Critical analysis of graft loss and death in kidney transplant recipients treated with mTOR inhibitors

Análise crítica da perda do enxerto e óbito em receptores de transplante renal tratados com inibidores da mTOR

Authors

Luis Gustavo Modelli de Andrade [1]

Helio Tedesco-Silva [2]

[1] Universidade Estadual Paulista.

[2] Universidade Federal de São Paulo.

ABSTRACT

Registry studies and systematic reviews have shown higher risk for mortality and graft loss in patients in use of mTOR inhibitors (mTORi) compared to calcineurin-based (CNI) immunosuppressive regimens. The majority of these studies pooled data from early trials using different strategies such as "*de novo*" combination of the high dose mTOR inhibitors with standard dose of CNI or high dose mTORi combined with mycophenolate. The large heterogeneity of these initial exploratory studies, many of them no longer in use, turns difficult any comparison with a well-defined standard of care regimen. The new strategies using concentration controlled reduced exposure of mTORi and CNI or early conversion from CNI to mTORi use have shown comparable patient and graft survival. Nevertheless, considering the central role of mTOR in health and disease states, more research is necessary to mitigate the adverse events and to explore further the potential beneficial effects of mTOR inhibitors.

Keywords: mortality; kidney transplantation; serine-threonine kinases; sirolimus.

RESUMO

Estudos de registro e revisões sistemáticas mostraram um aumento de mortalidade e perda do enxerto nos pacientes em uso dos inibidores da mTOR (imTOR) em comparação a regimes baseados nos inibidores de calcineurina (iCN). A maioria destes estudos reuniu dados de ensaios clínicos iniciais utilizando diferentes estratégias, tais como a combinação "de novo" de altas doses de imTOR com doses padrão de iCN ou altas doses de imTOR combinado com micofenolato. A grande heterogeneidade destes estudos exploratórios iniciais, muitos deles não mais em uso, tornam difícil qualquer comparação. As novas estratégias que utilizam a concentração controlada e reduziram a exposição tanto de imTOR quando de iCN mostraram sobrevida do paciente e enxerto comparáveis. No entanto, considerando o papel central dos imTOR nos estados de saúde e doença, é necessária mais investigação para mitigar os eventos adversos e explorar melhor seus potenciais efeitos benéficos.

Palavras-chave: mortalidade; serina-treonina quinases TOR; sirolimo; transplante de rim.

INTRODUCTION

The clinical use of mammalian target of rapamycin (mTOR) pathway inhibitors following kidney transplantation remains challenging even after more than 20 years of clinical trials. During these years, several analyses using database registration data indicate that patients receiving mTOR inhibitors(imTOR), mainly sirolimus (SRL), are at increased risk of mortality and graft loss compared to patients receiving cyclosporine (CSA) or tacrolimus (TAC) in combination with mycophenolate (MMF)[1-8] (Table 1).

The first study, published in 2004, used data from the United States transplant registry, including 23,016 kidney transplants performed between 1998 and 2003, of which 1999 (8.7%) received CSA/SRL, and 21,017 (91.3%) who received CSA/MMF.[1] A 4-year graft survival was lower among patients receiving CSA/SRL (74.6% *vs*. 79.3%, HR = 1.22).

The second study, published in 2005, also analyzed data from the United States

							TABLE 1	DESCRIPTION OF THE MAIN STUDIES THAT SHOWED A HIGHER RISK OF GRAFT LOSS OR DEATH IN RENAL TRANSPLANT RECIPIENTS WHO USED imTOR	

Ref	Study type	Year Inclusion Time Follow up	iCN	imTOR with iCN n (%)	imTOR without iCN n(%)	imTOR use	imTOR Group characteristics	Graft survival	Patient survival
(1)	SRTR Registry	1998 to 2013 4 years	21,017	1999	-	*De novo*	CsA/SRL: lower use of indication in the group	79.3% CsA/ MMF 74.6 CsA/ SRL (HR = 1.22)	ns.
(2)	SRTR Registry	2000 to 2004 3 years	44,915	5393	-	*de novo*		85.9% TAC/ MMF 85.3% CsA/ MMF (HR = 1.15) 82.2% CsA/ SRL (HR = 1.38) 80.3% TAC/ SRL (HR = 1.47)	92.2% TAC/ MMF 91.0% CsA/ MMF (HR = 1.22) 90.0% CsA/ SRL (HR = 1.49) 89.9% TAC/ SRL (HR = 1.41)
(3)	SRTR Registry	2000 to 2005 5 years	49,412	6394 (73%)	2325 (27%)	*de novo*	SRL/MMF: higher donor age, higher rate of deceased donor	73.8% TAC/ MMF 71.8% CSA/ MMF (HR = 1.16) 68.9% TAC/ SRL (HR = 1.38) 67.6% CSA/ SRL (HR = 1.37) 57.7% SRL/ MMF (HR = 2.01)	TAC/MMF CSA/MMF (HR = 1.17) TAC/SRL (HR = 1.33) CSA/SRL (HR = 1.49) SRL/MMF (HR = 1.75)
(4)	Hungary cohort	2007 3 years	1241	37 (37%)	64 (63%)	conversio	Higher number with neoplasia and diabetes	ns.	ns. Patients with a history of neoplasia (HR = 2.6-5.6)
(5)	UNOS Registry	1999 to 2010 2-8 years	125,623	10,510 (76.4%)	3,237 (23.5%)	*de novo*	imTOR without iCN: higher number with a history of malignancy, diabetes and kidney from expanded-criterion donor; higher PRA and TIF	iCN/imTOR (HR = 1.07) imTOR/ MMF (HR = 1.17)	iCN/imTOR (HR = 1.13) imTOR/ MMF (HR = 1.25)
(6)	Systematic review	1999 to 2013 1 year to 4 years	2,600			*de novo* 4,717 (80.3%) conversion 1,159 (19.7%)	unreported	Not analyzed	imTOR (HR = 1.43)

