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Incidence of Malignancies in Patients Treated With Sirolimus Following Heart Transplantation

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ABSTRACT

BACKGROUND Malignancy is a major cause of late post-heart transplantation (HT) mortality. Sirolimus (SRL) exerts antiproliferative properties and its long-term use in HT as primary immunosuppression (IS) is associated with decreased mortality risk that is not fully explained by attenuation of cardiac allograft vasculopathy progression.

OBJECTIVES This study sought to examine whether conversion from calcineurin inhibitor (CNI)-based to SRL-based IS was associated with decreased risk of malignancy post-HT.

METHODS Overall, 523 patients underwent HT between 1994 and 2016 at a single institution. The main outcomes included incidence of overall de novo malignancies (excluding non-melanoma skin cancers [NMSCs]), post-transplantation lymphoproliferative disorders (PTLD), and first and subsequent primary occurrences of NMSC post-HT.

RESULTS The study identified 307 patients on SRL-based and 216 on CNI-based maintenance IS. Over a median follow-up of 10 years after HT, overall de novo malignancies (non-NMSC) occurred in 31% of CNI patients and in 13% of SRL patients (adjusted hazard ratio [HR]: 0.34; 95% confidence interval [CI]: 0.18 to 0.62; $p < 0.001$). The incidence of the first NMSC was similar in the SRL and CNI groups (HR: 0.92; 95% CI: 0.66 to 1.28; $p = 0.62$). However, conversion to SRL was significantly associated with a decreased risk of subsequent primary occurrences of NMSC compared with that of CNI (adjusted HR: 0.44; 95% CI: 0.28 to 0.69; $p < 0.001$). The adjusted PTLD risk was significantly decreased in the SRL group (HR: 0.13; 95% CI: 0.03 to 0.59; $p = 0.009$). Late survival post-HT was markedly decreased in patients who developed non-NMSC, PTLD, or non-PTLD compared with patients who did not develop these malignancies, whereas NMSC had no significant effect on survival.

CONCLUSIONS Conversion to SRL was associated with a decreased risk of all de novo malignancies, PTLD, and subsequent primary occurrences of NMSC after HT. These findings provided further explanation of the late survival benefit with long-term SRL use. (J Am Coll Cardiol 2019;73:2676–88) Published by Elsevier on behalf of the American College of Cardiology Foundation.

With the introduction of better immunosuppression (IS) therapy, survival following heart transplantation (HT) has markedly improved over the last 2 decades. Approximately 50% of HT recipients survive >13 years, and the number of patients who survive >20 years post-HT continues to rise (1). Because of the resulting longer exposure to IS therapy, in conjunction with an increasing population of patients who are undergoing transplantation at an older age and with a higher risk profile in the current era, further improvement in late survival is blunted by a greater risk of de novo

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malignancy following HT. Malignancy remains the most frequent cause of death after the first 5 years post-HT (2). A large international registry study has shown a significant temporal increase in rates of overall de novo malignancy from 2000 to 2005 compared with 2006 to 2011, with >10% of the patients who underwent transplantation in the recent period developing de novo malignancy between 1 and 5 years after HT, which was associated with increased mortality (3). Long-term IS therapy has been associated with ≥5-fold increase in incidence of post-transplantation non-melanoma skin cancer (NMSC), development of post-transplantation lymphoproliferative disorders (PTLDs), and other solid cancers (2,4). HT patients experience especially high rates of malignancy, largely due to a greater intensity of IS compared with other solid organ transplantations that require lower IS intensity (e.g., kidney transplantation) (5). Although recipient-related risk factors, such as sun exposure, viral infections (mainly Epstein-Barr virus [EBV]), and local immune reactions against the graft, have emerged as risk factors for malignancy after HT, the type and duration of IS remains as important determinants of malignancy risk (5,6). For instance, the use of calcineurin inhibitors (CNIs) has been implicated in a dose-dependent increase in risk of different types of cancers that might be attributed to increased levels of transforming growth factor-β and pro-angiogenic factors (7,8).

