De novo cancer avoidance after renal transplantation: A case–control study on low-dose sirolimus combined with a calcineurin inhibitor

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KEYWORDS
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Background/purpose: Full-dose sirolimus (SRL) therapy without a calcineurin inhibitor (CNI) reduces the incidence of malignancy after renal transplantation, but with significant side effects. We hypothesized that de novo therapy with low-dose SRL combined with a CNI could still prevent cancer in renal transplant recipients.

Methods: A retrospective case–control study was performed to assess the cancer incidence among renal transplant patients who had undergone surgery in our transplant centers between January 2000 and June 2012. Patients who received low-dose SRL and a CNI (SRL group, n = 189) were compared with patients receiving conventional CNI-based therapy in the same hospitals (Conventional group, n = 271).

Results: The 5-year graft and patient survival rates were comparable between the two groups. Seven patients in the SRL group and 24 patients in the Conventional group developed malignancies during mean follow-up periods of 68.2 ± 37.5 months and 81.7 ± 51.4 months, respectively. The cancer incidence at 5 years was significantly lower in the SRL group (1.9%), than that in the Conventional group (6.7%; p = 0.04). By multivariate analyses, SRL therapy...
Introduction

Renal transplantation is associated with an increased risk of cancer, which is probably caused by prolonged immunosuppression due to the use of calcineurin inhibitors (CNIs).\(^1\)\(^2\) Research and clinical studies have reported that sirolimus (SRL), a macrocyclic lactone inhibitor of the mammalian target of rapamycin (mTOR) signaling pathway, exerts both anticancer and immunosuppressive effects.\(^3\)\(^6\) SRL therapy, after early CNI withdrawal, reduced the incidence of both skin and nonskin malignancies in renal transplant patients after 5 years.\(^7\)\(^8\) Furthermore, SRL prevented secondary skin cancer in high-risk renal transplant recipients.\(^9\)\(^10\) However, SRL-related adverse events occurred frequently, sometimes resulting in the discontinuation of SRL treatment. For example, Campbell et al\(^11\) reported that 46.2% of patients taking full-dose SRL (trough levels > 8 ng/mL) without a CNI discontinued treatment during their follow-up. We reported recently that low-dose SRL (targeting trough levels of 4–8 ng/mL) combined with either tacrolimus (TAC) or cyclosporine (CsA) CNIs might have a reduced SRL discontinuation rate (15%).\(^12\)

Although low-dose SRL and CNI combinations reduce the recurrence of hepatocellular carcinoma after liver transplantation, the efficacy of this drug combination has not yet been assessed thoroughly in renal transplant patients.\(^13\)\(^14\) Previously, Kreis et al\(^15\) demonstrated that, compared with placebo, the combination of SRL and CsA resulted in a significantly lower 2-year incidence of skin cancer. In addition, a low incidence of malignancy (mostly skin tumors) was reported in SRL/CsA-treated renal transplant recipients when analyzed using the Surveillance, Epidemiology, and End Results (SEER) database of the general United States population.\(^16\) In a large, long-term study performed by Wimmer et al,\(^17\) mTOR inhibitor-based regimens did not significantly reduce the risk of de novo malignancies after renal transplantation. By contrast, Kauffman et al\(^18\) used the Organ Procurement and Transplantation Network/United Network for Organ Sharing (OPTN/UNOS) database to document the association between mTOR inhibitors and reduced post-transplant malignancies, but did not report the timing and doses of SRL used.

A primary dose of 2 mg/d of SRL is commonly used in combination with a CNI.\(^18\)\(^19\) We previously performed several prospective trials to assess the efficacy of de novo SRL therapy (2 mg/d, trough levels of 4–8 ng/mL) against acute rejection.\(^12\)\(^20\)\(^22\) We hypothesized that low-dose SRL and a CNI might help prevent cancer. Therefore, we performed a case–control study to assess the cancer incidence in long-term renal transplant patients treated with or without de novo SRL.

