REVIEW

mTOR inhibitors and risk of chronic antibody-mediated rejection after kidney transplantation: where are we now?

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SUMMARY

Antibody-mediated rejection (AMR) usually starts with generation of donor-specific anti-HLA antibodies (DSAs), arising from a B-cell response to antigen recognition. *In vitro* and preclinical data demonstrate that mammalian target of rapamycin (mTOR) inhibition attenuates the mTOR-mediated intracellular signaling pathway involved in AMR-related kidney damage. The limited available data from immunological studies in kidney transplant patients, however, have not shown such effects *in vivo*. In terms of clinical immunosuppression, the overriding influence on rates of *de novo* DSA (dnDSA) or AMR—regardless of the type of regimen—is patient adherence. To date, limited data from patients given mTOR inhibitor therapy with adequate concurrent immunosuppression, such as reduced-exposure calcineurin inhibitor (CNI) therapy, have not shown an adverse effect on the risk of dnDSA or AMR. Early switch to an mTOR inhibitor (<6–12 months post-transplant) in a CNI-free regimen, in contrast, can increase the risk of dnDSA, especially if adjunctive therapy is inadequate. Late conversion to CNI-free therapy with mTOR inhibition does not appear to affect the risk of dnDSA. More data, from prospective studies, are required to fully understand that association between use of mTOR inhibitors with different types of concomitant therapy and risk of dnDSA and AMR.

Transplant International 2017; 30: 647–657

Key words
antibody-mediated rejection, donor-specific anti-HLA antibodies, everolimus, mammalian target of rapamycin, sirolimus, transplantation

Received: 19 December 2016; Revision requested: 13 February 2017; Accepted: 21 April 2017

Introduction

Late graft loss after kidney transplantation remains a major clinical issue. Acute T-cell-mediated rejection has been largely controlled, helping to increase short-term survival rates to over 90% [1], but only around half of all grafts are still functioning after 10 years [1]. In recent years, there has been a growing awareness that inadequate control of the humoral component of a recipient's immune response is pivotal in many cases of chronic graft dysfunction and failure [2]. Antibody-mediated rejection (AMR), overlooked for decades, is now recognized as a leading cause of late kidney graft loss [3]. Chronic AMR has a complex pathophysiology with a highly variable course that can manifest in various clinical forms [2]. It is frequently detected only after irreversible damage has developed [4], and the patient presents with deteriorating graft function. As a

doi:10.1111/tri.12975

result, it is notoriously difficult to treat, with 15–20% of grafts failing within a year after diagnosis [5]. Preventative strategies, which aim to block the generation of *de novo* donor-specific anti-HLA antibodies (dnDSAs), should therefore be the priority of physicians in charge of transplanted patients.

The natural history of AMR starts with generation of high-affinity alloantibodies directed against protein antigens in the graft, usually DSAs, which are largely responsible for chronic graft deterioration [2]. The introduction of solid-phase single antigen bead technology to detect and characterize HLA antibodies has prompted a new phase of research in this field. Solid-phase immunoassays, notably Luminex®, are more sensitive than earlier complement-dependent lymphocytotoxicity (CDC) assays and Luminex® testing for DSA in transplant candidates—with regular DSA monitoring of high-risk patients—is now recommended [6]. The resulting insights into the impact of DSA on graft outcomes, and the ability to evaluate levels of DSA under specific immunosuppressive regimens, have drawn attention to the question of which drug classes may influence the risk of generation of DSA [7].

The mammalian target of rapamycin (mTOR) inhibitors everolimus and sirolimus exert highly complex effects on the immune system due to the multiple roles of the mTOR signaling pathway in the immune cascade [8]. It has long been known that their potent immunosuppressive action is founded on suppression of the proliferation and clonal expansion of antigen-specific T cells, but ongoing research has shown that this is accompanied by alterations in the balance of memory and helper T-cell subsets [8] and, more recently, by potential effects on B cells, plasma cells, and natural killer (NK) cells [9].

