REVIEW ARTICLE

Inhibition of the Mechanistic Targets of Rapamycin Beyond Transplant Immunosuppression: A Mini-Review

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ABSTRACT

It has been about thirty years since identifying the target of rapamycin and using mTOR inhibitors in organ transplantation. There was a promise that they would replace calcineurin inhibitors with fewer nephrotoxic effects and better graft survival. Unfortunately, with time, the enthusiasm for using them in transplantation decreased due to the unpleasant profile of adverse events and limited evidence of tangible clinical benefit.

With more understanding of physiology and judicious clinical application, new venues for mTOR inhibitors emerged. The mTOR pathway regulates primary cellular functions, including cell growth, metabolism, proliferation, and survival, and is critical for autophagy induction. Thus, as a master regulator, mTOR inhibitors emerged as anticancer therapies. In addition, such action proved beneficial for native or post-transplant malignancies.

Signaling through components of the mTOR pathway is an essential regulator of normal cardiac growth and pathological hypertrophy. mTOR inhibitors are effective in reducing left ventricular thickness and mass. It could be an add-on benefit in kidney transplant recipients with high cardiovascular risk or attenuate cardiac allograft vasculopathy in heart transplant recipients.

The mTOR inhibitors may help manage viral infections like cytomegalovirus, human herpesvirus 8-related Kaposi sarcoma, and possibly the BK virus. Furthermore, the mTOR pathway is modulated in many RNA viruses. Based on these facts, the idea of using mTOR inhibitors to treat COVID-19 infection has been evaluated. Accordingly, a new therapeutic role for mTOR inhibitors for treating COVID-19 infection t has emerged through reducing viral replication, and autophagocytosis, improving T cells function and preventing cytokine storm.

This paper will review these applications of mTOR inhibitors beyond the horizon of transplant immunosuppression.

Keywords: Autophagy, Cancer, mTOR inhibitors, Transplant, Viral infection

Background:

The mammalian target of rapamycin (mTOR) is a serine/ threonine kinase that regulates many eukaryotic cellular functions. It is found in the cell as part of 2 multi-protein complexes: mTOR complex 1 (mTORC1) and mTORC2. mTORC1 is implicated in protein synthesis, lipid metabolism, cellular growth, and proliferation. Stimulation of mTORC1 blocks autophagy. mTORC2 controls cell survival, cytoskeleton organization, lipogenesis, and gluconeogenesis. 1,2,3. Figure 1.
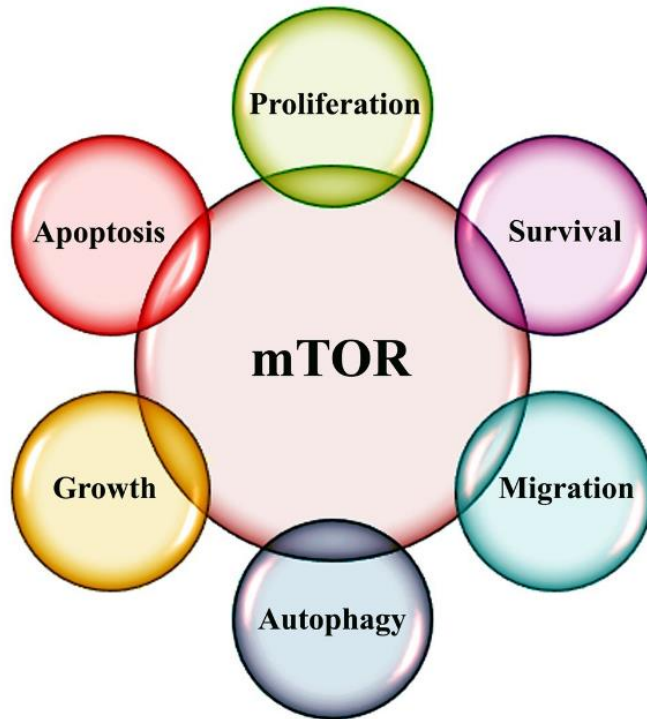

Figure 1: Various Functions of mTOR signaling. 3

Dysregulation of the mTOR pathway occurs in various human pathologies, including neurological disease, cancer, diabetes, and cardiac disease. 4 Therefore, several mTOR inhibitors have been developed, including rapamycin (Sirolimus) and its analogs. 5

