking S6 kinase action (S6 kinase assumes a part in mRNA interpretation in the cell), and consequently it hinder cell replication in erythroid cell lines and alter erythroid cell development.

Additionally Thauat et al. had reported in 2005 a close relationship between chronic inflammatory status and mTOR-I-related anemia. Equally, others reported low serum iron levels and micro-inflammation in patients got converted from CNI to EVR. An alternative hypothesis of the myelosuppressive influence of SRL is due to the inhibition of the signal transduction via the gp-130 [beta] chain shared by a variety of cytokine receptors, including interleukin-11, granulocyte colony stimulation factor, and erythropoietin, which stimulate the production of platelets, leukocytes, and erythrocytes, respectively [3,15].

Leukopenia was noticed in 5-39% with both SRL and EVR, necessitating the use of granulocyte colony-stimulating factors in few patients [3-5,15].

Significant thrombocytopenia was reported in 10%-20% of renal transplant recipients and thought to be dose dependent. Thrombocytopenia is reversible within 2 weeks after discontinuation of the drug; most of those require dose reduction and few needs temporary drug withdrawal. No patient requires permanent discontinuation of therapy [5,15].

TTP/TMA/HUS:

m-TOR I may increase the risk of CNI-induced haemolytic uraemic syndrome/ thrombotic thrombocytopenic purpura/ thrombotic microangiopathy if used in combination with CNI [26].

Respiratory Symptoms (2-11%)

Several varieties of pulmonary injury have been reported in the literatures, including lymphocytic interstitial pneumonitis, lymphocytic alveolitis, bronchiolitis obliterans with organizing pneumonia, focal pulmonary fibrosis, dyspnea and cough.

A standout amongst the most serious symptoms is the development of SRL- induced interstitial pneumonitis. This is usually dose dependent, with an onset of symptoms between 1 to 51 months after the initiation of SRL/EVR therapy. Diffuse alveolar hemorrhage has been accounted too.

The pathogenic mechanism of pulmonary toxicity is still unclear. A cell-mediated autoimmune response was suggested. Lung biopsy had shown several histological features such as intra-alveolar non-necrotizing epitheloid granuloma, lymphocytic interstitial inflammation, and focal pattern of organizing pneumonia [3,4,15,29].

Post-Transplantation Diabetes (3-33%)

To date, just a small number of clinical studies had claimed that m-TOR-I can cause PTDM, the mechanisms are not clearly understood, though, a few speculations were made: [15,42]

- SRL can induce ectopic triglyceride deposition prompting insulin resistance.
- Impairment of insulin-mediated suppression of hepatic glucose generation.
- Direct toxic impact on pancreatic cells.
- AKT activation with impairment of insulin receptor substrate signaling.
- Another interesting hypothesis is that mTOR is involved in insulin signaling, and its inhibition may impede insulin related gene transcription and expression, including glucose transporters, leading to inhibition of GLUT1mRNA increase, resulting in failure of insulin to stimulate glucose uptake.
- Similarly, mTOR is an inducer of ribosomal S6 kinase (S6K), and SRL blocks S6K activation or induces S6K inactivation through inhibition of T389 phosphorylation interfering with the transcript of insulin. This action may impact blood sugar levels.

Wound Complications

in up to 20-50% of patients, which is significantly higher compared with other immunosuppressive drugs. These include wound dehiscence, lymphocele development, delayed wound healing, incisional hernias, and infections.

Lymphocele formation has an incidence that varies according to different publications, e.g. in (Langer and Kahanshows) study it had reached up to 38% of patients [3,15].

Wound complications are likely identified with SRL capacity to debilitate signal transduction of fibroblast and endothelial growth factors.

Risk factors for wound complications includes older age recipients, obesity, Caucasian race, diabetics, corticosteroid utilization, and higher dose of SRL at early post-transplant period.

Approaches to diminish wound complications consist of avoidance of loading doses, and modification of surgical techniques, for example, suction drain position, usage of non-absorbable sutures while closing, and perhaps delay the

introduction of the drug for several weeks particularly for the high risk group (patients with a BMI > 30, Caucasian, and elderly) [1,3,4,15,29].

Lymphedema

Lymphedema is a relatively unusual adverse consequence of mTOR-I treatment. The underlying mechanism is not totally clarified.