Modulation of B-cell activity could influence AMR by inhibiting differentiation to plasma cells, as well as suppressing their immunoregulatory functions and presentation of antigens to T cells. This article considers the current evidence regarding a potential influence of the non-T-cell immunological effects of mTOR inhibitors on development of DSA and AMR following kidney transplantation.

An overview of the pathophysiology of AMR

The pathological processes underlying AMR are not fully elucidated, although recent experimental studies have provided new insights [10]. The cascade of events begins with the generation of DSA, a relatively frequent event after kidney transplantation. Prospective studies have shown the incidence of dnDSA, that is, DSA in patients without DSA at time of transplant, to be 15–20% by 5 years after transplantation [11–13], with the highest rate of development during the first post-transplant year [12]. The presence of dnDSA is a well-recognized risk factor for chronic AMR and graft loss [2]. Complement-binding dnDSA and anti-HLA DQ DSA are particularly unfavorable for risk of AMR and graft failure [14–17].

Donor-specific anti-HLA antibodies bind to mismatched polymorphic HLA antigens on the graft endothelium, or to other targets such as polymorphic minor histocompatibility antigens, initiating antigen-mediated allograft injury. If complement-activating DSAs are present, binding of circulating DSA to endothelial cells can trigger activation of the classical complement pathway. This ultimately leads to deposition of C4d in graft tissue and cleavage of C3 to C3a and C3b, resulting in leukocyte recruitment and endothelial cell activation [2,10]. Activation of the classical complement pathway is fundamental for acute AMR, but chronic AMR can develop in its absence, arising from non-complement-fixing DSA [17,18]. Chronic AMR, characterized by chronic vascular inflammation, and vascular lesions such as transplant glomerulopathy, which gradually become irreversible and eventually cause progressive tissue destruction and loss of graft function, may be more dependent on NK cells [19]. It has also been proposed that DSA may directly induce activating signals in endothelial cells, prompting the expression of adhesion molecules and growth factors [20].

The characteristics of DSAs (high affinity, mutated immunoglobulins) indicate that they arise from a thymo-dependent B-cell response to recognition of its cognate antigen, which takes place within secondary lymphoid organs, that is, the spleen and lymph nodes. After entering the germinal center, activated B cells then proliferate and differentiate toward antibody-secreting long-lived plasma cells, which generate circulating DSA, or toward memory B cells [21]. If mTOR inhibitors restrict B-cell activity by blocking the mTOR-mediated downstream signaling triggered by antigens binding to surface B-cell receptors, it could potentially be highly relevant for prevention of dnDSA after transplantation.

mTOR inhibition & mediators of AMR: *in vitro* & preclinical data

The mTOR signaling pathway exerts highly diverse effects on immune cell proliferation, function, and

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interactions [22,23]. Research has tended to focus on the mTOR complex 1 (mTORC1), the principal target of everolimus and sirolimus, in mediating T-cell responses. *In vitro* and preclinical data are now starting to accumulate regarding the role of the mTOR pathway within B cells and other immune cell types involved in the pathogenesis of AMR [9]. Understanding the implications of mTOR inhibition, for B-cell activity in particular, is by no means a simple challenge, as the relative activity of regulatory and effector B cells appears critical [24]. Although B-cell depletion can improve graft survival rates in patients being treated for AMR, B cells can also exert a protective effect on the graft by secretion of protective antibodies or via lowering of inflammatory response via secretion of IL-10, as well as by direct inhibition of effector T cells [24].

The conclusions of murine experimental models cannot always be translated directly to patients due to the fact that (i) the biology of mice differs from that of humans, (ii) laboratory mice live in an abnormally hygienic environment, which detrimentally impacts on memory effector development [25], and (iii) genetic engineering leads to complete extinction of the mTOR pathway in a specific cell subset rather than the partial blockade in all cell types observed during treatment with mTOR inhibitors. Yet murine experimental models are useful to dissect the role of the mTOR pathway in B-cell biology. Laboratory mice are inbred and genetically homogeneous, can be genetically manipulated, allow kinetic tissue analyses to be carried out from the onset of disease, and permit the use of tractable disease models. Comparably reductionist experiments are neither technically nor ethically possible in humans. Therefore, where clinical studies can only establish correlations (often blurred by many confounding factors), murine experimental models allow precise dissection of the molecular mechanism of immune responses and establishment of causality.