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The mammalian target of rapamycin (mTOR) pathway is a key regulator of cell growth and survival, and this pathway is frequently dysregulated in various types of malignancies (9). Sirolimus (SRL) and its derivative, everolimus, are mTOR inhibitors that suppress tumor growth in animal models (9,10) and have been successfully used in treating selective types of cancers (11,12). In the HT population, studies assessing the effect of mTOR inhibition on the development of de novo malignancy are lacking due to the relatively small pool of HT recipients treated with mTOR inhibitors to date (13). Recently, we showed that early conversion to a maintenance SRL-based IS regimen, with complete withdrawal of CNI therapy, was associated with remarkable attenuation of cardiac allograft vasculopathy (CAV) progression and improvement in cardiac outcomes and late survival after HT compared with continued CNI use over a mean follow-up of 8.9 years from time of HT (14). However, this improved late survival with long-term use of SRL is not fully explained by attenuated CAV progression and reduced CAV-associated events. In the present study, we sought to examine, in a large cohort of HT recipients with prevalent use of SRL for primary IS, whether conversion from CNI- to SRL-based IS was associated with decreased rates of overall de novo malignancies, thus providing an additional explanation for the survival benefit seen with long-term SRL maintenance therapy. Furthermore, we sought to examine the effects of conversion to SRL on the incidence and rates of primary and subsequent primary occurrences of NMSC, a common but not directly life-threatening malignancy, in this HT population.

METHODS

DATA SOURCE. We retrospectively analyzed a cohort of 523 patients who underwent HT between January 1994 and December 2016 and were treated either with a CNI-based regimen (CNI group; n = 216) or were converted to a SRL-based regimen without CNI (SRL group; n = 307) as primary IS, at the Mayo Clinic (Rochester, Minnesota). We included HT patients who survived HT surgery. Seven patients were excluded because they did not provide research authorization to participate in the study. The Institutional Review Board of Mayo Clinic College of Medicine approved the study protocol.

IMMUNOSUPPRESSION. In our institution, all patients received induction therapy mainly with antithymocyte globulin (ATG), and a minority of patients received muromonab-CD3 (OKT3) in the first 5 years of the study period. All HT recipients received maintenance IS, including a CNI (tacrolimus or cyclosporine), an antimetabolite (mycophenolate mofetil [MMF]or azathioprine), and tapering doses of prednisone. According to our new protocol (14), we used rabbit ATG (dose of 1.5 mg/kg) at the time of HT and continued dosing based on CD4 and CD8 T-cell counts until tacrolimus was in the goal range of 10 to 14 ng/ml, in addition to MMF (goal dose of 1,000 to 1,500 mg twice daily) and steroid therapy. In the old HT era, patients received induction therapy with low-dose OKT3, which was dosed similarly and based on CD3 T-cell subset counts, as well as maintenance IS with cyclosporine, azathioprine (1 to 3 mg/kg), and steroids. Since July 2006, a routine conversion protocol from CNI to SRL was introduced in our institution. Post-HT stable patients without evidence of rejection and on stable doses of antimetabolites and prednisone received gradually increasing doses of SRL to achieve levels 10 to 14 ng/ml, and then the CNI dose was gradually titrated during conversion without changes in the antimetabolite and steroid

ABBREVIATIONS AND ACRONYMS

ATG = anti-thymocyte globulin
CAV = cardiac allograft vasculopathy
CMV = cytomegalovirus
CNI = calcineurin inhibitor
EBV = Epstein-Barr virus
HT = heart transplantation
IS = immunosuppression
MMF = mycophenolate mofetil
mTOR = mammalian target of rapamycin
OKT3 = muromonab-CD3
PTLD = post-transplantation lymphoproliferative disorders
SRL = sirolimus