CONTINUATION TABLE 1.

(7)	Australia and New Zealand Registry	1996 to 2012 7 years	7,801	398 (25.5%)	1,162 (74.5%)	de novo 481 early conversion 504 late conversion 567	Higher number of Caucasians and history of neoplasia. Lower number of patients with a history of diabetes and cardiovascular disease	ns.	imTOR (HR = 1.47)
(8)	SRTR Registry	2000 to 2013 5 years	56,764	2,167 (44.9%)	2,659 (55.1%)	de novo	African-American recipient; expanded criterion donor with coronary disease	TAC/MMF TAC/SRL (HR = 1.38) SRL/MMF (HR = 1.41)	TAC/MMF TAC/SRL (HR = 1.59) SRL/MMF (HR = 1.44)

transplant registry of 44,915 adult kidney transplants performed between 2000 and 2004.[2] In this analysis, 3,524 (7.8%) patients received TAC/SRL; 27,007 (60.1%) TAC/MMF; 1,869 (4.2%) CSA/SRL and 12,515 (27.9%) CSA/MMF. The number of patients receiving CT/MMF increased from 42.1% in 2000 to 74.5% in 2004. No differences were found in the incidence of acute rejection between the groups (11.5-12.6%) during the first six months of transplantation. Graft survival over 3 years was 85.9% (TAC/MMF); 85.3% (CsA/MMF); 82.2% (CsA/SRL) and 80.3% (TAC/SRL).

The relative risk of graft loss was higher for patients who received TAC/MMF (HR = 1.47) or CsA/SRL (HR = 1.38). Patients' survival at 3 years was 92.2% (TAC/MMF); 91.0% (CsA/MMF); 90.0% (CsA/SRL) and 89.9% (TAC/SRL) among the patients who received SRL. These effects were more evident in recipients who received kidneys with pre-existing structural or functional damage - more vulnerable to the nephrotoxic action of calcineurin inhibitors (iCN).

The third study, published in 2007, again evaluated data from 58,131 renal transplant recipients from the United States transplant registry between 2000 and 2005.[3] Five immunosuppressive regimens were evaluated, with 62% of patients receiving TAC/MMF, 23% CSA/MMF, 7% TAC/SRL, 4% CSA/SRL and 4% SRL/MMF. The use of CT/MMF increased from 41.6% in the year 2000 to 80.4% in 2005, while no large variations were found in the SRL groups.

Compared with TAC/MMF, patients from the SRL/MMF group received kidneys from donors of a higher average age (36.8 vs. 41.4 years), which proportion of deceased donors was higher (58 vs. 66%). Compared with TAC/MMF, the only group with a higher risk of acute rejection was the one that received SRL/MMF (HR = 1.53). The 5-year graft survival was 73.8% (TAC/MMF); 71.8% (CSA/MMF); 68.9% (TAC/SRL); 67.6% (CSA/SRL) and 57.7% (SRL/MMF). Compared with the TAC/MMF group. There was a progressive increase in the risk of graft loss in 5 years in patients under CSA/MMF (HR = 1.16); TAC/SRL (HR = 1.38); CSA/SRL HR = 1.37) and SRL/MMF (HR = 2.01).

The fourth study, published in 2012, evaluated 993 renal transplant recipients who, in August 2007, had a median follow-up time of 72 months after transplantation.[4] At the initial visit, 101 patients (10.8% of the cohort) were using imTOR (SRL 78.2% and EVR 21.2%), combined (37%) or not (63%) with iCN.

The main determining reasons for the use of imTOR were a history of malignancy and the suspicion of nephrotoxicity by iCN. At the initial visit, the patients in the imTOR group had a higher mean age, a higher proportion of diabetes (15% vs. 3%), a higher proportion of malignancy history (33% vs. 2%), and a higher number of B and DR -loci incompatibilities, higher Charlson comorbidities index, lower glomerular filtration rate (47 vs. 51 ml/min/1.73 m²), higher cholesterol and triglyceride concentrations, and lower albumin concentration.

