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The case for the therapeutic use of mechanistic/mammalian target of rapamycin (mTOR) inhibitors in xenotransplantation

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Abstract

The mechanistic/mammalian target of rapamycin (mTOR) is one of the systems that are necessary to maintain cell homeostasis, such as survival, proliferation, and differentiation. mTOR inhibitors (mTOR-Is) are utilized as immunosuppressants and anti-cancer drugs. In organ allotransplantation, current regimens infrequently include an mTOR-I, which are positioned more commonly as alternative immunosuppressants. In clinical allotransplantation, long-term efficacy has been established, but there is a significant incidence of adverse events, for example, inhibition of wound healing, buccal ulceration, anemia, hyperglycemia, dyslipidemia, and thrombocytopenia, some of which are dose-dependent. mTOR-Is have properties that may be especially beneficial in xenotransplantation. These include suppression of T cell proliferation, increases in the number of T regulatory cells, inhibition of pig graft growth, and anti-inflammatory, antiviral, and anti-cancer effects. We here review the potential benefits and risks of mTOR-Is in xenotransplantation and suggest that the benefits exceed the adverse effects.

Keywords

graft growth; immunosuppression; inflammation; mTOR inhibitor; neoplasia; Tregs; viral infection; xenotransplantation

1 | INTRODUCTION

Initially, rapamycin was studied as a macrolide antibiotic.[1] During these studies, the mechanistic/mammalian target of rapamycin (mTOR) was identified, and rapamycin was found to inhibit the cell cycle at the G1 phase.[1] Although there are now a number of mTOR inhibitor (mTOR-I) compounds, some of which are used therapeutically, their basic mechanism of action is the same.[2] However, pharmacokinetic parameters and sub-effects are different.[3] Therefore, considering the limited information available, it may be necessary to

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CONFLICT OF INTEREST STATEMENT

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compare them and consider switching to a different one depending on the situation. Today, mTOR-Is are well-known as immunosuppressants or anti-cancer drugs.

Although not administered to the patient who received the first pig heart transplant,[4] there has been significant experience of mTOR-Is in pig-to-nonhuman primate (NHP) models[5–10] (Table 1). The barriers to successful xenotransplantation include several that an mTOR-I might help overcome (Table 2). mTOR-Is have many qualities that make them advantageous in xenotransplantation (Table 3A).

1.1 | Suppression of the immune and inflammatory responses

The main purpose of using an mTOR-I in organ transplantation is to suppress “signal III” in T cell activation. Signal III is the stimulation by interleukin (IL)-2 that triggers T cell proliferation.[11] mTOR is downstream of the IL-2 and IL-15 receptors.[11] IL-15 in tissues can promote $CD4^+$T helper cell-mediated immunity and provide co-stimulatory signals to effector cytotoxic T cells.[12] Inflammatory IL-15 has a variety of effector functions against memory $CD8^+$T cells, such as proliferation, cell survival, enhanced cytotoxicity, and trafficking into non-lymphoid tissues.[13] The immunosuppressive effect of mTOR-Is is by blockade of IL-2 and IL-15 induction of T cell proliferation[14] (not by blocking all IL-2 signaling). Inhibition of mTOR also leads to suppression of B cell activation, proliferation, and differentiation into antibody-producing cells, and antibody production.[14,15]

The mTOR network consists of two main complexes, mTOR complex-1 (mTORC1) and −2 (mTORC2).[16] mTORC1 is sensitive to an mTOR-I, and mTORC2 is inhibited by prolonged administration of an mTOR-I.[17,18] mTORC2 is important for peripheral B lymphocyte maturation, homeostasis, function, and survival[19] (low-dose rapamycin may increase IgM production against influenza vaccines, while maintaining inhibition of antibody class switch[20]). The mTOR-I, PP242 (not used clinically), inhibits mTORC1 and mTORC2 at low concentrations (without the need for prolonged administration) and increases the fraction of B cells undergoing antibody class switching.[21] The use of PP242 at high concentrations inhibits B cell proliferation and differentiation.[21]