Patients and methods

Study group

A case–control study was performed to assess the beneficial effect of low-dose SRL and a CNI on cancer prevention after renal transplantation. Data from renal transplant patients who met the criterion of our previous studies for SRL of de novo therapy were collected and reviewed. We excluded: patients who had received antibody induction therapy or multiple solid organ transplants; patients who had tested positive for hepatitis B virus surface antigen or anti-hepatitis C virus antibodies; and patients with ABO incompatibility or positive lymphocytotoxicity.\(^12\)\(^20\)\(^22\) Patients with < 6 months of follow-up, secondary transplants, or pretransplant cancers were also excluded. The outcomes of patients who underwent renal transplantation with the combination therapy of low-dose SRL and a CNI between January 2000 and June 2012 (SRL group) were compared with outcomes of those who received conventional CNI-based regimens in the same transplant centers (Conventional group). The clinical and research activities reported comply with the ethical standards laid down in the Declaration of Helsinki and its later amendments.

Immunosuppressive regimens

The immunosuppressive regimens in the SRL group included SRL, corticosteroids, and a CNI (either CsA or TAC). SRL was administered at a loading dose of 6 mg within 48 hours after graft reperfusion, followed by a maintenance dose of 2 mg/d. The initial target trough levels of the CNIs were 100–200 ng/mL for CsA and 4–8 ng/mL for TAC.\(^22\) The trough levels of SRL were measured regularly and adjusted if side effects occurred. The target blood levels at 12 months were 50–150 ng/mL for CsA, 3–6 ng/mL for TAC, and 4–8 ng/mL for SRL. In the Conventional group, the CNI doses were initially adjusted to the target trough levels of 200–400 ng/mL for CsA and 816 ng/mL for TAC. The target blood levels at 12 months were 100–200 ng/mL for CsA and 5–8 ng/mL for TAC. In addition, mycophenolate mofetil or mycophenolate sodium was prescribed at an initial dose of 1–2 g/d or 720–1440 mg/d, respectively. White blood cell counts were maintained between 4 × 10^9/L and 6 × 10^9/L unless intolerance developed or the maximum dose was reached.
Corticosteroids, including intravenous methylprednisolone and oral prednisolone, were administered, consistent with standard practices. The dose of prednisolone was reduced to 2.5–5 mg/d at 12 months and could then be discontinued if significant side effects were reported. To prevent cytomegalovirus disease, valganciclovir (450 mg/d) was administered to patients with a functioning graft for 3 months, unless the patient had leukopenia.

Statistical analyses

Intent-to-treat analyses were performed using NCSS 2008 for Windows software (Kaysville, UT, USA). Graft and patient survival, and cancer incidence were estimated using the Kaplan–Meier method. For patients with multiple cancers, only the first cancer was recorded for statistical analyses. All values were expressed as the mean ± standard deviation. Unpaired two-tailed t tests and Fisher’s exact tests were used for normally distributed continuous variables and categorical variables, respectively. Univariate analysis using the log-rank test was used to determine the statistical significance of the effects of SRL de novo therapy, recipient sex, CNI (CsA or TAC) therapy, donor type, and acute rejection on cancer incidence. Cox regression analysis was used to assess the statistical significance of recipient age at transplantation and HLA mismatches on cancer occurrence. Finally, multivariate Cox’s regression analysis was employed to examine the independent effect of each factor that exhibited statistical significance in univariate analyses.

Results

Patient demographics

Patient demographics and outcomes of the SRL and Conventional groups are summarized in Table 1. The mean ages of patients in the SRL (42.5 ± 13.0 years) and Conventional groups (40.5 ± 13.5 years) at transplantation were comparable (p = 0.10). There were more female patients in the Conventional group (53.9%, 146/271) than in the SRL group (49.7%, 139/189), but the difference was statistically insignificant (p = 0.39). The number of deceased-donor transplants (73.5%, 139/189) included in the SRL group was higher than that included in the Conventional group (62.0%, 168/271; p = 0.01). The mean number of HLA mismatches between donors and recipients was also higher in the SRL group (3.2 ± 1.5 vs. 2.6 ± 1.4, p < 0.001). In addition, the number of patients in the SRL group who received TAC (82.0%, 155/189) was higher than the number of patients in the Conventional group who received TAC (56.1%, 152/271; p < 0.001).