Considering the study design, several types of complex statistical analyzes were performed, yielding inconsistent results. However, a higher risk of death

was found only in the population using imTOR, analyzing separately those patients with no history of malignancy (OR = 2.9, n = 943), but not in the total population.[4]

The fifth study, published in 2013, again used information from the United States transplant registry database, including data from 139,370 adult or pediatric recipients of the first kidney transplant performed between 1999 and 2010.[5] Three groups were analyzed, one with patients receiving iCN without imTOR (iCN, n = 125,623, 90.1%); another with imTOR without iCN (imTOR, n = 3237, 2.3%) and the last one using imTOR and iCN (iCN/imTOR, n = 10,510, 7,5%).

Compared with the iCN group, the group without imTOR was composed of patients with a higher proportion of diabetes (32.1% *vs*. 29.8%); cardiovascular disease (18.7% *vs*. 16.9%), PRA > 10% (18.8% *vs*. 16.4%), longer cold ischemia time (14.5h *vs*. 12h), and higher proportion of expanded criterion donor (14.2% *vs*. 10%).

During the first 2 years after transplantation, the imTOR group had a higher risk of graft loss and death compared to the iCN group. Likewise, the imTOR group was independently associated with a higher risk of death (HR = 1.25) and loss of graft (HR = 1.17) in fully adjusted analyses for the second and eighth year after transplantation. In this paper the iCN regimen was not systematically compared with the iCN/imTOR regimen.

The sixth study, published in 2014, consisted of a systematic review of the literature, including 21 papers published between 1999 and 2013.[6] A total of 5,876 patients using SRL, either again (n = 4717) or in conversion (n = 1159) were found. In this meta-analysis, the use of SRL was associated with a reduction in the risk of neoplasia (HR = 0.60) and non-melanoma skin cancer (HR = 0.44). The risk of death was higher among patients who used SRL (OR = 1.43) again (OR = 1.39), in conversion (OR = 1.59) or at a high dose (> 10 ng/ml, OR = 1.53), but not a low dose of sirolimus (< 10 ng/ml, OR = 1.07).

The seventh study, published in 2016, analyzed transplant registry data from Australia and New Zealand,[7] including a longitudinal cohort of 9,353 adult patients who underwent 9,558 kidney transplants between 1996 and 2012, with graft survival ≥ 1 year and followed up for a median time of 7 years.

The groups analyzed included patients who used imTOR from the start of transplantation (n = 481, 5%, of these: 83% combined with iCN), in early conversion (n = 504, 5.3%) or late conversion (n = 567.5, 9%), compared with patients receiving iCN without imTOR (iCN, n = 7801, 82%). Among patients who received imTOR, there was a higher number of white patients (87.3% *vs*. 81%) and history of neoplasia (37.6% *vs*. 29%), but a lower percentage of diabetics (10% *vs*. 13%) and history of cardiovascular disease (11.3% *vs*. 15%). The use of imTOR, regardless of the strategy, was associated with a higher risk of death (HR = 1.47).[7]

The eighth study, published in 2016, again used data from the United States transplant registry.[8] 61,300 kidney transplant recipients were enrolled between 2000 and 2013, 2,167 (3.5%) receiving TAC/SRL; 2,659 (4.3%) were given SRL/MMF and 56,764 received CT/MMF (93.2%).

Several demographic characteristics of donors and recipients were different among the three groups. The number of transplants performed in the initial period between 2000 and 2009 was higher for patients who received TAC/SRL (83.9%) and SRL/MMF (94.7%) compared to TAC/MMF (58.7%). The risk of death was higher among patients who received TAC/SRL (OR = 1.38) and SRL/MMF (OR = 1.41) compared to those who received CT/MMF. In recipients with negative pre-transplant serology for the Epstein-Barr virus, cytomegalovirus, hepatitis B and C the risk of neoplasia was lower in those who received SRL compared to those who received CT/MMF.

CRITICAL ANALYSIS OF MORTALITY STUDIES AND GRAFT LOSSES WITH imTOR USE

Several aspects should be considered by analyzing this set of published studies. The first is that, of the eight studies cited, five used the same database of transplant data from the United States, with small variations of the cohort analyzed[1-3,5,8] (Table 1).

Thus, it is not surprising that the outcomes were similar. The Australian and New Zealand registry study,[7] the observational study from Hungary[4] and the meta-analysis[6] with individual patient data from 21 published studies also showed equivalent outcomes. However, in these studies, there is a high heterogeneity in the patients' demographics, in the strategies regarding the use of new or conversion, in the combinations of SRLs with iCN or MMF and

in the doses or concentrations of imTOR and iCN (Table 1).

The second aspect is the huge disproportion of patients receiving imTOR. In all the analyses, less than 10% of the population received an immunosuppressive regimen with imTOR, compared to standard regimens such as CSA/MMF or CT/MMF.[6] In addition, in these analyses, groups of patients receiving CSA/MMF or TAC/MMF are more contemporary due to the increasing use of these combinations.