Knock-in mTOR hypomorph mice models in which mTOR transcription is neo-inserted then partially disrupted have been used to explore the effect of mTOR deficiency. Using this approach, Zhang and colleagues demonstrated that mTORC1/mTORC2 inhibition not only lowered T-cell counts (particularly memory T cells), with lower cytokine levels and greater FoxP3 expression, but suppressed B cells to an even greater degree [26]. A partial block of B-cell development was detected, with reduced proliferation, antibody production, and migration to cytokines. Consistent with this, Jones *et al.* [27] observed that abrogation of mTORC1 in a similar model prevented the generation of antibody-secreting plasma cells. Additionally, newly formed plasma cells in the spleen and bone marrow were ablated—an effect replicated by acute sirolimus administration—although long-lived bone marrow plasma cells were unaffected [27]. Using the same knock-in technology, Jindra *et al.* [28] showed that knockdown of either mTORC1 and mTORC2 blocked endothelial cell proliferation induced by HLA Class I. In a murine model where mice were transplanted with allogeneic hepatocytes, administration of mTOR inhibitors suppressed alloantibody production by alloprimed IgG1 B cells, an effect that was not observed after calcineurin inhibitor (CNI) administration [29].

In vitro cultures of human B cells have also provided evidence concerning the effects of mTOR inhibitors on B-cell proliferation, activation, and differentiation [30–32]. Both everolimus [30] and sirolimus [33] have been shown to inhibit $CD19^+$ B-cell proliferation and differentiation into plasma cells, even at low doses. Experiments with everolimus [30,31] and sirolimus [32] have shown that B-cell activation and production of IgG antibodies are profoundly attenuated in a dose-dependent manner. Sirolimus exerted a more potent effect on cultured B cells than tacrolimus [30,32], cyclosporine (CsA) [30,32], or steroids [30], while mycophenolic acid (MPA) strongly influenced B-cell function [30–32]. The picture is complex, however. Traitanon and colleagues, when assessing the effect of sirolimus on stimulated human B cells, found that $CD27^+$ memory B cells were suppressed more than naïve B cells, but that the residual B cells acquired an activated phenotype, and induced proliferation of $CD4^+CD25^-$ T cells with a shift to the type 1 T helper cells (Th1) phenotype. Additionally, the residual B cells showed enhanced expression of HLA DR [33].

In addition, there is preliminary evidence that mTOR inhibition may disrupt T-cell infiltration of endothelial cells. By interrupting the mTORC2 pathway, sirolimus can inhibit expression of vascular cell adhesion molecule-1(VCAM-1) by activated endothelial cells [34].

Overall, the available evidence from *in vitro* and murine models indicates that mTORC1 and mTORC2 inhibition can suppress B-cell activation, immunoglobulin production, proliferation and differentiation into plasma cells, and attenuate the mTOR-mediated intracellular signaling pathway involved in AMR-related kidney damage [9] (Fig. 1).

One further area of interest is a potentially beneficial impact of mTOR inhibition in patients who have