A recent study regarding SARS-CoV-2 mRNA vaccination showed that kidney transplant recipients who received an mTOR-I achieved higher IgG titers and specific T cell-derived interferon (IFN)-γ against SARS-CoV-2.[22] This result is contrary to a report regarding influenza vaccination.[20] There are some differences between the two reports such as the type of vaccine (e.g., mRNA or inactivated vaccine) and the timing of vaccination. The effect of mTOR-Is on antibody production after vaccination is influenced by the concentration of the mTOR-I, the type of antigen, and the amount and frequency of antigen exposure. In a clinical allotransplant study, de novo donor-specific antibody (DSA) tended to be lower when the immunosuppressive regimen included an mTOR-I.[23]

Collectively, these reports suggest that maintaining a sufficient concentration of mTOR-I for a prolonged period would help prevent AMR by sufficiently inhibiting both mTORC1 and mTORC2.

Evidence is that the current target trough levels for mTOR-Is are in the optimal range. Caution should be exercised immediately after the initiation of administration of an mTOR-I or when switching to another type of immunosuppressant, as there may be a period of decreased blood concentration. In addition, rapamycin and everolimus have different strengths of interaction on mTORC2.[3] Therefore, their effects may differ.

Dendritic cells (DCs) are also influenced by an mTOR-I.[24] mTOR-Is impair endocytosis.[25,26] Additionally, expression of the major histocompatibility complex (MHC)-I and -II, co-stimulatory molecules (including CD40), and antigen uptake receptors in DCs are decreased.[26–28] It can be assumed that an immune response initiated by DC uptake of donor antigens by endocytosis (indirect recognition pathway) in allotransplantation is suppressed.[29] Subsequently, DC-induced T cell stimulation and proliferation are reduced by an mTOR-I.[26–28] T cell-primed mTOR-I-treated DCs decrease proinflammatory cytokines, for example, IL-2 and IFN-γ.[27,30] Thus, mTOR-Is have the potential to suppress antigen presentation and the production of proinflammatory cytokines. They also block IL-6 and vascular endothelial growth factor (VEGF) production after reperfusion.[31] Macrophages treated with an mTOR-I down-regulate the production of cytokines, including IL-6.[32] In addition, mTOR-Is suppress the mTOR-signal transducer and activator of transcription (STAT) 3 pathway and reduce protein and mRNA levels of IFN-γ and IL-17 in splenocytes.[33] In human vein endothelial cells, mTOR inhibition reduces IFN-γ-induced human leukocyte antigen (HLA)-class II expression.[34]

These results suggest that the effects of mTOR inhibition in suppressing the immune response and inflammation are exerted not only on the recipient's immune system, but also on the donor graft. mTOR-Is have anti-inflammatory effects and subsequent suppression of the immune response.

In xenotransplantation, early upregulation of recipient IL-6 may result in the activation of coagulation.[35] Additionally, increases in serum pig-IL-6 and baboon-IL-6 correlated with graft dysfunction.[36] mTOR inhibition partially inhibited human, baboon, and pig IL-6/IL-6 receptor (IL-6R)α/STAT3 pathways and suppressed inflammatory gene expression.[37]

An mTOR-I is able to suppress pig endothelial cell-induced responder T cell proliferation.[38] mTOR-I-preconditioned pig endothelial cells do not stimulate T cell proliferation.[38]

The prevention of systemic inflammation in xenograft recipients (SIXR) is beneficial in prolonging graft survival in xenotransplantation.[35,39,40] Therefore, mTOR-Is have the potential to prevent SIXR and contribute to achieving long-term xenograft survival.

