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REVIEW

Can mTOR inhibitors reduce the risk of late kidney allograft failure?

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Summary

The most frequent causes of late kidney allograft failure are chronic rejection, nonalloimmune injury and death, all of which may depend on the characteristics of the donor and recipient, but may also be influenced by the type of immunosuppression. Combining calcineurin inhibitors (CNIs) and corticosteroids offers potent immunosuppression, but may also cause side effects leading to progressive graft dysfunction or an increased risk of death. New immunosuppressive strategies may come from the availability of inhibitors of mTOR, a downstream effector of phosphatidylinositol-3 kinase that provides the signal for cell proliferation by phosphorylating a cascade of kinases. Recent trials have shown that it is possible to minimize the dose or withdraw CNIs a few weeks after transplantation when they are combined with mTOR inhibitors and their combination may also make it possible to minimize or avoid the use of corticosteroids. Moreover, by inhibiting the signal for cell proliferation, mTOR inhibitors may reduce the replication of cytomegalovirus inside host cells, prevent transplant vasculopathy, and exert anti-oncogenic activity. All of these characteristics offer a ray of hope for reducing the risk of long-term allograft failure.

Despite continuing advances in immunosuppression and supportive therapy, there has been only a small improvement in long-term cadaver kidney graft survival [1]. This is partly due to the poorer 'quality' of donors and recipients in comparison with the recent past as many kidneys are now harvested from older cadaver donors with preexisting renal diseases and/or renal dysfunction [2,3] and many patients who were previously excluded from transplant programmes on the grounds of older age, long-term dialysis and/or comorbidity are now considered suitable candidates for renal transplantation [4–6]. The choice of immunosuppressive therapy also has a considerable impact on the long-term results.

The 2005 Banff Conference [7] led to the etiological subdivision of chronic graft dysfunction into cases of chronic rejection, nonalloimmune events and specific chronic diseases. Chronic rejection may be caused by cell immunity [8] or, more frequently, donor-specific antibodies [9] and a number of factors may contribute to its development. Poor HLA compatibility and the presence of anti-HLA antibodies before transplantation are well-known risk factors [10]. Long-term graft survival is poorer in patients who have experienced an acute [11,12] or subclinical rejection [13] and humoral [14] and late rejections [15] are particularly harmful. Poor adherence to prescription is an under-rated cause for graft dysfunction and failure [16,17] and is often caused by the aesthetic disfigurements or other side effects of calcineurin inhibitors (CNIs) and steroids. Cytomegalovirus infection is also associated with shorter graft survival [18] because it may favour the development of early acute rejection [19] or expose patients to late allograft dysfunction [20].

There are many cases of late progressive allograft dysfunction because of nonalloimmune factors such as the donor and recipient characteristics mentioned above, and nonspecific factors of progression such as arterial

hypertension, glucose intolerance, hyperlipidaemia, atherosclerosis, which may be caused or aggravated by CNIs and steroids [21,22]. And the nephrotoxicity of CNIs gives rise to particular concerns.

Clinico-pathological conditions caused by identifiable factors (i.e. *de novo* or recurrent thrombotic microangiopathy, polyoma BK virus nephritis, recurrent or *de novo* glomerulonephritis and vasculitis, chronic ureteral obstruction, bacterial infection, etc.) should be considered separately as specific chronic diseases.

The three leading causes of post-transplantation death are cardiovascular diseases, infections and tumours [23]. Cardiovascular diseases are more frequent in elderly patients, smokers, recipients who have been on long-term dialysis, and those who have experienced pretransplant cardiovascular events [24], and their post-transplant development may be favoured by arterial hypertension, diabetes, hyperlipidaemia and accelerated atherosclerosis. These risk factors are often caused or aggravated by CNIs and/or corticosteroids and there is increasing evidence that CMV infection can increase the risk of atherosclerosis [25–27]. Cancer is a major cause of morbidity and mortality in renal transplant recipients, and the greater the immunosuppression, the higher the risk of cancer. NonHodgkin lymphomas are particularly frequent, and the risk of developing a post-transplant lymphoproliferative disease is greater in patients with CMV infection [28] and those treated with thymoglobulins and OKT3 [29].

Theoretically, on the basis of the above, to prevent late graft dysfunction and reduce post-transplant mortality, minimizing the risk of rejection should be accompanied by: (i) reducing CNI doses; (ii) avoiding or minimizing the use of corticosteroids; (iii) preventing the development of CMV infection; (iv) preventing vascular proliferation and (v) reducing the risk of cancer.

i) Is it possible to avoid or minimize the use of CNIs?