Aboujaoude et al. have hypothesized that the lymphedema could be connected with increased lymph flow along with lymphatic interruption in the affected extremities secondary to the surgical procedures, in addition to the well-identified effect of SRL in increasing vascular permeability and vasodilation [95].

Moreover, Huber et al. had demonstrated an anti-lymphangiogenic activity of mTOR-I, which is not restricted to a specific mTOR-I, rather it is a general phenomenon of the whole class.

Before establishing the diagnosis of lymphatic disease caused by mTOR-I, it is essential to rule out other causes such as neoplasia, infection, and venous obstruction. Unfortunately, reduction or discontinuation of the drug therapy is the only treatment for such patients [4,15].

Cutaneous Adverse Effects

This includes lymphedema (described above), acne, epistaxis, vasculitis and nail disorders [1,3,15]:

- Acne, folliculitis: Acne is reported in 15% to 25% in recipients treated with SRL. Predominantly in male patients suggesting hormonal etiology.
- Chronic peripheral edema (8% to 64%), affecting primary lower limbs.
- Angioedema (acute subcutaneous edema), found in 15% of patients, developed within few hours and disappeared in less than 4 days. They were non-pruritic, non-erythematous, and localized mainly on the face, with oral cavity involvement.
- Nail involvement: includes fragile and thin nails, longitudinal ridging, distal onycholysis, and erythema.
- Skin and scalp hair abnormalities include mild alopecia or hypertrichosis of the face.

These adverse events were not serious in most cases; however, in 12% of patients it was necessary to withdraw the therapy [1,3,15].

Gonadal Effects

There are several publications highlighted the effects of m-TORI particularly SRL on male gonads, these studies have focused on sex hormones production, erectile function, and fertility.

It was found that SRL therapy had significantly lowered testosterone levels while it had increases the gonadotrophic hormones (FSH and LH). Additionally, a substantial reduction of total sperm quantity was noticed.

Nevertheless, patients receiving a sirolimus-based regimen had a significant decreased fathered pregnancy rate, compared with those administered sirolimus-free regimen (5.9 vs 92.9 pregnancies/1000 patient-years). Thus, men who desire to father children must be well educated of the risks and benefits associated with exposure to sirolimus. The underlying mechanisms still remains obscure. Feng et al. had found that SRL assumes a focal inhibitory part on a stem cell factor (SCF)/c-kit-dependent process in spermatogonial proliferation via the PI3-K/AKT/p70S6K pathway in the animal models [96-100].

In females, sirolimus may cause infertility, menstrual irregularities, secondary amenorrhea, and increases in luteinizing hormone and follicle-stimulating hormone. Boobes et al. were the first to demonstrate the suppressive effect of SRL on the ovaries of renal transplant recipients. Further study by Yu et al. had proved that inhibition of mTORi in granulosa cells and ovarian follicles resulted in compromised granulosa proliferation and reduced follicle growth. Moreover, Yu and his colleagues detected increased fraction of cells that underwent anomalous mitotic events during mTOR inhibition in animal models [100].

The potentiality for everolimus to cause infertility in male and female patients is not known yet, however, male infertility and secondary amenorrhea have been observed [95].

Renal Nephrotoxicity

Although m-TOR-I were considered for long time as non-nephrotoxic drugs, however, experimental studies had shown that mTOR-inhibitors can influence renal structure and function, though their nephrotoxicity is much less than CNIs nephrotoxicity [1,4,29].

Following effects were noted with m-TOR-I:

1. mTOR-inhibitors can impairs recovery from ischemia in animal models of renal transplantation.
2. mTOR-inhibitors has synergistic effects if combined with CNI, and can worsen the CNI toxicity.
3. Delayed graft function is often seen with mTOR-inhibitors. Literatures have reported that patients on SRL stayed on dialysis for longer time compared to others and this was attributed to increase frequency of acute tubular necrosis, however, recent literature suggested that the hazard risk may be lower with EVR, due to the differences in pharmacokinetics between each and the lack for the loading doses in EVR.
4. New-onset proteinuria has been accounted for in two-thirds of patients converted to SRL. Histological lesions in biopsied patients resemble focal segmental glomerulosclerosis; in spite of the fact that it is not clear whether this is recurrent primary kidney disease, chronic allograft damage or happens as a result of CNI withdrawal and/or SRL introduction, therefore baseline proteinuria should be measured before any change regimen. It is conceivable that withdrawal of CNIs prompts hyperfiltration on the grounds that these medications have constrictive impacts on the renal vasculature. Reasons for proteinuria caused by SRL therapy could be explained by

hemodynamic changes, podocyte injury, tubular dysfunction and antagonism of vascular endothelial growth factor.