1.2 | Induction of T regulatory cells (Tregs) and tolerance

mTOR-Is can suppress $CD4^+$T helper cells and effector cytotoxic T cells, but may also have a beneficial effect on the induction of Tregs. Donor-specific tolerance is one of the goals of transplantation. $CD4^+CD25^+Foxp3^+$ Tregs[41] can suppress the immune response, and contribute to the induction of tolerance.[41,42] Tregs help to maintain stable graft function and reduce the incidence of both acute and chronic rejection.[42]

Organ graft recipients who were treated with an mTOR-I had a higher frequency of Tregs compared with those treated with a calcineurin inhibitor (CNI).[43] Low IL-2 receptor signaling acts to promote the maturation of immature Tregs ($CD4^+CD25^{low}Foxp3^{low}$) to $CD4^+CD25^{hi}Foxp3^{hi}$Tregs.[44] IL-2 is required for Tregs, whereas CNIs inhibit the transcription of IL-2.[45] Thus, it is suggested that mTOR inhibition maintains Tregs better than therapy with a CNI.

Some studies indicate that mTOR inhibition can expand the number of Tregs regardless of antigen levels.[46–49] In a transforming growth factor-β (TGF-β)-dependent manner, an mTOR-I enhanced the expression of Foxp3.[50] Tregs that are expanded by an mTOR-I suppress the proliferation of both syngeneic and allogeneic $CD4^+$ and $CD8^+$ T cells in vitro and prevent allograft rejection in vivo.[46,51] Similarly in an in vitro study, purified Tregs enriched from $CD4^+$ cells in the presence of an mTOR-I suppressed baboon anti-pig immune responses more strongly than freshly-isolated natural Tregs.[52]

The CD40/CD154 pathway is one of the co-stimulatory pathways between antigen-presenting cells (APCs) and T cells.[53] Anti-CD154 (anti-CD40 ligand) monoclonal antibody (mAb) is attractive as an immunosuppressant, and may lead to tolerance.[54] The mechanism by which anti-CD154 mAb induces tolerance is by inducing Tregs.[55–57] In contrast, another report suggested that monotherapy with an anti-CD154mAb cannot induce tolerance and is not sufficient to prevent chronic graft vasculopathy.[58] Thus, the combination of an mTOR-I and an anti-CD154 mAb may have additive or synergistic effects. As supportive information, in islet-kidney allotransplantation in NHPs, the combination of an mTOR-I and an anti-CD40mAb achieved long-term islet and kidney allograft survival.[59] An mTOR-I combined with blockade of the CD40/CD154 pathway may therefore be an efficient regimen.

A regimen including an mTOR-I may therefore have advantages in the establishment of donor-specific tolerance.

1.3 | Effect on viral infection

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) cause well-known infections after allotransplantation.[60–67] After kidney allotransplantation, BK polyomavirus (BKV) is associated with rejection and kidney graft dysfunction.[68] Furthermore, SARS-CoV-2 (COVID-19) has caused a pandemic since 2019.[69–71] Although infections with CMV, EBV, BKV, and COVID-19 are not limited to immunosuppressed patients, the influence of immunosuppressants on viral infection should be considered.

CMV is one of the most frequent infectious agents seen in organ transplant recipients and is associated with higher risks of graft rejection/loss and patient morbidity and mortality.[60–65] An mTOR-I reduced the incidence of human CMV viremia after allotransplantation.[72] In xenotransplantation studies, pig CMV (pCMV) infection was associated with rejection and reduced survival.[62,63,65,73–75] pCMV transmission was also associated with increased levels of IL-6 and TNFα.[75] Anti-CMV agents, for example, ganciclovir or valganciclovir, are generally administered in clinical CMV infection,[76] but these agents are much less effective in pCMV infection.[64]

There is increasing evidence that mTOR-Is have anti-CMV properties.[23,77–79] They have indirect anti-viral activity and reduce the rate of CMV infection in CMV-seropositive organ transplant recipients.[80] mTOR-Is inhibit proliferation of infected cells by activating specific signaling pathways.[81] The mechanism of suppression of CMV infection by mTOR-Is has been reported as reinvigorating $\alpha\beta$ and $\gamma\delta$ T-cell function.[82]