In one randomized controlled trial (RCT), the patients assigned to daclizumab, mycophenolate mofetil (MMF) and steroids experienced significantly more rejections and nonsignificantly fewer 1-year graft survivals than those receiving the same regimen plus standard- or low-dose cyclosporine (CsA) [30].

Good short-term results have been obtained by combining sirolimus, MMF and steroids [31]. A controlled trial comparing sirolimus, MMF and steroids with tacrolimus, MMF and steroids (with both arms being given thymoglobulins for induction purposes) found that graft function and survival were similar in the two groups, but 1-year control renal biopsies showed fewer chronic vascular changes in the sirolimus group [32]. However, a retrospective review of the Scientific Registry of Renal Transplant Recipients found that a combination of MMF, sirolimus and steroids was associated with poorer graft survival and an increased incidence of acute rejections than regimens based on CNIs [33]. Moreover, combining sirolimus and MMF may increase the risk of gastrointestinal complications [34], anaemia [35], thrombocytopenia and other minor side effects including oral ulcers [36]. Regimens based on polyclonal anti-lymphocyte antibodies, MMF and steroids have shown acceptable acute rejection rates, but high rates of CMV and opportunistic infections [37]. There have been reports of profound leucopenia, pulmonary toxicity and a high rate of acute rejection (including some irreversible humoral rejections) in patients treated with alemtuzumab followed by CNI-free immunosuppression [38].

The common feeling is that CNIs are still needed to prevent rejection [39], but some investigators are concerned about the risk of progressive renal lesions caused by nephrotoxicity. Nankivell *et al.* [21] performed protocol kidney biopsies for up to 10 years in kidney and pancreas transplant recipients and, after 10 years, observed chronic allograft nephropathy in 58%, with sclerosis in 37% of glomeruli; once established, the tubulo-interstitial and glomerular damage was irreversible and led to declining renal function and graft failure. However, 10-year graft survival was excellent (95%) and mean serum creatinine levels remained stable at about 1.6 mg/dl.

A few RCTs have explored the possibility of minimizing CNIs while using mTOR inhibitors. One investigated the withdrawal of CsA a few weeks after transplantation when the risk of acute rejection is less: the 525 patients received CsA, sirolimus and steroids for 3 months and were then randomized to continue triple therapy or to stop CsA while increasing the sirolimus dose (95 patients were not randomized because of delayed graft function or rejection). Protocol-mandated biopsies were performed at engraftment and after 12 and 36 months and 484 biopsies were blindly assessed by two pathologists using the Chronic Allograft Damage Index (CADI). After 36 months, the mean CADI score of the patients with serial biopsies was significantly lower in those treated with sirolimus and steroids, as was the mean tubular atrophy score [40]. After 4 years, the mean glomerular filtration rate (GFR) for any quartile of the patients receiving sirolimus and steroids was significantly higher than in the patients on triple therapy and the benefit was more marked if baseline GFR was ≤45 ml/min; the rates of mortality and graft loss were not significantly different between the two groups [41]. These data are interesting even though they come from a selected population, moreover the patients in the control group were penalized because they received sirolimus together with standard

CsA doses, an association that is now known to increase the nephrotoxicity of CsA [42] as a result of an increased expression of pro-fibrotic TGFβ-1 [43].

A review of studies involving conversion from a CNI to sirolimus in kidney transplantation patients yielded five randomized and 25 nonrandomized trials. In the former, the conversion to sirolimus improved short-term creatinine clearance in comparison with the controls and, in the nonrandomized studies, renal function improved or stabilized in 66% of the cases and cholesterol and triglyceride levels increased. Sirolimus was discontinued by 28% of the patients in the randomized trials and 17% in the nonrandomized trials. The authors concluded that adequately powered randomized trials with a longer follow-up of hard outcomes are needed to determine whether this strategy leads to a lasting benefit in the clinical care of transplant recipients [44].

A different approach may be to combine an mTOR inhibitor with low doses of CsA. One RCT gave renal transplant recipients standard or low doses of CsA aimed at maintaining trough blood drug levels of CsA between 50 and 100 ng/ml as well as basiliximab for induction, everolimus at a dose of 3 mg/day and steroids. After 3 years, failures (death, graft loss, acute rejection, loss to follow-up) were significantly less frequent in the patients receiving low-dose CsA (17% vs. 36%), as were graft losses, and acute and chronic rejections, as well as discontinuations and serious adverse events; mean creatinine clearance was also better [45]. Another RCT compared two different doses of everolimus in 420 patients given low-dose CsA and steroids and found a cumulative 1-year graft survival of 94.3% with a mean creatinine clearance of 64 ml/min [46].

ii) Is it possible to avoid or minimize the use of steroids?