Sirolimus is a natural macrolide; it was isolated from a strain of fungus called Streptomyces hygroscopicus and was initially recognized for its potent anti-fungal activity. Later, it is shown to be a potent immunosuppressant and antiproliferative agent. In post-transplantation patients, sirolimus is used clinically to prevent kidney and heart rejection and was the first mTOR inhibitor approved for kidney transplantation. 1,6

Everolimus is structurally similar to sirolimus, with an extra hydroxyl-ethyl chain in position 40. However, it has better oral bioavailability and was approved for solid organ transplantation by the US Food and Drug Administration in 2010. 1

Temsirolimus is a prodrug of sirolimus with better stability and solubility; it is available as an intravenous drug and was approved by Food and Drug Administration in 2007 for treating renal cell carcinoma and by European Medicines Agency in 2009 for relapsed and refractory mantle cell lymphoma. 7

Deforolimus or Ridaforolimus has been proposed to treat estrogen-receptor-positive breast tumors and other solid cancers. 8

Another class of mTOR inhibitors, known as TORKinibs, seem to have better antiproliferative properties. This class has been tested in animal cancer and mouse heart transplantation models. 9

mTOR inhibition in organ transplantation:

In 1990, mTOR inhibitors appeared as a new class that could replace calcineurin inhibitors (CNI) in kidney transplantation. Initially, they were believed not to cause nephrotoxicity. Furthermore, this class has antitumor potential and inhibitory effects on vascular smooth muscle proliferation that may prevent chronic rejection. mTOR inhibitors have been evaluated for use in kidney transplantation as an addition to CNI-based therapy and as a substitute for CNI. 10

It has been reported that mTOR inhibitors will suit the best risk/benefit profile for elderly kidney transplant recipients if added to low-dose CNIs and steroids after induction with thyroglobulin. [11] The famous ELITE-Symphony Study reported a higher rejection rate in renal transplant recipients under mTOR inhibitors–based regimen without CNI compared with standard CNI-based therapy. [12] However, the TRANSFORM study revealed that everolimus was non-inferior to mycophenolic acid (MPA) in kidney transplant recipients with mild to moderate immunological risks. [13]

Several side effects limited the use of mTOR inhibitors. These include pulmonary toxicity, hematological disorders, dysmetabolism, lymphedema, stomatitis, cutaneous adverse effects, and gonadal toxicity. [14]

From a nephrocentric perspective, proteinuria is a detrimental side effect of mTOR inhibitors. It is observed in 20-40% of kidney transplant recipients and is associated with poor graft survival. [15] Although how mTOR inhibitors induce or exacerbate proteinuria is not fully understood. Some studies have shown that mTOR is crucial to maintain glomerular podocyte morphology and function. Sirolimus and everolimus may alter the integrity of the actin cytoskeleton, decrease cell adhesion, disturb podocyte function, and lead to podocyte necrosis. It has also been suggested that mTOR inhibition is associated with reduced VEGF secretion and blockage of the VEGF signaling pathway. A disrupted VEGF balance at the podocyte level could contribute to proteinuria. [15,16]

Emerging Uses of mTOR Inhibitors:
   I.   Autophagy:

Autophagy is a genetically regulated intracellular nutrient recycling process involving lysosomal degradation of unwanted cellular proteins and defective organelles. In other words, It plays a critical role in cellular homeostasis. [17,18,19] mTOR critically regulates the balance between growth and autophagy in response to cellular physiological conditions and environmental stressors. mTORC1 has been described as a central negative regulator of autophagy; the role of mTORC2 in the regulation of autophagy is less studied despite mounting evidence that this complex may regulate autophagy differently from that of mTORC1. [20]

Autophagy is often upregulated in cardiomyocytes within a failing heart. Due to insufficient protein degradation, numerous forms of HF are implicated by the accumulation of aberrant proteins. [21] Rapamycin is cardioprotective in pressure-overloaded and ischemic heart diseases by regulating the mTOR signaling pathway. In chronic HF models, sirolimus reduced cardiomyocyte apoptosis and promoted autophagy. This is through regulating the crosstalk between the mTOR and the endoplasmic reticulum (ER) stress pathways. [22]