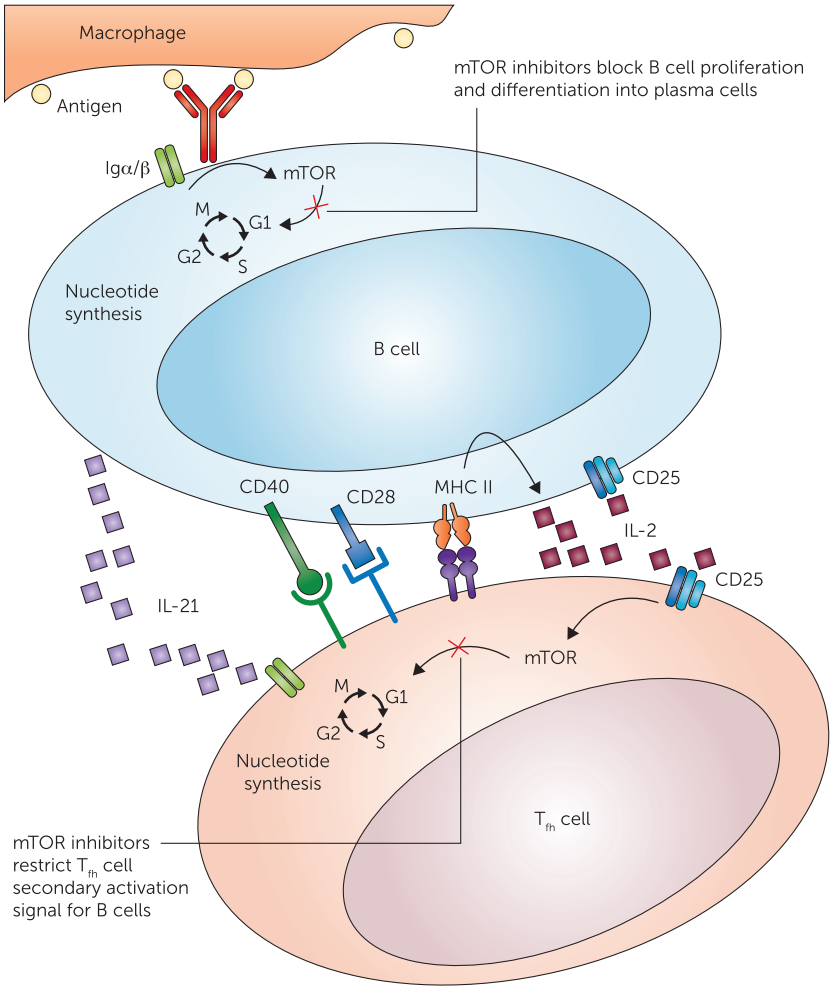

Figure 1 Potential role of mammalian target of rapamycin (mTOR) inhibition in suppression of B-cell function. Binding of the cognate antigen to immunoglobulin (Ig) on the surface of B cells triggers activation of the mTOR pathway, which stimulates production of nucleotides. Inhibition of the mTOR pathway blocks nucleotide production, thus restricting B-cell proliferation and differentiation to plasma cells. The second activation signal for B cells is generated by T follicular helper (T_{fh}) cells. Internalization and processing of cognate antigen by B cells are followed by presentation of selected antigen peptides to the B-cell surface on MHC class 2 (MHC II) molecules, leading to coupling of the B cell to a T_{fh} cell. The T_{fh} cell then activates the B cell by cell surface costimulatory ligand interactions (e.g., CD40) and by releasing directional cytokine production (IL-2 and IL-21). IL-2 receptor stimulation on the B cells and on the T_{fh} cells stimulates mTOR-mediated nucleotide production. mTOR inhibition can thus restrict the extent and effect of the Tfh cell-mediated second activation signal.

preformed DSA at the time of transplant, or who develop dnDSA. Awareness that complement-independent endothelial cell injury can contribute to AMR lesions has led to an exploration of the proinflammatory and proproliferation mechanisms involved [20,35]. DSAs have been shown to directly trigger activating signals in endothelial cells by cross-linking with major histocompatibility complex I (MHC-I) [20], a process in which the mTOR pathway plays a major role [35,36]. Furthermore, activation of the innate immune effectors, such as neutrophils and monocytes, which are involved in the development of antibody-dependent cell-mediated cytotoxicity, is also dependent on the mTOR pathway [37,38]. One could postulate that inclusion of mTOR inhibitors in the immunosuppressive regimen for DSA-positive transplant recipients might attenuate complement-independent AMR endothelial damage, but this possibility is beyond the scope of the present review and will not be discussed herein.