A meta-analysis of RCTs found that CsA-treated patients who stopped taking corticosteroids had a significantly higher rate of acute rejection and graft failure than patients who did not [47]. On the contrary, a multicentre RCT with a long-term follow-up found that the patients assigned to receive CsA alone had a higher incidence of acute rejection but better 9-year graft survival than the patients given CsA together with steroids; the patients assigned to steroid-free immunosuppression also showed a significantly lower incidence of cardiovascular disease, cataracts and osteoporosis [48]. A more recent meta-analysis of studies in which transplant recipients were treated with tacrolimus or CsA microemulsion confirmed a significantly higher incidence of rejection but a reduced risk of hypercholesterolemia in the patients who stopped taking steroids [49]. Reviewing the data of the Collaborative Transplant Study, Opelz *et al.* [50] reported significantly better 7-year patient and pure graft survival rates with significantly improved risk factors among the patients who stopped steroids than in those who continued them. In another RCT, 150 kidney recipients treated with basiliximab CNI and MMF or sirolimus stopped steroids on the second day and 150 continued them: 3-year graft survival was 79% in the controls and 78% in the steroid-free group. The acute rejection rate and serum creatinine levels were similar in the two groups [51]. The feasibility of avoiding steroids in the early postoperative days has also been confirmed by recent RCTs with short -term follow-ups [52–54].

All of the above RCTs used standard CNI doses and few data are available concerning the possibility of avoiding steroids in regimens based on low CNI doses. In one RCT [55], 113 renal transplant recipients received basiliximab, everolimus 3 mg/day and CsA targeted to keep trough blood levels of between 50 and 100 ng/ml and were randomized to stop steroids within the first post-transplant week or to continue with prednisone. After 2 years, there was a higher risk of rejection among the steroid-free patients but the difference was not significant. Two-year graft survival was 95% in the patients randomized to stop steroids and 87% in those who continued. In another study [56], 96 patients received thymoglobulin induction, sirolimus, arginine and omega-3 fatty acids. MMF was discontinued within 2 years and CsA was given at reduced doses for 4, 6 or 12 months. After 3 years, 79% of the patients were rejection free; furthermore, 90% of the 84 patients at risk at the end of the study were steroid free and 87% were off CNI. In a non randomized study [57], 82 renal transplant patients received thymoglobulin plus tacrolimus, MMF and prednisone for 6 days, and then maintenance therapy with sirolimus, MMF and tacrolimus minimization: 91% of the kidney recipients with functioning grafts remained steroid free.

iii) Is it possible to reduce the risk of CMV infection?

Monocytes and macrophages play a key role in disseminating CMV to host tissue. Blood monocytes do not allow viral replication but, if they extravasate into host tissue monocytes, may subsequently differentiate into permissive macrophages. Human CMV up-regulates the phosphatidylinositol-3kinase (PI-3K) activity that is essential for the transendothelial migration of infected monocytes and the activated monocytes express a number of inflammatory mediators via PI-3K signalling [58]. The administration of sirolimus or everolimus (which inhibit the activity of mTOR, the downstream effector of PI-3K) may therefore inhibit the translation and proliferation signals coming

from the cascade of kinases governed by PI-3K. However, mTOR may be found in two complexes that differ in their binding partner: rictor or raptor. Although the activity of the raptor complex is normally inhibited by sirolimus, this inhibition can be circumvented because human CMV can induce an alternative phosphorylation pathway [59]. Human CMV infection also activates the rictor complex, which is more significant for viral infection: this phosphorylation is insensitive to mTOR inhibitors but, in the case of raptor and rictor depletion, the rictor complex becomes sensitive to sirolimus [60]. These data suggest that the rictor- and raptor-containing complexes can be modified by factors such as cell stress, substrate specificities, etc. and that their sensitivity to mTOR inhibitors can be altered. On the other hand, inhibition of the PI-3K pathway can modulate a viral IL-10 homologue [61] that is developed by CMV to circumvent its detection and destruction by the host immune system [62].