Although the statistical tests used can adjust for these differences, including demographic differences, residual risk factors not included in the statistical models may still interfere with the interpretation of the outcomes found. For example, a registry data analysis of the Collaborative Transplant Study (CTS) demonstrated an association between dose of steroid and mortality, either cardiovascular or infectious.[9] Steroid doses were not included as a risk factor in analyses of transplant registry data comparing the use of imTOR and iCN.

Another important point is that these analyses were performed correctly by intention to treat. However, changes in immunosuppressive regimens, common after renal transplantation, are not analyzed in these statistical models. In addition, in several of these analyses, experimental practices were analyzed as early and late conversion strategies for iCN for imTOR, either systematically or based on varying perceptions of risk, or for the patient - as in the case of neoplasia, or for the graft, as in the case of chronic nephrotoxicity. In these circumstances, it is difficult to establish the risk factors associated with unfavorable outcomes, which are usually attributed to any new therapeutic intervention implemented.

The two initial strategies combining imTOR with iCN or MMF clearly present efficacy and safety concerns. The combination of imTOR/iCN is known to be nephrotoxic, at least at the previously used doses/concentrations, which constitute most cohorts analyzed in the registry data studies. In the 4-large prospective and randomized clinical trials, two using SRL[10,11] and two using EVR[12,13] in combination with standard doses of cyclosporine, renal function was lower in the groups receiving SRL or EVR compared to the control groups.

The association between renal function, patient[14] and graft.[15] survival is well known. Even then, in one of the recent transplant registries in the United States,[5] patients using iCN/IMT had death and graft loss outcomes very close to those observed in patients receiving iCN without imTOR. Studies

have only recently been conducted to identify the dose/concentration combinations of the two drug classes (imTOR and iCN) associated with a better relationship between efficacy and safety.

On the other hand, extensive clinical information from prospective and randomized clinical trials indicates that the combination of imTOR/MMF does not present sufficient efficacy for the prevention of acute rejection in the first year of transplantation, even using more effective induction therapies.[16-18] Furthermore, there is an overlap of adverse reactions associated with this regimen, mainly hematological and gastrointestinal toxicities.

The lack of efficacy and safety found in iCN conversion studies for imTOR, whether early or late, has been attributed to the use of imTOR.[4,7] While the early iCN-to-imTOR conversions in the first 6 months after transplantation are associated with increased risk of acute rejection and development of anti-HLA-specific donor antibodies,[19] late conversions after the first year of transplantation were not associated with improvements in renal function and still produced an increase in the incidence of adverse reactions.

Recent data indicates that lack of efficacy may be associated with discontinuation of iCN, as this is the most effective class of drugs to inhibit memory T-cell proliferation[20] and, therefore, to prevent all rejection phenotypes, including early or late ones, mediated or not by specific anti-HLA antibodies against donors.[21] On the other hand, lack of safety stems from the overlap of adverse reactions from the combination of relatively high doses of imTOR/MMF, due to perceived higher risk of rejection associated with this immunosuppressive regimen.

Although specific causes of death and graft loss have not been studied systematically, adverse events typically associated with the use of imTORs such as proteinuria,[22-24] *diabetes mellitus* after transplantation[25-27] and dyslipidemia[27] may be involved in these findings.

These complications, however, are associated with the relatively high blood concentrations of iCN and imTOR initially used. In contrast, preliminary data suggests that the use of imTORs is associated with a reduction in left ventricular hypertrophy, independent of blood pressure response and use of angiotensin converting enzyme inhibitors.[28-30]

A less often used strategy was the use of CSA/SRL with interruption of the use of CSA after 3 months of transplantation. In the original study, 215 renal transplant recipients were randomized to discontinue the use of CSA and 215 maintained the CS/SRL combination.

After 4 years of follow-up, the group of patients who interrupted presented better renal function (58.3 vs. 43.8 ml/min, $p < 0.001$) and longer graft survival (91.5% vs. 84.2% $p = 0.024$) compared to the group that maintained CSA use, with no differences in mortality (4.7% vs. 7.9%). The difference in biopsy-proven acute rejection incidence after randomization (10.2% vs. 6.5%) was not significant.[31]

The major criticism of this study is the lack of a control group of patients receiving CSA/MMF, since the survival of the CSA/SRL group may have been negatively influenced by the combination of nephrotoxic doses of iCN with imTOR. The small increase in the incidence of acute rejection following discontinuation of CSA in this study encouraged the development of subsequent clinical trials comparing the efficacy and safety of a CSA dose reduction or discontinuation with adjustments based on the blood concentration of the two drugs,[32,33] giving rise to more contemporary strategies for minimizing iCN in combination with imTOR.

MORTALITY AND GRAFT LOSS OUTCOMES IN STUDIES INVOLVING NEW PROPOSALS FOR imTOR USE

The most recent proposals for the use of imTORs are based on the use of reduced doses of imTORs and iCNs adjusted to maintain blood concentrations within predetermined therapeutic ranges, either in combination from the start of transplantation[34-37] or in early conversion strategies of iCN for imTOR[38-42]. In these studies, the incidence of adverse reactions typically associated with imTORs was lower than that observed in previous studies, resulting in a lower incidence of treatment discontinuation, especially when imTORs were combined with iCNs.