Sirolimus is a potent inhibitor of animal and human vascular smooth muscle cell proliferation and migration and significantly affects cell-matrix synthesis. Such a potential will be reflected in the inhibition of neointimal hyperplasia in rapamycin-eluting stents. [23] Interestingly, despite their related risk of increasing serum lipids, mTOR inhibitors are associated with an overall lower risk of atherosclerosis. In addition, sirolimus reduces oxidized-LDL adhesion and uptake to endothelial cells and can promote its autophagic degradation. [24]

There are conflicting reports on mTOR inhibitors' efficacy in reducing the LV mass index in kidney transplant recipients. However, the favorable signal for sirolimus came from reducing cardiac fibroblast proliferation and collagen secretion. Furthermore, most studies may have been underpowered to detect differences in cardiovascular outcomes in kidney transplants. [23,24] In addition, everolimus was very effective in preventing neutrophil adhesion and cytokine release in heart transplants, which are essential for developing and progressing cardiac allograft vasculopathy. [24,25]

Recently, increasing attention has been given to a potential link between abnormal lysosomal function, neurodegeneration, and the induction of autophagy as a potential therapeutic target for neurological diseases. Diseases that share the aggregation of pathogenic proteins are potential targets, like Huntington's disease, Parkinson's disease, and Alzheimer's disease. [26]

It is interesting to have therapeutic approaches that achieve the balance between the activation of MTOC1 and MTORC2 and, in turn, maintain the balance between autophagy and cell survival. Figure 2.
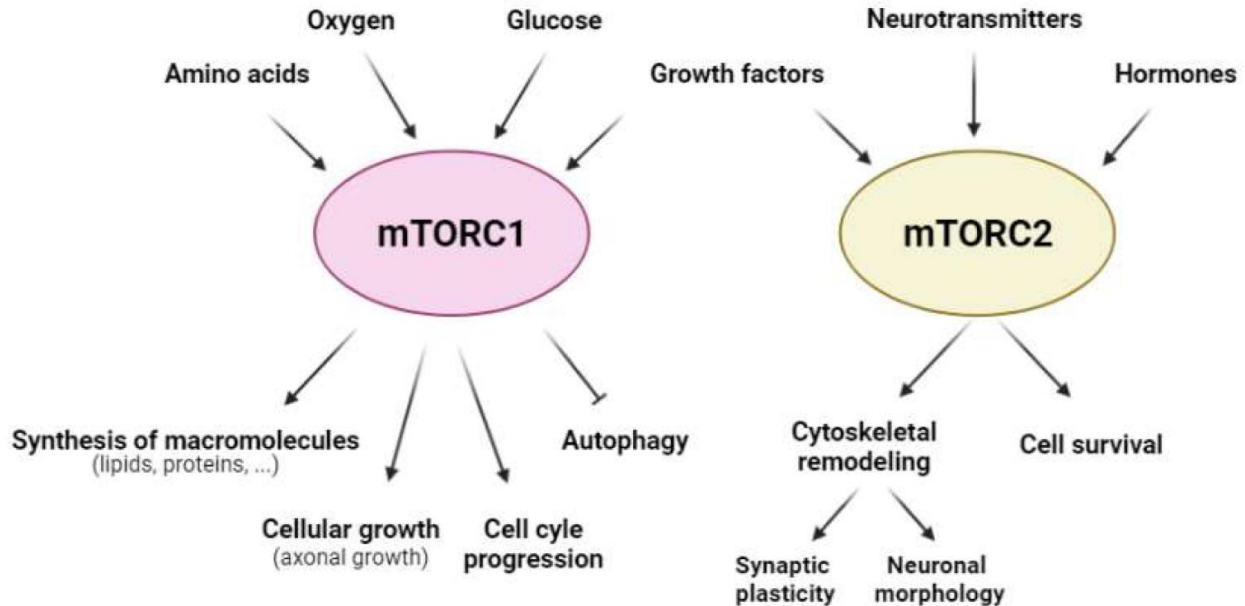
Figure 2: Differential actions and consequences of activation of mTOR complexes 1 and 2. 27

II. Anticancer therapy:

While mTOR is necessary for normal human physiology, cancer cells take advantage of mTOR signaling to drive their neoplastic growth and progression. 28 mTORC1 pathway activation has been reported in a wide variety of solid tumors; therefore, drugs that bind mTORC1 selectively and specifically were anticipated to hinder cancer cell metabolism and downstream protein and lipid synthesis, thereby eliciting anticancer effects. 6 Studies indicate that the mTOR signaling pathway can promote the occurrence and progression of tumors by regulating autophagy and apoptosis of tumor cells.