EBV is a human oncovirus and is associated with the development of post-transplant lymphoproliferative disorders (PTLD).[67] An mTOR-I decreased EBV^+ B cell proliferation.[83] After allotransplantation, the levels of EBV-DNA were significantly lower in patients receiving an mTOR-I than in those receiving a mycophenolate mofetil-based regimen.[84] Thus, there is evidence that the risk of PTLD may be reduced in patients receiving an mTOR-I.[7]

Most adults have been exposed to BKV[85] and this is particularly problematic after kidney transplantation.[68] However, therapy with an mTOR-I reduced urinary BKV titers[86] and the incidence of BK viremia.[72]

Although COVID-19 infection has been common recently,[69–71] there are reports that the administration of an mTOR-I may reduce the incidence through a mechanism that may involve inhibition of viral replication.[87]

Therefore, although it is not definitively known whether mTOR inhibition reduces viral infections in xenotransplantation, we suggest that the anti-viral effect of mTOR-Is may be beneficial in xenotransplantation.

1.4 | Effect on growth of a pig graft

mTOR coordinates eukaryotic cell growth,[88] and mTOR inhibition inhibits the signal transduction pathways required for cell growth and proliferation.[88,89] This inhibitory effect has been applied in various diseases.[90] The observation that the growth of pig kidneys in NHPs receiving rapamycin[8,9] appeared to be less than in those that did not[91] suggested a beneficial effect on pig organ growth. In a cardiac xenotransplantation study, mTOR inhibition appeared to suppress growth,[92] but its immunosuppressive effect may also have contributed to reduced cell infiltration, edema, and interstitial hemorrhage (thus reducing the apparent “growth” of the organ).

We suggest, therefore, that inclusion of an mTOR-I may inhibit graft growth and subsequent dysfunction.

1.5 | Effect on de novo neoplasia

As a central regulator of cell growth, proliferation, differentiation, and survival, mTOR is also thought to play an important role in controlling tumor cell motility, invasion, and metastasis.[93] There are increasing reports on the effects of mTOR inhibition on several types of cancer,[94–97] indicating the efficacy of mTOR-Is against cancer. For example, immunosuppression with a CNI increases the risk of hepatocellular carcinoma,[98,99] whereas an mTOR-I reduces it.[99–101] In a human kidney transplantation study, mTOR inhibition

protected from non-melanoma skin cancer and solid organ malignancies.[102] Additionally, as mentioned above, an mTOR-I may contribute to preventing PTLD associated with EBV.[83,84]

1.6 | Adverse effects

The potential beneficial effects of mTOR-Is are offset by potential adverse effects (Table 3B), which are related to the inhibition of mTOR-induced pathways.[103]

Impaired wound healing is related to several processes (hemostasis, inflammation, proliferation, and remodeling),[104] and can be problematic as it is often accompanied by the development of a lymphocele.[103,105,106] mTOR inhibition is associated with a higher frequency than is mycophenolate mofetil (MMF),[107,108] but the absolute incidence trends lower with everolimus.[108] A lower initial dosage of the mTOR-I and/or its delayed introduction may prevent this complication.[103–106] Of note, in our pig-to-NHP kidney transplantation model, we have never experienced any wound healing problems. This may, of course, be associated with the fact that, in contrast to patients undergoing organ transplantation, the recipient animals are healthy, with no pre-transplant comorbidities.

There is a higher incidence of proteinuria than when a CNI is administered.[109] In kidney xenotransplantation, proteinuria may be problematic even in the absence of an mTOR-I,[110] but mTOR inhibition may exacerbate it. Podocyte cytotoxicity[111] or reduced albumin endocytosis[112] may be seen in mTOR-I-associated proteinuria. Angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers may contribute to reversing it.[112]

Oral ulceration/stomatitis[103,105] is more frequent than with a CNI.[113] Combination therapy with an mTOR-I and mycophenolate mofetil tends to amplify the incidence.[106] Although ulceration may be self-limiting,[114] high-potency topical steroids (e.g., clobetasol), non-steroidal anti-inflammatory drugs (NSAIDs), and anesthetics (e.g., viscous lidocaine) can reduce pain and promote healing.[105,106,115]