The systematic review by Knoll et al.[6] demonstrated higher mortality only in the studies using high doses of SRL, but not in those using low doses, suggesting that these newer strategies with reduced doses of imTOR combined with iCN may not be associated with increased risk of death. Indeed, in these more recent studies, with follow-up ranging from 2 to 8 years, no differences were found in the incidence of graft loss or death, either with the combined use of imTOR/iCN[34,43,44] or with early conversion strategies from iCN to imTOR.[40,42,45,46]

Similarly, in a single center retrospective analysis including 581 patients who participated in 10 clinical trials comparing SRL or EVR combinations or AZA or MMF in combination with iCN and antimetaboles, no difference was found in the incidence of acute rejection, graft loss, and patient death within 10 years of follow-up[47] (Table 2).

TABLE 2		RANDOMIZED CLINICAL TRIALS WITH MEDIUM TERM ASSESSMENTS USING MORE RECENT IMMUNOSUPPRESSION WITH imTOR				
Ref.	N	Regimens	Follow up time	Acute rejection (%)	Graft loss (%)	Death (%)
(40)	162	CSA → SRL/MMF (n =77) CSA/MMF (n = 85)	4 years	2.6 2.6	5.2 2.4	2.6 0
(43)	60	BAS/TAC/MMF (n = 30) BAS/CSAr/EVE (n = 30)	3 years	17 23	6.6 6.6	3 0
(34)	833	BAS/EVE (3-8 ng/ml)/CSAr (n = 277) BAS/EVE (6-12 ng/ml)/CSAr (n = 279) BAS/CSA/MPS (n = 277)	2 years	19.9 15.1 19.1	5.8 6.1 4.0	3.2 3.6 2.9
(46)	300	CSA → EVR/MMF (n = 155) CSA/MMF (n = 145)	5 years	13.6 7.5	2.6 2.1	2.6 2.6
(42)	182	CSA → CSA/MMF (n = 90) EVR/MMF (n = 92)	3 years	13 11.1	1.1 3.3	1.1 3.3
(44)	99	rATG (7,5mg/kg)/SRL/MMF rATG(7,5mg/kg)/CSA/MMF	8 years	nr nr	14 14.8	11 8
(45)	128	iCN → SRL/MMF (n = ?) iCN/MMF (n = ?)	8 years	22.7 14.5	15.2 19.4	7.6 9.7
(47)	581	iCN/SRL (n = 347) iCN/EVE (n = 128) iCN/AZA-MPA (n = 124)	10 years	22.2 22.7 22.6	19 18 23	12 10 13

Conclusions

The importance and central role of mTOR in various physiological and pathophysiological processes has become a pharmacological target for the treatment of a range of diseases, from neoplasms to immunosuppression after solid organ transplantation.[48] Despite this, the clinical use of imTOR has been complicated by the diversity of population heterogeneity, immunosuppression regimens, transplantation period, drug combinations, the doses/concentrations used and the potential for pharmacokinetic and pharmacodynamic interaction.

The highest incidence of death and graft loss found in the data analysis of transplant registries is not confirmed in more recent prospective studies. Due to its central role in multiple intracellular processes, the possibility of adverse reactions is higher - a fact that has limited its clinical use and probably the determining factor of the higher incidence of treatment discontinuation.

Advances in knowledge will be fundamental for the improvement of current strategies, considering the diversity of renal transplant recipients, changes in time after transplantation, the unpredictability of innumerable complications and the limited number of drug options, further aggravated by the absence of new compounds in advanced stages of development for clinical use in the coming years.

References

1. Meier-Kriesche HU, Steffen BJ, Chu AH, Loveland JJ, Gordon RD, Morris JA, et al. Sirolimus with neoral *versus* mycophenolate mofetil with neoral is associated with decreased renal allograft survival. Am J Transplant 2004;4:2058-66. DOI: http://dx.doi.org/10.1111/j.1600-6143.2004.00624.x

2. Meier-Kriesche HU, Schold JD, Srinivas TR, Howard RJ, Fujita S, Kaplan B. Sirolimus in combination with tacrolimus is associated with worse renal allograft survival compared to mycophenolate mofetil combined with tacrolimus. Am J Transplant 2005;5:2273-80. DOI: http://dx.doi.org/10.1111/j.1600-6143.2005.01019.x

3. Srinivas TR, Schold JD, Guerra G, Eagan A, Bucci CM, Meier-Kriesche HU. Mycophenolate mofetil/sirolimus compared to other common immunosuppressive regimens in kidney transplantation. Am J Transplant 2007;7:586-94. DOI: http://dx.doi.org/10.1111/j.1600-6143.2006.01658.x

4. Cortazar F, Molnar MZ, Isakova T, Czira ME, Kovesdy CP, Roth D, et al. Clinical outcomes in kidney transplant recipients receiving long-term therapy with inhibitors of the mammalian target of rapamycin. Am J Transplant 2012;12:379-87. DOI: http://dx.doi.org/10.1111/j.1600-6143.2011.03826.x