A. Non-transplant-associated malignancies:

mTOR inhibitors have shown clinical efficacy against many solid malignancies. 29 For example, mTOR inhibitors represented a window opportunity for non-small cell lung cancer patients who benefited from temsirolimus, some patients had a confirmed partial response, and 27% had stable disease. 30 In addition, everolimus and sirolimus showed apparent G1 cell cycle arrest effects and suppressed proliferation in gastric cancer cell lines. 31,32

The US FDA has authorized many renal cell carcinoma (RCC) drugs. Temsirolimus and everolimus partially inhibit mTORC1 activation, leading to modest survival benefits in advanced RCC patients, according to the results of the phase III Global ARCC trial. 33,34

As reported from a phase II study, everolimus demonstrated mild antitumor effects in metastatic urinary bladder cancer (UBC) patients

resistant to chemotherapy. 35 In another phase II study, only a small portion of patients with advanced UBC responded to everolimus. 36 It seems that rapalogs utilized as a monotherapy are not as effective as expected in the treatment of urinary bladder cancer.

mTOR signaling activation is associated with enhanced tumor progression, invasion, and frequently reduced survivability in breast cancer patients. Everolimus has been proven to treat hormone receptor-positive, HER2-negative breast cancer. In addition, mTOR inhibitors have been utilized with other immune therapies in many clinical trials of breast cancer treatments. 37,38,39

Rapamycin, initially regarded as a specific inhibitor of mTORC1, was found to suppress both mTORC1 and mTORC2 in head and neck squamous cell carcinoma (HNSCC) cells. Moreover, in a study of newly diagnosed HNSCC patients, rapamycin achieved effectiveness, as most patients responded, and one patient got a complete response. 40

Tuberous-Sclerosis Complex (TSC) is an autosomal dominant disorder that involves multiple human systems and presents with complex clinical features. Activation of the mTOR signaling pathway plays an essential role in the multisystem involvement of TSC. 41 Renal angiomyolipoma (AML) is a common cause of TSC-related mortality. The CAST trial was the first prospective clinical trial that evaluated the therapeutic effects of rapamycin in patients with AML. After 12 months of treatment, the mean AML volume decreased to 53.2%±26.6% of the baseline value. 42 In the EXIST-2 trial, the response rate of AML to everolimus was 42% compared with the placebo, demonstrating the

remarkable efficacy of everolimus in treating AML. [43]

B. Transplant-associated malignancies:

The incidence of most common malignancies such as lung, breast, prostate, and colorectal cancers is 2-4 fold higher in kidney transplant recipients compared to the general population. [44] In the CONVERT trial, a CNI-free regimen with sirolimus was associated with a significantly lower overall incidence of malignancies; after the 2-year follow-up, the incidence of Nonmelanoma skin cancers (NMSCs) was also low. [45]

NMSC is the most common malignancy in kidney transplant recipients. It affects around half of the transplant recipients. [46] However, in a single-center randomized controlled trial, renal transplant recipients with pre-malignant skin lesions who were switched to a sirolimus-based regimen, less progression of these lesions were noted and even regression in some cases. [47] In addition, randomized controlled trials have documented reduced squamous cell carcinoma (SSC) burden with sirolimus treatment. However, the recurrence-free survival was restricted to patients with only one previous invasive cutaneous SCC and was observed up to 1 year and two years after conversion, respectively. [46,48] Furthermore, switching CNI to sirolimus has led to complete histological remission of post-kidney transplant Kaposi's sarcoma with preservation of graft function. [49]

Although everolimus and sirolimus have been shown to inhibit the growth of human PTLD-derived cell lines and Epstein–Barr virus-transformed B lymphocytes, the evidence is limited to support the use of mTOR inhibitors in the management of PTLD. Adding Phosphoinositide 3-kinases (PI3Ks) inhibitors or an antiapoptotic protein kinase pathway (Akt) inhibitor can be more efficacious than rapamycin alone for treating EBV-associated PTLD while simultaneously promoting allograft survival. Such action is possible by inhibiting the constitutive activation of multiple nodes within the PI3K/Akt/mTOR pathway in EBV+ PTLD-derived cell lines. [50,51]

III. Polycystic kidney disease:

Studies confirmed the role of mTOR in cyst pathogenesis, and mTOR inhibitors have been shown to slow cyst development in animal models. [52] However, randomized controlled trials of mTOR inhibitors in non-transplanted ADPKD patients have failed to demonstrate efficacy in preventing cyst formation. [53,54]

In human patients with ADPKD who received a kidney transplant, mTOR inhibition was associated with a reduction in hepatic cysts in some subjects. [55] In addition, Shillingford et al. [56] found that rapamycin treatment largely reduces native polycystic kidney size in transplant recipients with ADPKD.