Immunoregulatory effects of mTOR inhibitors: clinical findings

Different immunosuppressant classes exert various—or indeed no—direct effects on B-cell or plasma cell activity (Table 1). Studies in kidney transplant patients have consistently demonstrated that the proportion of CD4 + $CD25^{high}$ FOXP3-expressing regulatory T cells in kidney transplant patients expands under sirolimus compared to CNI therapy in patients with or without prior lymphocyte-depleting induction therapy [51–55], but this does not appear to protect against chronic allograft injury [52]. Data regarding the effects of mTOR inhibitors on B-cell or plasma cell activity *in vivo* are sparse. A small study of 19 kidney transplant patients who underwent profound T-cell depletion with rabbit antithymocyte globulin (rATG), followed by maintenance immunosuppression based on sirolimus or CsA, reported that reconstitution of $CD19^{-}IgD^{+/-}CD27^{+}$ memory B cells was expanded under sirolimus, with

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Table 1. Overview of the impact of immunosuppressive agents & classes on B-cell ontogeny.

Immunosuppressive agent/class		Impact on B-cell/plasma cell biology
Antithymocyte globulin	Inhibition of T-B cooperation	*In vitro* B- and plasma cell apoptosis [39]
Rituximab		Depletes memory B cells [40,41]
Bortezomib		Inhibits activated B cells [42] Induces plasma cell apoptosis [43]
Alemtuzumab		B-cell repopulation with protolerogenic profile [44]
IL2R antagonists		No direct action
Corticosteroids		Apoptosis of activated B cells [45,46] Prevent B-cell differentiation to plasma cells *in vitro* [30]
Calcineurin inhibitors		No direct action [47,48]
mTOR inhibitors		Inhibition of B-cell activation [30–32], proliferation [30,33], and differentiation to plasma cells [30,33] *in vitro*
Belatacept		Expansion of transitional B cells [49]
Mycophenolic acid		Inhibition of B-cell proliferation and generation of plasma cells [50]

mTOR, mammalian target of rapamycin.

fewer naïve B cells [55]—an effect that would be expected to promote AMR. In a study of 36 kidney transplant patients, Latorre and colleagues observed that both mTOR inhibitors and CNI agents significantly reduced the number of B_{reg} cells versus healthy controls [54], which again could potentially contribute to antibody-induced injury. However, Gong *et al.* [53] assessed 18 recipients of a related living-donor kidney and found that neither sirolimus nor tacrolimus therapy affected the proportion of B_{regs} compared to controls. Studies reporting plasma cell counts or T cell-independent antibody production in transplant patients receiving mTOR inhibitors are lacking.

mTOR inhibition in the context of overall immunosuppression

A regimen that provides adequately potent immunosuppression, regardless of its constituent drugs, is essential to minimize risk of DSA and AMR-related graft loss—although of course this must be balanced against potentially fatal complications of over-immunosuppression, notably infections, and malignancies. Nonadherence to the prescribed regimen [4,11], or overly mild immunosuppression [56,57], very substantially increase rates of both DSA and AMR. Wiebe *et al.* [11] studied risk factors for dnDSA in a series of 315 consecutive kidney transplants and found nonadherence to have the greatest predictive effect of any variable, increasing risk almost nine-fold. One series of 23 patients with late AMR (>6 months) found that 17% had documented nonadherence, while in 69% of the cases the treating physician had reduced immunosuppression prior to AMR [56]. Another retrospective analysis, in a cohort of 27 patients with late AMR, found that 56% were either nonadherent or had suboptimal immunosuppression [57]. In a prospective study, Sellarés and colleagues found that among 36 kidney transplant patients who progressed to graft failure after rejection (all of whom had evidence of AMR), 47% had been assessed as nonadherent [3]. The evidence for nonadherence as the dominant risk factor for AMR is compelling. Pertinent to this, the prevalence of adverse events associated with mTOR inhibitors, including early mild stochastic symptoms triggered by a paradoxical inflammatory response due to destabilization of the inflammatory cytokine balance [58,59], can prompt nonadherence although today's lower exposure levels have ameliorated side effects [60]. The *in vitro* and preclinical evidence demonstrating a potential benefit for mTOR inhibition in controlling humoral alloimmunity may be attenuated by nonadherence, dose reductions or, indeed, discontinuation of mTOR inhibitors due to intolerance.