5. Isakova T, Xie H, Messinger S, Cortazar F, Scialla JJ, Guerra G, et al. Inhibitors of mTOR and risks of allograft failure and mortality in kidney transplantation. Am J Transplant 2013;13:100-10. DOI: http://dx.doi.org/10.1111/j.1600-6143.2012.04281.x

6. Knoll GA, Kokolo MB, Mallick R, Beck A, Buenaventura CD, Ducharme R, et al. Effect of sirolimus on malignancy and survival after kidney transplantation: systematic review and meta-analysis of individual patient data. BMJ 2014;349:g6679. PMID: 25422259 DOI: http://dx.doi.org/10.1136/bmj.g6679

7. Badve SV, Pascoe EM, Burke M, Clayton PA, Campbell SB, Hawley CM, et al. Mammalian Target of Rapamycin Inhibitors and Clinical Outcomes in Adult Kidney Transplant Recipients. Clin J Am Soc Nephrol 2016 Jul 21. [Epub ahead of print] DOI: http://dx.doi.org/10.2215/CJN.00190116

8. Santos AH, Casey MJ, Wen X, Womer KL. Association of Baseline Viral Serology and Sirolimus Regimens with Kidney Transplant Outcomes: A Fourteen Year Registry-Based Cohort Study in the US. Transplantation 2017;101:377-86. DOI: http://dx.doi.org/10.1097/TP.0000000000001520

9. Opelz G, Döhler B. Association between steroid dosage and death with a functioning graft after kidney transplantation. Am J Transplant 2013;13:2096-105. DOI: http://dx.doi.org/10.1111/ajt.12313

10. Kahan BD, Julian BA, Pescovitz MD, Vanrenterghem Y, Neylan J. Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in caucasian recipients of mismatched primary renal allografts: a phase II trial. Rapamune Study Group. Transplantation 1999;68:1526-32. DOI: http://dx.doi.org/10.1097/00007890-199911270-00016

11. MacDonald AS; RAPAMUNE Global Study Group. A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. Transplantation 2001;71:271-80. DOI: http://dx.doi.org/10.1097/00007890-200101270-00019

12. Vítko S, Margreiter R, Weimar W, Dantal J, Viljoen HG, Li Y, et al. Everolimus (Certican) 12-month safety and efficacy *versus* mycophenolate mofetil in de novo renal transplant recipients. Transplantation 2004;78:1532-40. PMID: 15599319 DOI: http://dx.doi.org/10.1097/01.TP.0000141094.34903.54

13. Lorber MI, Mulgaonkar S, Butt KM, Elkhammas E, Mendez R, Rajagopalan PR, et al.; B251 Study Group. Everolimus *versus* mycophenolate mofetil in the prevention of rejection in de novo renal transplant recipients: a 3-year randomized, multicenter, phase III study. Transplantation 2005;80:244-52. DOI: http://dx.doi.org/10.1097/01.TP.0000164352.65613.24

14. Meier-Kriesche HU, Baliga R, Kaplan B. Decreased renal function is a strong risk factor for cardiovascular death after renal transplantation. Transplantation 2003;75:1291-5. DOI: http://dx.doi.org/10.1097/01.TP.0000061602.03327.E2

15. Hariharan S, McBride MA, Cherikh WS, Tolleris CB, Bresnahan BA, Johnson CP. Post-transplant renal function in the first year predicts long-term kidney transplant survival. Kidney Int 2002;62:311-8. PMID: 12081593 DOI: http://dx.doi.org/10.1046/j.1523-1755.2002.00424.x

16. Ekberg H, Tedesco-Silva H, Demirbas A, Vítko S, Nashan B, Gürkan A, et al.; ELITE-Symphony Study. Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med 2007;357:2562-75. PMID: 18094377 DOI: http://dx.doi.org/10.1056/NEJMoa067411

17. Flechner SM, Glyda M, Cockfield S, Grinyó J, Legendre Ch, Russ G, et al. The ORION study: comparison of two sirolimus-based regimens *versus* tacrolimus and mycophenolate mofetil in renal allograft recipients. Am J Transplant 2011;11:1633-44. DOI: http://dx.doi.org/10.1111/j.1600-6143.2011.03573.x

18. Flechner SM, Gurkan A, Hartmann A, Legendre CM, Russ GR, Campistol JM, et al. A randomized, open-label study of sirolimus *versus* cyclosporine in primary de novo renal allograft recipients. Transplantation 2013;95:1233-41. DOI: http://dx.doi.org/10.1097/TP.0b013e318291a269

19. Liefeldt L, Brakemeier S, Glander P, Waiser J, Lachmann N, Schönemann C, et al. Donor-specific HLA antibodies in a cohort comparing everolimus with cyclosporine after kidney transplantation. Am J Transplant 2012;12:1192-8. DOI: http://dx.doi.org/10.1111/j.1600-6143.2011.03961.x