Pneumonia/interstitial lung disease is a rare, but serious, adverse effect.[103,105] Due to differences in dosing regimens and the frequency of radiation therapy, it is more common in the oncology setting than in the transplant setting.[105] Early detection is desirable.[106] Improvement in pneumonitis is expected with the reduction or discontinuation of the mTOR-I,[116,117] or conversion from rapamycin to everolimus.[118,119]

Anemia is related to decreased iron availability, gastrointestinal absorption, and globin synthesis, and is dose-dependent,[120,121] and generally not severe.[121] The administration of iron and an agent that stimulates erythropoiesis, for example, erythropoietin, can be beneficial.[105,120,121]

Dyslipidemia, including hypercholesterolemia and hypertriglyceridemia, is a common side-effect of mTOR-Is,[103,105,122] though the increases are rarely more than modest.[106] Standard medications, for example, statins and fibrates, that prevent or reduce hypercholesterolemia and hypertriglyceridemia, are recommended to reduce cardiovascular events.[103,105,106,122,123]

Hyperglycemia or new-onset diabetes mellitus after transplantation (NODAT) has been reported to occur.[103,105,124] mTOR inhibition is independently associated with NODAT.[125] The mechanism of NODAT caused by an mTOR-I is multifactorial, but ultimately results in insulin resistance and/or defective insulin secretion.[126–128] Hyperglycemia needs to be controlled to decrease the risk of cardiovascular disease.[129,130] In islet allotransplantation, a regimen that included an mTOR-I without steroids[131,132] resulted in prolonged graft function and controlled HbA1c.[131,133,134] Thus, hyperglycemia may not result directly from the administration of an mTOR-I.

In transplantation, the trough levels of mTOR-Is are regularly monitored, and close monitoring can reduce the incidence and severity of adverse events,[135] and yet ensure prolonged graft survival.

1.7 | Target trough levels of rapamycin (sirolimus)

In clinical allotransplantation (when rapamycin is combined with cyclosporine and prednisolone), the target level of rapamycin is frequently 5–15 ng/mL.[136] When rapamycin is combined with azathioprine and prednisolone, the trough levels may be maintained at 30 ng/mL for the first 2 months posttransplant, then reduced to 15 ng/mL.[137,138] In xenotransplantation studies in NHPs, the target levels of rapamycin have generally been lower, for example, 8–20 ng/mL.[5,7–9]

In human whole blood, 95% of sirolimus is found in the RBCs, and the blood/plasma ratios are approximately 30. Furthermore, 97% of sirolimus is extensively bound to albumin. According to these data, it would be expected that the trough level of sirolimus depends on the RBC count (e.g., in anemia) and albumin concentration (e.g., proteinuria, or when there is suboptimal nutrition) in the recipient. However, in pharmacokinetics studies in kidney allotransplant recipients, the hematocrit, RBC count, and serum albumin levels were excluded from covariates that could affect blood concentrations.[139,140] Therefore, factors affecting pharmacokinetics need to be further examined.

2 | DISCUSSION AND CONCLUSIONS

An mTOR-I may exhibit a variety of beneficial effects, including (i) suppression of effector T cells, B cells, DCs, and antibody production, (ii) increases in Tregs, and (iii) anti-inflammatory, anti-viral, anti-cancer, and anti-growth effects. mTOR-Is have the potential to become valuable immunosuppressive agents in xenotransplantation, although it will be essential to ensure that appropriate blood levels are maintained and adverse effects minimized. It is possible that the adverse effects of mTOR-Is might not be observed in a healthy experimental animal but are more common in a debilitated patient.

