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

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.

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 mTOR

<table>
<thead>
<tr>
<th>Ref</th>
<th>Study type</th>
<th>Year inclusion</th>
<th>Time follow up</th>
<th>iCN</th>
<th>iCN (%)</th>
<th>imTOR without iCN (%)</th>
<th>imTOR use</th>
<th>imTOR Group characteristics</th>
<th>Graft survival</th>
<th>Patient survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>SRTR Registry</td>
<td>1998 to 2013</td>
<td>4 years</td>
<td>21,017</td>
<td>1999</td>
<td>-</td>
<td>De novo</td>
<td>CsA/SRL: lower use of indication in the group</td>
<td>79.3% CsA/MMF 74.6 CsA/SRL (HR = 1.22)</td>
<td>ns.</td>
</tr>
<tr>
<td>(2)</td>
<td>SRTR Registry</td>
<td>2000 to 2004</td>
<td>3 years</td>
<td>44,915</td>
<td>5393</td>
<td>-</td>
<td>de novo</td>
<td>85.9% TAC/MMF 85.3% CsA/MMF (HR = 1.15)</td>
<td>82.2% CsA/SRL (HR = 1.38)</td>
<td>80.3% TAC/SRL (HR = 1.47)</td>
</tr>
<tr>
<td>(3)</td>
<td>SRTR Registry</td>
<td>2000 to 2005</td>
<td>5 years</td>
<td>49,412</td>
<td>6394 (73%)</td>
<td>2325 (27%)</td>
<td>de novo</td>
<td>SRL/MMF: higher donor age, higher rate of deceased donor</td>
<td>73.8% TAC/MMF 71.8% CSA/MMF (HR = 1.16)</td>
<td>68.9% TAC/SRL (HR = 1.38)</td>
</tr>
<tr>
<td>(4)</td>
<td>Hungary cohort</td>
<td>2007</td>
<td>3 years</td>
<td>1241</td>
<td>37 (37%)</td>
<td>64 (63%)</td>
<td>conversio</td>
<td>Higher number with neoplasia and diabetes</td>
<td>ns.</td>
<td>Patients with a history of neoplasia (HR = 2.6-5.6)</td>
</tr>
<tr>
<td>(5)</td>
<td>UNOS Registry</td>
<td>1999 to 2010</td>
<td>2-8 years</td>
<td>125,623</td>
<td>10,510 (76.4%)</td>
<td>3,237 (23.5%)</td>
<td>de novo</td>
<td>iCN/imTOR: higher number with a history of malignancy, diabetes and kidney from expanded-criterion donor; higher PRA and TIF</td>
<td>iCN/imTOR (HR = 1.07)</td>
<td>imTOR/MMF (HR = 1.17)</td>
</tr>
<tr>
<td>(6)</td>
<td>Systematic review</td>
<td>1999 to 2013</td>
<td>1 year to 4 years</td>
<td>2,600</td>
<td>-</td>
<td>-</td>
<td>de novo</td>
<td>4,717 (80.3%) conversion 1,159 (19.7%)</td>
<td>unreported</td>
<td>Not analyzed</td>
</tr>
</tbody>
</table>
transplant registry of 44,915 adult kidney transplants performed between 2000 and 2004. In this analysis, 3,524 (7.8%) patients received TAC/MMF; 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. 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. 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 2010.4 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 analyzed1-3,5,8 (Table 1).

Thus, it is not surprising that the outcomes were similar. The Australian and New Zealand registry study,7 the observational study from Hungary4 and the meta-analysis6 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 SRL10,11 and two using EVR12,13 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, patient14 and graft,15 survival is well known. Even then, in one of the recent transplant registries in the United States,1 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 proliferation20 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 transplantation25-27 and dyslipidemia27 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.

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, 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 or in early conversion strategies of iCN for imTOR. 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. 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 or with early conversion strategies from iCN to imTOR.

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 antimetabolites, no difference was found in the incidence of acute rejection, graft loss, and patient death within 10 years of follow-up (Table 2).

### Table 2: Randomized Clinical Trials with Medium Term Assessments Using More Recent Immunosuppression with imTOR

<table>
<thead>
<tr>
<th>Ref.</th>
<th>N</th>
<th>Regimens</th>
<th>Follow up time</th>
<th>Acute rejection (%)</th>
<th>Graft loss (%)</th>
<th>Death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(40)</td>
<td>162</td>
<td>CSA → SRL/MMF (n = 77)</td>
<td>4 years</td>
<td>2.6</td>
<td>5.2</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CSA/MMF (n = 85)</td>
<td></td>
<td>2.6</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>(43)</td>
<td>60</td>
<td>BAS/TAC/MMF (n = 30)</td>
<td>3 years</td>
<td>17</td>
<td>6.6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BAS/CSAr/EVE (n = 30)</td>
<td></td>
<td>23</td>
<td>6.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BAS/EVE (3-8 ng/ml)/CSAr (n = 277)</td>
<td></td>
<td>19.9</td>
<td>5.8</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BAS/EVE (6-12 ng/ml)/CSAr (n = 279)</td>
<td></td>
<td>15.1</td>
<td>6.1</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BAS/CSA/MPS (n = 277)</td>
<td></td>
<td>19.1</td>
<td>4.0</td>
<td>2.9</td>
</tr>
<tr>
<td>(46)</td>
<td>300</td>
<td>CSA → EVR/MMF (n = 155)</td>
<td>5 years</td>
<td>13.6</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CSA/MMF (n = 145)</td>
<td></td>
<td>7.5</td>
<td>2.1</td>
<td>2.6</td>
</tr>
<tr>
<td>(42)</td>
<td>182</td>
<td>CSA → CSA/MMF (n = 90)</td>
<td>3 years</td>
<td>13</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EVR/MMF (n = 92)</td>
<td></td>
<td>11.1</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>(44)</td>
<td>99</td>
<td>rATG (75mg/kg)/SRL/MMF</td>
<td>8 years</td>
<td>nr</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rATG(75mg/kg)/CSA/MMF</td>
<td></td>
<td>nr</td>
<td>14.8</td>
<td>8</td>
</tr>
<tr>
<td>(45)</td>
<td>128</td>
<td>iCN → SRL/MMF (n = ?)</td>
<td>8 years</td>
<td>22.7</td>
<td>15.2</td>
<td>7.6</td>
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<td></td>
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<td>iCN/MMF (n = ?)</td>
<td></td>
<td>14.5</td>
<td>19.4</td>
<td>9.7</td>
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<tr>
<td></td>
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<td>iCN/SRL (n = 347)</td>
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<td>22.2</td>
<td>19</td>
<td>12</td>
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<td></td>
<td>iCN/AZA-MPA (n = 124)</td>
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<td>22.6</td>
<td>23</td>
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</table>

(jbranefrol 2017;39(1):70-78)
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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. Despite this, the clinical use of mTOR 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.

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