5. Recent literatures had suggested that mTOR-I-induced proteinuria through reduction of glomerular nephrin expression in transplanted patients. It's advisable to add either ACEIs or ARBs when convert patients from CNIs to sirolimus for their vasoconstrictive properties on glomerular efferent arterioles [1,3,4,29,42].
6. Everolimus has similar effects on increasing proteinuria, and reversibility has been observed when patient discontinued the medicine and switched back to CNI therapy [26,27].
7. Renal graft thrombosis: An increased risk of kidney arterial and venous thrombosis, resulting in graft loss, has been reported, mostly within the first 30 days post-transplantation [26,27].
8. Tubular dysfunction: biochemical disorders like hypokalemia, hypophosphatemia, hypocalcaemia, and hypouricaemia was noted in some patients following switching from CNI to sirolimus, suggesting chronic tubular toxicity. Sirolimus therapy might alter tubular handling of K, phosphate and uric acid by renal tubules [1,3,15]. Renal magnesium wasting and potentially significant hypomagnesemia was also noted. **Viorica Bumbea** et al, interestingly had found that the renal phosphate threshold and uric acid clearance were altogether lower in patients taking sirolimus compared to those taking everolimus, despite similar PTH levels [3].

Special Considerations

Vaccinations

Immunosuppressants may affect the response to vaccination, rendering it to be less effective. Usage of live vaccines should be avoided [26].

Impact of mTORi on pregnancy

There are inadequate data regarding the use of m-TOR I in pregnant women. Studies in animals have shown reproductive toxicity including embryo/foetotoxicity. The potential risk for humans is unknown.

There are only 11 cases of transplanted women who received SRL during pregnancy were reported in literatures, hence the real safety has not yet been determined and there is no real existing data to strongly confirm that SRL/EVL are teratogenic. In one of those cases, SRL treatment was introduced at 24 weeks of pregnancy and the infant was born with cleft lip-palate and microtia. The first reported case of successful delivery in a renal-transplant female receiving SRL (in association with CNI) through all gestation was reported by Guardia et al. in 2006, subsequently in 2008 and 2011 two cases of successful delivery in a renal transplant females treated with SRL were reported by Chu et al. and Framarino-dei-Malatesta respectively. With regards to EVR there is, as SRL, little experience about its role

during pregnancy. Only two cases of successful pregnancies in 2 kidney-transplant recipients treated with EVR during pregnancy were reported. All these cases demonstrate that either SRL or EVR might not be an absolute contraindication for pregnancy, however, due to limited available data most nephrologists prefer to avoid m-TOR I therapy completely during pregnancy and advise to be discontinued and switched to CNI at least 8-12 weeks prior to attempted conception unless the potential benefit outweighs the potential risk for the fetus [24,26-28,100].

Impact of mTORi on breast-feeding

Due to the potentiality for adverse reactions in the nursing infant, breast-feeding is not recommended by the manufacturer during therapy and for 2 weeks following the last dose [26,27].

Conclusion

mTOR-inhibitors are very promising immunosuppressive agents, It has different mode of action with different adverse effect profile and can be used as de novo therapy or as a substitute immunosuppressant mainly to CNI.

The promising effect of reduced nephrotoxicity compared to CNI, hypertension and lower incidence of post-transplant malignancy in addition to the lower viral rate infections; all had make them a good alternative to CNI therapy, however, delayed wound healing, lymphoceles and hematological disorders remained a great challenge with this therapy.

Furthermore, mTOR I has different implication outside transplant field, though their use remained off labeled in most. There is accumulating evidence that the mTOR pathway is involved in the pathophysiology of a number of neurological, genetic and inflammatory diseases that brand mTOR I as promising and novel agent in such diseases, however in most; this evidence is derived from basic science and animal models.

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