Transplant outcomes

The 5-year graft and patient survival rates of the two groups were comparable, although there was a lower incidence of acute rejection at 1 year in the SRL group than that in the Conventional group (15.3% vs. 19.2%; p = 0.32). Seven patients in the SRL group and 24 patients in the

### Table 1 Patient demographics and outcomes.

| Characteristics | SRL group (n = 189) | Conventional group (n = 271) | p *
|-----------------|---------------------|-----------------------------|---
| Age at transplantation (y) | 42.5 ± 13.0 | 40.5 ± 13.5 | 0.10
| Sex (M:F) | 95:94 | 125:146 | 0.39
| Donor type (D:L) | 139:50 | 168:103 | 0.01
| HLA mismatches | 3.2 ± 1.5 | 2.6 ± 1.4 | <0.001
| Initial CNI (TAC:CsA) | 159:34 | 152:119 | <0.001
| 1-y acute rejection | 29/189 (15.3%) | 52/271 (19.2%) | 0.32
| 5-y graft survival | 86.5% | 84.7% | 0.25
| 5-y patient survival | 97.0% | 97.5% | 0.43
| 5-y cancer incidence | 1.9% | 6.7% | 0.04
| Follow-up (mo) | 68.2 ± 37.5 | 81.7 ± 51.4 | 0.002

CNI = calcineurin inhibitor; CsA = cyclosporine; D = deceased; F = female; L = living; M = male; SRL = sirolimus; TAC = tacrolimus.

* Two-tailed Fisher’s exact test was used for categorical variables; two-tailed unpaired t-test was used for continuous variables; log-rank test was used for survival analysis.

Conventional group developed malignancies during mean follow-up periods of 68.2 ± 37.5 months and 81.7 ± 51.4 months, respectively. During the follow-up period, 22 SRL group patients stopped taking SRL at 2 years (11.6%, 22/189). The incidences of de novo cancers at 5 years post-transplantation in the SRL and Conventional groups were 1.9% and 6.7%, respectively (Fig. 1). The difference in post-transplant cancer incidence between the two groups was statistically significant (p = 0.04).

Urothelial carcinoma was the most common post-transplant malignancy, accounting for 57.1% (4/7) and 54.2% (2/3) of the pathological diagnoses in the SRL and Conventional groups, respectively. Skin cancer was relatively rare in our study population: only one and two cases
of nonmelanoma skin cancer were reported in the SRL and Conventional groups, respectively. There were two cases of post-transplant lymphoproliferative disease in the Conventional group but none in the SRL group. Even though patients with hepatitis B and C viral infections were excluded, there was one case of hepatocellular carcinoma in the Conventional group. The pathological diagnoses of post-transplant malignancy are summarized by immunosuppressive regimens in Table 2.

Univariate and multivariate analyses

Univariate analyses were performed to identify risk factors for post-transplant de novo malignancies. Data revealed that SRL de novo therapy ($p = 0.04$), sex ($p = 0.04$), and age at transplantation ($p = 0.02$) were significant prognostic factors. Patients in the SRL group had a significantly lower risk of post-transplant cancer than those in the Conventional group. Interestingly, male patients were found to have lower incidence of cancer after renal transplantation than female patients. Besides, the risk ratio of post-transplant cancer was found to be 1.038 per year of increased age in the univariate analysis. Donor type, CNI therapy, acute rejection, and follow-up duration were not significantly associated with the development of post-transplant cancer. Results of the univariate analyses are shown in Table 3. Cox’s multivariate regression analysis revealed that de novo SRL therapy ($p = 0.04$), sex ($p = 0.04$), and age at transplantation ($p = 0.01$) were significant factors for post-transplant malignancies. De novo SRL therapy (risk ratio = 0.38) and male sex (risk ratio = 0.43) were independently associated with decreased risk of cancer development, whereas older age at transplantation increased the risk of malignant tumors by a ratio of 1.04 per year (Table 4).