Although the overall intensity of immunosuppression remains the key determinant for risk of dnDSA, the specific immunological actions of immunosuppressant agents may also be influential [7]. As transplant recipients are only rarely maintained on a single agent, disentangling whether specific agents affect DSA development is challenging. A recent review [61] concluded that induction with rATG may achieve a short-term decrease in dnDSA production in moderately sensitized patients [62]. Although randomized trials are lacking, this is consistent with prolonged depletion of $CD19^+$ B cells

observed in a prospective clinical trial of kidney transplant patients given rATG [63]. MPA or early steroid withdrawal [64] in standard-risk patients does not appear to modify the risk of dnDSA [61]. The fusion protein belatacept appears to lower rates of dnDSA compared to CsA [65]. CNI administration, however, reduces risk of dnDSA versus regimens based solely on antimetabolite agents and steroids [61]. As one would expect, the level of exposure to CNI agents is highly influential: one retrospective analysis of 749 liver transplant patients found the risk of dnDSA at 1 year post-transplant to be increased by 2.66-fold if tacrolimus trough concentration was <3 ng/ml or CsA concentration was <75 ng/ml [66]. Clearly, any assessment of mTOR inhibitors must take into account the other component of the immunosuppressive regimen.

Clinical evidence regarding dnDSA & AMR under mTOR inhibition

De novo DSA

Until recently, the protocols for trials of mTOR inhibitors and other immunosuppressants only rarely included DSA measurement. Table 2 summarizes the key randomized clinical trials of mTOR inhibitors in which rates of dnDSA were captured. Regretfully, data on DSA were not collected in the large A2309 study, in which patients received everolimus with reduced-exposure CsA, or standard-exposure CsA with MPA, from the time of transplant [67]. Robust information on rates of dnDSA in patients given an mTOR inhibitor with reduced-CNI therapy is still lacking.

For patients who are switched from CNI therapy to mTOR inhibition, the evidence is mixed. The SPIESSER [68] and CENTRAL [69] studies showed no meaningful effect on dnDSA rates following withdrawal of CsA and introduction of sirolimus or everolimus (Table 2). In the large ELEVATE trial, 709 participants were randomized at 10–14 weeks post-transplant to start everolimus with CNI withdrawal or to continue standard CNI therapy (either tacrolimus or CsA), both with MPA and steroids [70]. In the subset of patients with DSA data available at year 2 post-transplant, dnDSA against HLA Class I was detected in more everolimus patients than in control patients (8.3% vs. 2.4%). Perhaps unexpectedly, the difference was largely due to low rates of anti-HLA Class I DSA in the subset of patients treated with CsA (0/52). The incidence of DSA against HLA Class II antigens was similar between the everolimus and control groups (6.3% vs. 7.0%) [70].

Two studies have reported a significantly higher rate of dnDSA after switch to mTOR inhibition [72,73] and merit discussion. In the CERTITEM study, the authors attributed this effect to overwhelming under-immunosuppression [72]. Everolimus exposure was relatively low (with ~35% having a trough concentration below the minimum threshold of 6 ng/ml) and, by protocol, was administered with half-dose MPA compared to full-dose MPA in the CNI treatment arm. Secondly, a *post hoc* analysis of 127 patients randomized in either the ZEUS or HERAKLES studies at a single center, published by Liefeldt *et al.* [73], reported that after a median follow-up of 3.5 years, dnDSA was detected in 23.0% of patients (14/61) who switched from CsA to everolimus, compared to 10.8% of patients (7/65) who continued CsA [hazard ratio (HR) = 2.43; $P = 0.048$]. The time to onset was shorter in the everolimus-treated patients (median 551 vs. 1173 days). It seems likely that the everolimus group was again under-immunosuppressed: 59% of patients in the everolimus group were steroid-free, and the mean dose of enteric-coated mycophenolate sodium was 1212 mg/day. Lastly, de Sandes-Freitas *et al.* [71] observed a nonsignificant trend to higher rates of dnDSA at year 2 post-transplant when patients were switched from tacrolimus to sirolimus at month 3 post-transplant, both with MPA and steroids (Table 2). In this study, tacrolimus exposure was low by protocol from time of transplant (mean 6 ng/ml at month 3), and only 36% received any induction therapy, so despite the low-risk population the overall intensity of immunosuppression may have been inadequate. Data on DSA in patients treated with sirolimus and belatacept versus belatacept-MPA or tacrolimus-MPA are too limited to draw firm conclusions [74]. The ongoing TRANSFORM study, in which over 2000 kidney transplants are randomized to everolimus with reduced-exposure CNI or to standard-exposure CNI and MPA, includes the incidence of DSA as an exploratory objective [75], providing high-quality data on this issue.