In some clinical trials, mTOR inhibitors cannot significantly decrease the total kidney volume, halt polycystic kidney growth, or slow the progression of renal impairment compared with placebo groups. [57]

An appealing pathophysiologic approach in ADPKD is to block the mTOR pathway, which is the driving force for the hyperproliferative phenotype of ADPKD cells, plus the cystic fibrosis transmembrane conductance regulator (CFTR), which mediates excessive fluid secretion into cyst lumen and promotes cyst enlargement in the kidney. PF-06409577 effectively down-regulated mammalian target of rapamycin pathway-mediated proliferation of cyst-lining epithelial cells and reduced cystic fibrosis transmembrane conductance regulator-regulated cystic fluid secretion. Overall, our data suggest that PF-06409577 holds therapeutic potential for ADPKD. [58]

In a recent systematic review and meta-analysis of 16 studies, mTOR inhibitors are effectively similar to tolvaptan, tyrosine kinase inhibitors, and somatostatin in reducing total kidney volume. However, only tolvaptan preserved kidney function compared to the placebo. [59]

IV. Antiviral effect:

The antiviral properties of mTOR inhibitors are attributed to a variety of mechanisms. The most prominent antiviral potential is the effect of mTOR inhibitors on memory T cells. Other proposed mechanisms focus on mTOR inhibitors' ability to inhibit viral cell growth through various pathways. Inhibition of mTOR blocks the downstream effects of the Akt pathway activation, repealing the growth of viral and tumor cells. Additionally, sirolimus has been shown to inhibit viral protein synthesis of pUL44 and pp65, essential proteins necessary for CMV replication in macrophages. Finally, mTOR inhibition interferes with virus-mediated transcriptional events. [60]

Polanco et al. showed that 56% of the patients had cleared the BK virus after discontinuation of MPA and conversion from TAC to Everolimus. In addition, more than a 95% decrease in viral load in the remaining cases. [61]

A meta-analysis of 28 studies revealed moderate- to high-quality evidence of reduced risk of cytomegalovirus infection in renal transplant recipients in the mTOR inhibitor-based compared with the CNI-based regimen. However, the evidence neither confirmed nor ruled out a reduction of BK

polyomavirus infection in the mTOR inhibitor-based group. 62

The early introduction of mTOR inhibitors to Low dose CNI or the transition from CNI to mTOR inhibitors will have a favorable outcome not only from clinical perspectives but a good economic long-term impact. Such impact could come from reducing the net immunosuppression 63 and avoiding costly antiviral therapy like valganciclovir. However, this should keep the delicate balance between the immunological and infection risks. Therefore, it seems appealing to low immunological-risk patients in resource-limited settings.

COVID-19 is associated with a poor prognosis in solid organ transplant recipients because of immunosuppression. However, studies suggested a potential therapeutic role of mTOR inhibitors in SARS-CoV-2 infection. For example, in a study of 371 kidney transplant recipients, 15% had SARS-CoV-2 disease. There were no differences among immunosuppressive therapies concerning the risk of acquiring SARS-CoV-2 infection. In contrast, the type of immunosuppressive therapy had a significant impact on the outcome of the disease. Patients who received mTOR inhibitors had a lower chance of developing a moderate or severe form of COVID-19 disease. 5,64

Conclusion

Although mTOR inhibitors did not achieve the desired goal as transplant immunosuppression agents, they still have a place in clinical medicine. The increasing understanding of the critical role of the mTOR pathway in cellular metabolism and growth has paved the way for their judicious use in different medical disciplines besides organ transplantation. The next challenge is achieving therapeutic strategies that balance the potential benefits of mTOR inhibitors and their side effects by balancing their differential actions on their complexes.

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