Risk factors for urothelial carcinoma

Since urothelial carcinoma accounted for most cancer pathology, survival analyses were further conducted to reveal prognostic factors for post-transplant urothelial carcinoma.

### Table 2 Cancer pathology grouped by immunosuppressive regimens.

<table>
<thead>
<tr>
<th>Cancer pathology</th>
<th>Sirolimus group</th>
<th>Conventional group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n = 7$</td>
<td>$n = 24$</td>
</tr>
<tr>
<td>Urothelial cancer</td>
<td>4/7 (57.1)</td>
<td>13/24 (54.2)</td>
</tr>
<tr>
<td>Skin cancer (nonmelanotic)</td>
<td>1/7 (14.3)</td>
<td>2/24 (8.3)</td>
</tr>
<tr>
<td>PTLD</td>
<td>0/7 (0.0)</td>
<td>2/24 (8.3)</td>
</tr>
<tr>
<td>Gastrointestinal carcinoma</td>
<td>1/7 (14.3)</td>
<td>1/24 (4.2)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>1/7 (14.3)</td>
<td>1/24 (4.2)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>0/7 (0.0)</td>
<td>1/24 (4.2)</td>
</tr>
<tr>
<td>Gynecological cancer</td>
<td>0/7 (0.0)</td>
<td>1/24 (4.2)</td>
</tr>
<tr>
<td>Others</td>
<td>0/7 (0.0)</td>
<td>3/24 (12.5)</td>
</tr>
</tbody>
</table>

Data are presented as n/N (%).

PTLD = post-transplant lymphoproliferative disease.

*In case of multiple cancers, only the first cancer was counted.

By log-rank tests and Cox’s regression, female sex was identified as the only risk factor ($p = 0.03$) for post-transplant urothelial carcinoma. Age at transplantation and SRL therapy were not significant factors for urothelial carcinoma, possibly because of inadequate patient and cancer numbers.

### Discussion

Determining the minimal dosage of SRL that could effectively prevent post-transplant cancers is worthwhile. Previous studies reported that high-dose SRL (trough levels > 8 ng/mL) without CNI was associated with an increased incidence of adverse events, which could lead to the discontinuation of SRL treatment. Nevertheless, the effects of SRL on cancer prevention were evident in patients who received SRL-based therapy initiated early post-transplantation.7,11 This mTOR inhibitor could be regarded as an agent for de novo cancer prevention, akin to ganciclovir for the prevention of cytomegalovirus, if the minimal effective dose of mTOR inhibitors could be identified. There might be an inverse relationship between the dose of mTOR inhibitor and the incidence of cancer after transplantation. However, the efficacy of SRL was difficult to assess in retrospective studies that included long-term follow-up of patients who were converted to variable doses of SRL at different time points post-transplantation.21 In addition, SRL was only transiently effective, or even ineffective, in treating renal transplant recipients with severe Kaposi’s sarcoma or urothelial carcinoma.24,25 De novo SRL therapy at 2 mg/d, which targeted a trough level of 4–8 ng/mL, could reduce the incidence of post-transplant malignancies in the current study, even in combination with a CNI.

The reduced cancer incidence in the current study might result from the antiproliferative effect of SRL, as well as the reduced doses of CNIs. A previous study reported that patients receiving CNI-based therapies had a significantly
higher risk of post-transplant cancer than those treated with azathioprine and steroids (ST).\textsuperscript{6} Campistol et al\textsuperscript{7} reported that immunosuppressive regimens converted from combined SRL (troughs of 5–15 ng/mL) with CsA and ST to high-dose SRL (troughs > 15 ng/mL) with ST reduced the rates of nonskin cancer from 9.6% to 4.0% at 5 years. However, the overall 5-year cancer incidence (1.9%) observed in our SRL group, which also included patients receiving the SRL + CsA + ST regimen, was much lower than the risk of nonskin cancer in patients treated with SRL + ST (4.0%) in the study by Campistol et al.\textsuperscript{7} This suggests that a SRL + CsA + ST regimen could still be effective for preventing nonskin cancer.