There is some clinical evidence that mTOR antagonists may at least partially inhibit CMV replication and inactivate the infected cells. In one RCT, Eisen *et al.* [63] found that the incidence of CMV infection was significantly lower in cardiac transplant recipients treated with standard doses of CsA, steroids and everolimus than in those treated with CsA, steroids and azathioprine. A 2% incidence of CMV infection was found in 150 liver transplant recipients who received sirolimus as primary immunosuppression [64]. In renal transplant recipients, three RCTs of everolimus in association with reduced CsA doses [45,55,65] found an incidence of 2–2.6%, and a meta-analysis comparing the risk of CMV infection in patients given a CNI in combination with an mTOR inhibitor or an inhibitor of nucleotide synthesis, found that the incidence of CMV infection was significantly lower in patients given mTOR inhibitors, with a relative risk of 0.49 [66].

iv) Is it possible to prevent transplant vasculopathy?

Allograft vasculopathy is a result of smooth muscle cell proliferation in the intima of kidney vessels, which leads to vessel occlusion and a restricted blood supply, and eventually to renal graft insufficiency. A key role in this process is played by endothelial cells and vascular endothelial growth factor (VEGF).

A number of events – including acute rejection, CNI nephrotoxicity, CMV infection and ischemia-reperfusion injury – may damage the endothelium of kidney allograft vasculature. The response is the recruitment of polymorphonuclear cells leading to inflammation, oxidative stress, senescence and the sloughing of endothelial cells into the circulation. To restore renal vascular integrity, endothelial progenitor cells (EPCs) are recruited from the bone marrow and they migrate to the inflamed tissues where they facilitate endothelial cell repair. If the injury persists, there is an excessive response that leads to the over-recruitment of leukocytes and EPCs, thus facilitating inflammation and angiogenesis under the influence of VEGF and as the angiogenetic reaction is itself pro-inflammatory, this process becomes self-sustaining [67]. Moreover, the recipient endothelial cells may process and present allogeneic peptides to T cells by means of mechanisms that are similar to the indirect pathway of allorecognition [68] and this may lead to further cell lysis and ongoing damage. In the long term, inflammation, neo-angiogenesis and rejection may increase the risk of chronic lesions with the development of allograft dysfunction. Recipient-derived lymphatic progenitor cells can also contribute to inflammation in renal transplants as they can transmigrate through the connective tissue stroma (presumably in the form of macrophages) under the influence of the lymphangiotrophic growth factor VEGF-C [69] .

Manipulating the response may protect against injury and the chronic disease processes. As PI-3K and its downstream effector mTOR are essential for modulating the effects of VEGF and providing the signal for endothelial cell proliferation and angiogenesis [70], both sirolimus and everolimus may prevent neo-angiogenesis by inhibiting mTOR. They may also induce EPC apoptosis [71] and inhibit the replacement of donor peritubular capillary endothelium by endothelial recipient cells, a mechanism that may lead to peritubular ischemia and consequent interstitial fibrosis [72]. Experimental studies have shown that sirolimus can prevent intimal thickening in different models of immune- and nonimmune mediated artery injury [73–75]. In clinical practice, coronary stents eluted with sirolimus or everolimus [76,77] may prevent neointimal hyperplasia and coronary restenosis, although the risk of early stent thrombosis is not significantly different between drug-eluting and bare-metal stents [78]. In the field of transplantation, RCTs and nonrandomized studies have clearly shown that everolimus can protect cardiac transplant recipients from transplant vasculopathy [62,79].

In brief, the available data suggest that mTOR inhibitors may: (i) reduce the intimal proliferation responsible for occlusive vasculopathy; (ii) inhibit the replacement of donor peritubular capillary endothelium by endothelial recipient cells; (iii) interfere with neo-angiogenesis and (iv) prevent the new production of recipient endothelial cells that may trigger indirect allorecognition.

v) Is it possible to reduce the incidence of tumours?

Although many factors may contribute to the etiopathogenesis of post-transplant cancer, the main causes are the

intensity [80] and duration of immunosuppression [81]. Among the drugs used to treat transplant recipients, mTOR inhibitors have proved to have anti-neoplastic properties and this is also generating increasing interest in oncologists [82]. A number of growth factors can activate PI-3K which, through the mediation of mTOR, phosphorylates various protein kinases (S6k, Cdk, 4EBP) that have an impact on cancer cell survival and proliferation. Physiologically, this pathway may be inhibited by the tumour suppressor gene PTEN [83] and there is now evidence that several oncoproteins may derive from an overactive PI-3K pathway [84,85] or the loss of PTEN [86].