Retrospective single-center studies have suggested that patients converted early to an mTOR inhibitor with CNI withdrawal (<12 months after kidney transplantation) [76] experience a higher rate of dnDSA than those maintained on CNI therapy, but that later conversion (>6–12 months post-transplant) [76–79], or treatment with everolimus and low-dose CNI [80], incurs no additional risk.

Antibody-mediated rejection

Few randomized trials of mTOR inhibitors have reported rates of AMR, since at the time the protocols

Table 2. *De novo* DSA and antibody-mediated rejection in randomized trials of mTOR inhibitors in kidney transplant recipients.

Reference	Time from tx to start of mTOR inhibitor	mTOR inhibitor group	Control group	Follow-up	*De novo* DSA, % (*n*/*N*)			AMR, % (*n*/*N*)			Comments
					mTOR inhibitor	Control group	*P* value	mTOR inhibitor	Control group	*P* value	
A2309 [67]	*De novo*	EVR 3–8 ng/ml or EVR 6–12 ng/ml Reduced CsA ±Steroids	CsA MPA ±Steroids	2 years	–	–	–	3.6 (10/277) or 4.3 (12/279)	5.3 (15/277)	n.s. for both EVR groups vs controls	DSA not recorded
SPIESSER [68]	*De novo*	SRL MMF ±Steroids	CsA MMF ±Steroids	8 years	13.0 (9/69)	10.9 (8/73)	0.520	n/a	n/a	–	Low immunological risk rATG induction High early SRL dosing
CENTRAL [69]	7 weeks	EVR EC-MPS Steroids	CsA EC-MPS Steroids	3 years	15.0 (9/60)	21.1 (12/57)	0.600	n/a	n/a	–	Low or moderate immunological risk
ELEVATE [70]	10–24 weeks	EVR EC-MPS Steroids	TAC or CsA EC-MPS Steroids	2 years	Class I: 8.3 (10/120) Class II: 6.3 (6/95)	Class I: 2.4 (4/168) Class II: 7.0 (9/138)	–	4.5% (16/353)	2.0 (7/356)	0.059	Low or moderate immunological risk
de Sandes-Freitas et al. [71]	3 months	SRL MPA Steroids	Reduced TAC MPA Steroids	2 years	17.8 (8/45)	7.3 (3/41)	0.201	10.4 (5/48)	4.4 (2/45)	–	Low immunological risk Low-exposure TAC in both groups to month 3 post-tx
CERTITEM [72]	3 months	EVR Low-dose EC-MPS Steroids	CsA EC-MPS Steroids	1 year	27.2 (22/81)	4.9 (4/82)	0.001	5.2 (5/96)	3.1 (3/98)	–	Low to moderate immunological risk Half-dose EC-MPS in the EVR group

Table 2. Continued.

Reference	Time from tx to start of mTOR inhibitor	mTOR inhibitor group	Control group	Follow-up	*De novo* DSA, % (*n*/*N*)			AMR, % (*n*/*N*)			Comments
					mTOR inhibitor	Control group	*P* value	mTOR inhibitor	Control group	*P* value	
Liefeldt *et al.* [73]	3–4.5 months	EVR EC-MPS Steroids	CsA EC-MPS Steroids	Median 2.9 years	23.0 (14/61)	10.8 (7/65)	0.048	13.1 (8/61)	3.0 (2/66)	0.036	Low to moderate immunological risk 5/8 EVR patients with AMR received reduced EC-MPS, 1/8 with reduced steroids, 2/8 steroid-free