20. Tsuda K, Yamanaka K, Kitagawa H, Akeda T, Naka M, Niwa K, et al. Calcineurin inhibitors suppress cytokine production from memory T cells and differentiation of naïve T cells into cytokine-producing mature T cells. PLoS One 2012;7:e31465. DOI: http://dx.doi.org/10.1371/journal.pone.0031465

21. Gaston RS. Our evolving understanding of late kidney allograft failure. Curr Opin Organ Transplant 2011;16:594-9. DOI: http://dx.doi.org/10.1097/MOT.0b013e32834c23a7

22. Stephany BR, Augustine JJ, Krishnamurthi V, Goldfarb DA, Flechner SM, Braun WE, et al. Differences in proteinuria and graft function in de novo sirolimus-based vs. calcineurin inhibitor-based immunosuppression in live donor kidney transplantation. Transplantation 2006;82:368-74. PMID: 16906035 DOI: http://dx.doi.org/10.1097/01.tp.0000228921.43200.f7

23. Wiseman AC, McCague K, Kim Y, Geissler F, Cooper M. The effect of everolimus versus mycophenolate upon proteinuria following kidney transplant and relationship to graft outcomes. Am J Transplant 2013;13:442-9. DOI: http://dx.doi.org/10.1111/j.1600-6143.2012.04334.x

24. Roodnat JI, Mulder PG, Rischen-Vos J, van Riemsdijk IC, van Gelder T, Zietse R, et al. Proteinuria after renal transplantation affects not only graft survival but also patient survival. Transplantation 2001;72:438-44. DOI: http://dx.doi.org/10.1097/00007890-200108150-00014

25. Johnston O, Rose CL, Webster AC, Gill JS. Sirolimus is associated with new-onset diabetes in kidney transplant recipients. J Am Soc Nephrol 2008;19:1411-8. DOI: http://dx.doi.org/10.1681/ASN.2007111202

26. Gyurus E, Kaposztas Z, Kahan BD. Sirolimus therapy predisposes to new-onset diabetes mellitus after renal transplantation: a long-term analysis of various treatment regimens. Transplant Proc 2011;43:1583-92. DOI: http://dx.doi.org/10.1016/j.transproceed.2011.05.001

27. Murakami N, Riella LV, Funakoshi T. Risk of metabolic complications in kidney transplantation after conversion to mTOR inhibitor: a systematic review and meta-analysis. Am J Transplant 2014;14:2317-27. DOI: http://dx.doi.org/10.1111/ajt.12852

28. Paoletti E, Amidone M, Cassottana P, Gherzi M, Marsano L, Cannella G. Effect of sirolimus on left ventricular hypertrophy in kidney transplant recipients: a 1-year nonrandomized controlled trial. Am J Kidney Dis 2008;52:324-30. PMID: 18585837 DOI: http://dx.doi.org/10.1053/j.ajkd.2008.04.018

29. Svensson M, Jardine A, Fellström B, Holdaas H. Prevention of cardiovascular disease after renal transplantation. Curr Opin Organ Transplant 2012;17:393-400. DOI: http://dx.doi.org/10.1097/MOT.0b013e3283560a3b

30. Paoletti E, Cannella G. Regression of left ventricular hypertrophy in kidney transplant recipients: the potential role for inhibition of mammalian target of rapamycin. Transplant Proc 2010;42:S41-3. PMID: 21095451 DOI: http://dx.doi.org/10.1016/j.transproceed.2010.07.007

31. Oberbauer R, Segoloni G, Campistol JM, Kreis H, Mota A, Lawen J, et al.; Rapamune Maintenance Regimen Study Group. Early cyclosporine withdrawal from a sirolimus-based regimen results in better renal allograft survival and renal function at 48 months after transplantation. Transpl Int 2005;18:22-8. DOI: http://dx.doi.org/10.1111/j.1432-2277.2004.00052.x

32. Baboolal K. A phase III prospective, randomized study to evaluate concentration-controlled sirolimus (rapamune) with cyclosporine dose minimization or elimination at six months in de novo renal allograft recipients. Transplantation 2003;75:1404-8. PMID: 12717239 DOI: http://dx.doi.org/10.1097/01.TP.0000063703.32564.3B

33. Tedesco-Silva H, Garcia VD, Contieri FL, De Boni Monteiro de Carvalho D, Noronha IL, Gonçalves RT, et al. Comparison of the safety and efficacy of cyclosporine minimization versus cyclosporine elimination in de novo renal allograft patients receiving sirolimus. Transplant Proc 2010;42:1659-66. PMID: 20620495 DOI: http://dx.doi.org/10.1016/j.transproceed.2010.02.083

34. Cibrik D, Silva HT Jr, Vathsala A, Lackova E, Cornu-Artis C, Walker RG, et al Randomized trial of everolimus-facilitated calcineurin inhibitor minimization over 24 months in renal transplantation. Transplantation 2013;95:933-42. DOI: http://dx.doi.org/10.1097/TP.0b013e3182848e03