The pathological diagnoses of post-transplant malignancies vary among different countries. Nonmelanoma skin cancer and lymphoproliferative disorders are prevalent in Western, but not Asian, countries.\textsuperscript{16,26} By contrast, urothelial carcinoma is relatively common among Asian renal transplant patients, especially when those with viral hepatitis are excluded.\textsuperscript{27} The underlying mechanism for this effect was suggested to be oncogenic viral infections with concurrent suppressed immune surveillance of tumor antigens.\textsuperscript{28} In addition, several nonviral risk factors, including age, sex, race, and duration of dialysis have been reported.\textsuperscript{29} Therefore, we chose to exclude patients with viral hepatitis from our study population to minimize bias toward cancer occurrence, although the true incidence of post-transplant cancer could be underestimated. Based on the substantial evidence suggesting that SRL has antitumor properties, a primary strategy to reduce cancer-related complications after renal transplantation might be \textit{de novo} therapy or early conversion to SRL (or another mTOR inhibitor), although conversion to SRL has not been proved effective in preventing recurrence of malignant cancers except for those derived from the skin.\textsuperscript{9,11}

Female recipients of renal transplantation, in this study, had a higher risk of post-transplant cancer, especially urothelial carcinoma. Actually, in the general population, the incidence of bladder cancer was similar in both sexes.\textsuperscript{30} Female sex was reported to be associated with higher grade and stage of urothelial carcinoma, although the transplant outcome of female patients (as we reported recently) seemed better than that of our male patients.\textsuperscript{31–33} The survival of female patients was lower after radical cystectomy for bladder urothelial carcinoma.\textsuperscript{34} While the contributing factors to worse prognosis of urothelial carcinoma in females remain to be identified, immunosuppressive therapy could possibly enhance the underlying oncogenic processes in female patients and uncover the sex differences in cancer biology. Future multicenter studies would determine if the prognosis of female renal transplant recipients with urothelial carcinoma is worse than that of male patients.

The early use of SRL, which can improve renal function and graft survival, for treating renal transplant recipients is controversial.\textsuperscript{35} There was a latent predisposition to use SRL in our study patients receiving transplants from deceased donors, although donor type was not identified as a significant factor for cancer occurrence in the univariate analysis. The possibility of wound complications, delayed graft function, and interstitial pneumonitis has concerned both patients and transplant surgeons.\textsuperscript{36} In addition, SRL exerts a paradoxical stimulatory effect on innate immunity; thus, a CNI might be indispensable for suppressing acute allograft rejection.\textsuperscript{37,38} Therefore, a regimen including low-dose SRL and a CNI, proposed by us and Campistol et al,\textsuperscript{39} might be an appropriate early immunosuppressive therapy for renal transplant recipients. However, it will be challenging to perform long-term randomized controlled trials, even in large-volume transplant centers, to address the role of immunosuppressive agents in post-transplant malignancies.\textsuperscript{16} As such, retrospective case–control cohorts are the method of choice.

In conclusion, compared with conventional CNI-based therapy, low-dose SRL combined with a CNI was associated with reduced risk of post-transplant cancer in renal transplant recipients. Accordingly, we propose a concept of \textit{de novo} cancer prevention using a low-dose proliferation signal inhibitor, such as SRL, for renal transplant recipients.

### References


<table>
<thead>
<tr>
<th>Table 4</th>
<th>Multivariate Cox’s regression analysis of the factors with statistical significance in the univariate analysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cox’s regression</td>
<td>Regression coefficient</td>
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<tr>
<td>Sirolimus therapy</td>
<td>-0.96</td>
</tr>
<tr>
<td>Male sex</td>
<td>-0.85</td>
</tr>
<tr>
<td>Age at transplantation</td>
<td>0.04</td>
</tr>
</tbody>
</table>

CL = confidence limit.
Cancer avoidance after renal transplantation