N, number of patients in whom data were available; AMR, antibody-mediated rejection; CsA, cyclosporine; DSAs, donor-specific anti-HLA antibodies; EC-MPS, enteric-coated mycophenolate sodium; EVR, everolimus; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; n/a, not available; n.s., non significant; rATG, rabbit antithymocyte globulin; SRL, sirolimus; TAC, tacrolimus; tx, transplant.

were developed endpoints focused on the occurrence and severity of cellular rejection. One recent exception was the A2309 trial, in which 833 *de novo* kidney transplant recipients were randomized from time of transplant to everolimus targeting either 3–8 ng/ml or 6–12 ng/ml with reduced-exposure CsA, or to standard CsA and MPA [67]. Both groups were given basiliximab induction and steroids were optional. Rates of AMR at two years' post-transplant were statistically noninferior in the two everolimus arms versus controls (3.6%, 4.3%, and 5.4%, respectively) (Table 2). The ELEVATE trial reported rates of histologically confirmed AMR in a cohort of 712 kidney transplant patients [70]. AMR at month 12 was more frequent in patients converted from CNI to everolimus at 10–24 weeks after kidney transplantation versus controls [3.7% (13/353) vs. 0.6% (2/356), $P = 0.004$]; at month 24, the rate of AMR remained numerically higher [4.5% with everolimus (16/353) vs. 2.0% with CNI (7/356) ($P = 0.059$) [70]]. Other major randomized trials in which patients were switched from CNI to mTOR inhibitor therapy during the first year post-transplant have not described AMR events. In the single-center analysis by Liefeldt *et al.* [73], the rate of AMR was higher after switch to mTOR inhibition than in controls who continued to receive CNI therapy (13.1% vs. 3.0%, $P = 0.036$). Of the eight everolimus-treated patients with AMR, however, five were receiving reduced-dose MPA, two had discontinued steroids, and one was on low-dose steroids.

More data are required, but based on these studies, everolimus with reduced-exposure CNI does not appear to increase risk of AMR but rates increase after early CNI withdrawal, particularly if adjunctive immunosuppression is inadequate.

Conclusion

Antibody-mediated rejection, the leading cause of kidney graft loss, represents the main unmet medical need in solid organ transplantation. In the absence of an effective treatment to block late events in the AMR cascade, clinicians should focus on primary prevention: that is, avoiding the generation of *dn*DSA. The influence of post-transplant immunosuppression on risk of dnDSA generation is very largely determined by the overall intensity of the regimen, and by the extent to which the patient adheres to that regimen. The overriding priority is to ensure that immunosuppressive tapering or withdrawal is gauged carefully to minimize the risk of dnDSA generation. The impact of a specific agent or class is secondary, but nevertheless of

considerable interest. Determining the contribution of an individual component is difficult since monotherapy is not widely used after kidney transplantation and the type and dose of adjunctive therapy vary. *In vitro* and preclinical data from mouse models suggest that mTOR inhibitors could potentially suppress development of *dn*DSA via effects on B-cell proliferation and differentiation but also act on late events in the AMR cascade including activation of innate immune effectors responsible for antibody-dependent cell-mediated cytotoxicity-mediated lesions and endothelial cells. The multiple functions of the mTOR pathway, however, mean that mTOR inhibition can also enhance certain B-cell responses or subpopulations [23], adding further complexity.

Well-designed studies are required to determine whether the B-cell modulation observed with mTOR inhibition *in vitro* is also manifested clinically. To date, limited data from patients given mTOR inhibitor therapy with adequate concurrent immunosuppression, such as reduced-exposure CNI therapy, has not shown an adverse effect on the risk of dnDSA or AMR. In contrast, an early switch from CNI therapy to mTOR inhibitor may increase the risk of dnDSA production, and it certainly seems prudent to maintain optimal MPA dosing and possibly continue steroids to minimize risk in this scenario. Early conversion to a CNI-free regimen should be followed by protocol biopsies and DSA monitoring. Late conversion from CNI therapy to an mTOR inhibitor, after the first year post-transplant, has not been shown to affect the risk of dnDSA development.

Funding

A medical writer funded by Novartis Pharma AG provided editorial assistance.

Conflict of interest

The authors have declared no conflicts of interest.

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