By inhibiting the downstream effector of PI-3K, mTOR inhibitors may interfere with the proliferation of a number of cancer cell lines [87]. PTEN-deficient cancer cells are highly sensitive to rapamycin, whereas cell lines with wild-type PTEN are at least 1000-fold less sensitive [88]. Both everolimus and sirolimus have also shown an anti-proliferative effect on EBV-transformed B cells in culture [89] and in mice [90] and further studies have validated them as a new treatment option for primary effusion lymphoma [91]. They may also interfere with neoplasms by means of other mechanisms. Vascular endothelial proliferation, survival and migration are controlled by VEGF, which operates through the mediation of the PI-3K kinase cascade [92]. The interference of sirolimus with VEGF-induced endothelial cell stimulation has led to anti-angiogenesis and delayed cancer progression in experimental models [93] and in human renal cancer metastases [94]. PI-3K/Akt and mTOR [95] also modulate the expression of hypoxia-inducible factor, a key regulator of cancer cell response to hypoxia.

Clinical trial results have shown that mTOR inhibitors are well tolerated and may induce prolonged stable disease and tumour regression in cancer patients [96]. Preliminary investigations seem to indicate that the use of mTOR inhibitors as immunosuppressive agents may reduce the risk of neoplasia in transplant recipients. Kahan *et al.* [97] have reported a low incidence of lymphoproliferative disorders (0.4%), renal cell carcinoma (0.2%) and skin cancer (1.9%) in renal allograft recipients treated with a combination of sirolimus and CsA. A meta-analysis of five multi-centre trials of sirolimus found that, 2 years after kidney transplantation, patients receiving sirolimus in combination with CsA had a significantly lower incidence of skin cancer than patients given CsA and placebo: the patients receiving sirolimus as base therapy had no malignancies compared with a 5% of those assigned to CsA [98]. Reviewing data from more than 30 000 primary kidney transplant recipients, Kauffman *et al.* [99] found that the relative risk of any *de novo* cancers in patients receiving an mTOR inhibitor alone or in combination with CNIs was 0.39 when compared with patients given CNIs but not mTOR inhibitors. Switching from a CNIs to everolimus was found to be safe and led to lesion regression or improvement in seven transplant patients with skin tumours and in one patient with post-transplant lymphoproliferative disorder [100] and Campistol *et al.* [101] reported the complete regression of Kaposi's sarcoma in two renal transplant recipients after conversion from CsA to sirolimus. Other investigators have confirmed the efficacy of sirolimus in reversing the cutaneous [102] and visceral lesions [103] of Kaposi's sarcoma, although it proved to be ineffective or only transiently effective in a minority of patients [104].

Side effects

Like other immunosuppressive agents, mTOR inhibitors are not devoid of side effects. Up to 80% of renal transplant recipients treated with sirolimus or everolimus may develop hypercholesterolemia and hypertriglyceridemia requiring treatment with statins [105,106]. Their effects on glucose metabolism are controversial. As phosphatase 2A in β cells may play a key role in insulin secretion, the inhibition of its activity caused by anti-mTOR agents might favour the onset of diabetes [107,108]. On the other hand, sirolimus and everolimus may protect from glucose intolerance as mTOR makes the insulin-receptor substrate unresponsive to insulin [109]. Thrombocytopenia and anaemia are frequent, although usually mild [110]. Within 1 year of converting from CsA to an mTOR inhibitor, up to 30% of renal transplant recipients may develop proteinuria [111], which might be due to increased intraglomerular pressure with glomerular hyperfiltration [112]; however, mTOR inhibitors may also cause proteinuria by interfering with protein endocytosis in tubular epithelial cells [113,114]. Mouth ulcers, joint pain and oedema can also occur and are usually dose-dependent. Retarded wound healing [115] and lymphocele [116] are other possible complications.

Conclusions

The fact that experimental and clinical studies have shown that mTOR inhibitors may help to solve some important problems related to post-transplant immunosuppression does not mean that we should abandon the drugs that have reduced the risk of rejection and improved graft survival. However, the introduction of mTOR inhibitors may allow the prevention of rejection while minimizing the doses of corticosteroids and CNIs, the agents mainly responsible for causing substantial side effects in renal transplant recipients. Furthermore, mTOR inhibitors can potentially protect against the development of malignancy, CMV infection, transplant vasculopathy and cardiovascular disease. The short-term results of anti-

mTOR-based regimens are encouraging with or even without low-dose CNIs and steroids. However, the main advantages of such immunosuppressive strategies should be seen in the long term, with a lower risk of developing CNI toxicity and a lower risk of death because of cardiovascular disease or tumours.

Conflicts of interest

C.P. is a consultant for Novartis, Italy.

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