There is one additional benefit associated with the administration of mTOR-Is that is gaining increasing attention, and that is their anti-aging effect.[141,142] It is now accepted that the effect is not simply associated with a reduced incidence of viral infection and/or cancer, but is a direct effect that slows the aging process. Rapamycin extends lifespan in all tested models from yeast to mammals. It is more effective at slowing down the aging process than reversing it. This effect should be of benefit to the recipients of xenografts.

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Abbreviations:

BKV	BK polyomavirus
CMV	cytomegalovirus
CNI	calcineurin inhibitor
DCs	dendritic cells
EBV	Epstein-Barr virus
mTOR	the mechanistic/mammalian target of rapamycin
mTOR-I	mTOR-inhibitor
NHP	nonhuman primate
NODAT	new-onset diabetes mellitus after transplantation

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Table 1:

Selected experience with mTOR-inhibitors in pig-to-baboon organ transplantation

Year (Reference)	Pig organ (Gene-editing)	Heterotopic/Life-supporting	Maximal graft survival (days) (Cause of termination of experiment)
2005 (McGregor et al)[4]	Heart (CD46)	Heterotopic	137 (Rejection)
2005 (Brandl et al)[5]	Heart (CD55)	Life-supporting	25 (Rejection)
2011 (Byrne et al)[6]	Heart (CD46/TPC)	Life-supporting	57 (Infection/PTLD)
2015 (Iwase et al)[7]	Kidney (GTKO/CD46/CD55/TBM/EPCR/CD39)	Life-supporting	136 (Infection)
2017 (Iwase et al)[8]	Kidney (GTKO/CD46/CD55/EPCR/ TFPI/CD47)	Life-supporting	260 (Infection)
2020 (Reichart et al)[9]	Heart (GTKO/CD46/TBM)	Life-supporting	195 (Study end-point)

EPCR = endothelial protein C receptor; PTLD = post-transplant lymphoproliferative disease; TBM = thrombomodulin; TFPI = tissue factor pathway inhibitor; TPC; polyethylene glycol α-Gal polymer.

Table 2:

Efficacy of mTOR-inhibitors in overcoming the barriers to successful pig-to-NHP organ transplantation

Barriers	Effect of mTOR-I	Focus	Targets	References
Innate immune response	Some effect	Recipient	Dendritic cells	24, 25, 26, 27, 28, 30
Adaptive immune response	Effective	Recipient	T cells, B cells Antibody producing cells	11, 14, 15, 20, 21, 22
Inflammatory response	Effective	Recipient Donor	Dendritic cells Endothelial cells	27, 30, 31, 32, 33, 34
Coagulation dysfunction	-	-	-	-
Viral infection	Effective	Recipient Donor	CMV, EBV, BKV, COVID-19	23, 72, 77, 78, 79, 80, 81, 82, 83, 84, 86, 87
Rapid growth of the pig organ	Effective	Donor	Graft	8, 9, 91, 92
De novo neoplasia	Effective	Recipient Donor	Tumor cells	83, 84, 99, 100, 101, 102

Table 3:

Potential advantages and disadvantages of administration of an mTOR inhibitor in xenotransplantation

A. Advantages
Suppresses both cellular and antibody-mediated rejection (T cells, B cells, and antibody-producting cells)
Associated with an increase in Tregs (especially when combined with CD40/CD154 co-stimulation pathway blockade)
Inhibits the primate and pig IL-6/IL-6Rα/STAT3 pathway and suppresses inflammatory gene expression
Decreases proinflammatory cytokines (e.g., IL-2, IFN-γ, and IL-6)
Reduces pig organ growth
Anti-viral/anti-cancer activity
B. Disadvantages
Increases IgM production (to specific antigens at low-concentration of mTOR-I)
May inhibit wound healing (though this has never been a problem in our pig-to-NHP model)
Not always tolerated by patients (but many side-effects are dose-dependent)
Can be associated with proteinuria (though we have not seen this)
May increase the risk of cardiovascular disease (e.g., dyslipidemia and hyperglycemia)
May need supportive medications (e.g., erythropoietin, statin/fibrate, and insulin)