35. Langer RM, Hené R, Vitko S, Christiaans M, Tedesco-Silva H Jr, Ciechanowski K, et al. Everolimus plus early tacrolimus minimization: a phase III, randomized, open-label, multicentre trial in renal transplantation. Transpl Int 2012;25:592-602. DOI: http://dx.doi.org/10.1111/j.1432-2277.2012.01465.x

36. Tedesco-Silva H, Felipe C, Ferreira A, Cristelli M, Oliveira N, Sandes-Freitas T, et al. Reduced Incidence of Cytomegalovirus Infection in Kidney Transplant Recipients Receiving Everolimus and Reduced Tacrolimus Doses. Am J Transplant 2015;15:2655-64. DOI: http://dx.doi.org/10.1111/ajt.13327

37. Qazi Y, Shaffer D, Kaplan B, Kim DY, Luan FL, Peddi VR, et al. Efficacy and Safety of Everolimus Plus Low-Dose Tacrolimus Versus Mycophenolate Mofetil Plus Standard-dose Tacrolimus in De Novo Renal Transplant Recipients: 12-Month Data. Am J Transplant 2016 Oct 24. [Epub ahead of print] DOI: http://dx.doi.org/10.1111/ajt.14090

38. Weir MR, Mulgaonkar S, Chan L, Shidban H, Waid TH, Preston D, et al. Mycophenolate mofetil-based immunosuppression with sirolimus in renal transplantation: a randomized, controlled Spare-the-Nephron trial. Kidney Int 2011;79:897-907. PMID: 21191361 DOI: http://dx.doi.org/10.1038/ki.2010.492

39. Guba M, Pratschke J, Hugo C, Krämer BK, Nohr-Westphal C, Brockmann J, et al. Renal function, efficacy, and safety of sirolimus and mycophenolate mofetil after short-term calcineurin inhibitor-based quadruple therapy in de novo renal transplant patients: one-year analysis of a randomized multicenter trial. Transplantation 2010;90:175-83. DOI: http://dx.doi.org/10.1097/TP.0b013e3181e11798

40. Lebranchu Y, Thierry A, Thervet E, Büchler M, Etienne I, Westeel PF, et al. Efficacy and safety of early cyclosporine conversion to sirolimus with continued MMF-four-year results of the Postconcept study. Am J Transplant 2011;11:1665-75. DOI: http://dx.doi.org/10.1111/j.1600-6143.2011.03637.x

41. Budde K, Becker T, Arns W, Sommerer C, Reinke P, Eisenberger U, et al. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. Lancet 2011;377:837-47. PMID: 21334736 DOI: http://dx.doi.org/10.1016/S0140-6736(10)62318-5

42. Mjörnstedt L, Schwartz Sørensen S, von Zur Mühlen B, Jespersen B, Hansen JM, Bistrup C, et al. Renal function three years after early conversion from a calcineurin inhibitor to everolimus: results from a randomized trial in kidney transplantation. Transpl Int 2015;28:42-51. DOI: http://dx.doi.org/10.1111/tri.12437

43. Favi E, Spagnoletti G, Salerno MP, Pedroso JA, Romagnoli J, Citterio F. Tacrolimus plus mycophenolate mofetil vs. cyclosporine plus everolimus in deceased donor kidney transplant recipients: three-yr results of a single-center prospective clinical trial. Clin Transplant 2013;27:E3597-67. DOI: http://dx.doi.org/10.1111/ctr.12141

44. Gatault P, Bertrand D, Büchler M, Colosio C, Hurault de Ligny B, Weestel PF, et al. Eight-year results of the Spiesser study, a randomized trial comparing de novo sirolimus and cyclosporine in renal transplantation. Transpl Int 2016;29:41-50. DOI: http://dx.doi.org/10.1111/tri.12656

45. Weir MR, Pearson TC, Patel A, Peddi VR, Kalil R, Scandling J, et al. Long-term Follow-up of Kidney Transplant Recipients in the Spare-the-Nephron-Trial. Transplantation 2017;101:157-66. DOI: http://dx.doi.org/10.1097/TP.0000000000001098

46. Budde K, Lehner F, Sommerer C, Reinke P, Arns W, Eisenberger U, et al.; ZEUS Study Investigators. Five-year outcomes in kidney transplant patients converted from cyclosporine to everolimus: the randomized ZEUS study. Am J Transplant 2015;15:119-28. DOI: http://dx.doi.org/10.1111/ajt.12952

47. de Paula MI, Medina Pestana JO, Nicolau Ferreira A, Pontello Cristelli M, Fabiano Franco M, Aguiar WF, et al. Long-Term Follow-Up of De Novo Use of mTOR and Calcineurin Inhibitors After Kidney Transplantation. Ther Drug Monit 2016;38:22-31. DOI: http://dx.doi.org/10.1097/FTD.0000000000000227

48. Liko D, Hall MN. mTOR in health and in sickness. J Mol Med (Berl) 2015;93:1061-73. DOI: http://dx.doi.org/10.1007/s00109-015-1326-7