TABLE 1 Baseline Clinical Characteristics

	All Group (N = 523)	CNI Alone (n = 216)	SRL Converters (n = 307)	p Value (CNI vs. SRL)
Age, yrs	50.0 ± 13.6	47.8 ± 14.0	51.5 ± 13.2	0.002
Male	354 (67.7)	138 (63.9)	216 (70.4)	0.122
Time from HT to SRL conversion, yrs			1.1 (0.58–3.2)	
Diagnosis				
ICM	140 (26.8)	55 (25.5)	85 (27.7)	0.571
DCM	165 (31.6)	60 (27.8)	105 (34.2)	0.118
CHD	49 (9.4)	26 (12.0)	23 (7.5)	0.082
Other	169 (32.3)	75 (34.7)	94 (30.6)	0.324
Combined transplants	122 (23.3)	56 (25.9)	66 (21.5)	0.230
Type of combined organ transplants				0.005
Heart and kidney	59 (11.3)	23 (10.6)	36 (11.7)	
Heart and liver	36 (6.9)	10 (4.6)	26 (8.5)	
Heart and lung	19 (3.6)	17 (7.9)	2 (0.65)	
Heart and BMT	1 (0.19)	1 (0.46)	0 (0.0)	
Heart, kidney, and liver	7 (1.3)	5 (2.3)	2 (0.65)	
Glucose, mg/dl	109.0 ± 28.6	107.9 ± 28.6	109.6 ± 28.7	0.521
Creatinine, mg/dl	1.4 ± 0.47	1.5 ± 0.44	1.4 ± 0.49	0.431
eGFR, ml/min/1.73 m^2	56.7 ± 22.1	57.1 ± 22.0	56.4 ± 22.1	0.725
Total cholesterol, mg/dl	208.5 ± 53.5	201.2 ± 52.8	213.0 ± 53.5	0.017
Triglycerides, mg/dl	168.5 ± 95.3	165.3 ± 98.5	170.5 ± 93.3	0.548
HDL cholesterol, mg/dl	60.0 ± 19.6	55.9 ± 18.8	62.5 ± 19.7	0.0002
LDL cholesterol, mg/dl	113.3 ± 40.7	110.2 ± 40.1	115.3 ± 41.0	0.178
Graft LVEF, %	62.5 ± 7.1	62.6 ± 7.4	62.3 ± 6.9	0.647
BMI, kg/m^2	26.1 ± 5.0	25.7 ± 5.1	26.4 ± 4.9	0.131
Ischemic time, min	171.7 ± 55.3	169.9 ± 53.9	172.9 ± 56.2	0.547
Donor age, yrs	32.3 ± 12.9	33.1 ± 13.2	31.8 ± 12.7	0.255
Hypertension	218 (41.7)	91 (42.1)	127 (41.4)	0.885
Diabetes	111 (21.2)	46 (21.3)	65 (21.2)	0.973
Baseline primary IS				0.60
Cyclosporine	292 (55.8)	131 (60.6)	161 (52.4)	
Tacrolimus	231 (44.2)	85 (39.4)	146 (47.6)	
AZA/MMF				0.066
AZA	167 (31.9)	79 (36.6)	88 (28.7)	
MMF	356 (68.1)	137 (63.4)	219 (71.3)	
Steroids	491 (93.9)	201 (93.9)	288 (93.8)	0.972

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regimens. Based on our protocol, CNI was typically used for the first 6 months after HT to avoid delayed wound healing that could occur with earlier introduction of SRL. Biopsies were generally performed 2 weeks following the conversion process, and a reduced dose of CNI was reintroduced if the biopsy was positive for rejection, with a second attempt to withdraw CNI therapy later if rejection subsided. Until July 2006, reasons for conversion included impaired renal function secondary to CNI (estimated glomerular filtration rate ≤50 ml/min/1.73 m^2 and lack of any other identifiable causes of renal dysfunction), CAV International Society for Heart and Lung Transplantation grade 2 or worse detected on annual coronary angiography, or intolerance of CNIs. We identified 307 patients who were converted to the SRL-based regimen with complete withdrawal of CNIs at a median of 1.1 years post-HT (interquartile range: 0.6 to 3.2 years). Seventy-five of these patients were converted before and 234 patients were converted after July 2006.

OUTCOMES. The main outcomes of our analysis were: 1) incidence of overall de novo malignancies excluding NMSC (hereafter denoted as non-NMSC malignancies); 2) incidence of NMSC; 3) rates of subsequent primary occurrences of NMSC; 4) incidence of PTLD; and 5) incidence of non-PTLD malignancies. Patient survival rates were analyzed comparing those who developed and those who did not develop a malignancy during follow-up, as well as comparing patients who were maintained on CNI and those who were converted to SRL as primary IS, based on patients who survived the first 6 months after HT.

STATISTICAL ANALYSIS. Categorical data were described by counts and percentages. Numerical data were described by mean ± SD or median (interquartile range), if noticeably skewed. Patient characteristics were compared between groups using the chi-square test for categorical variables and the independent Student's *t*-test for continuous variables for numerical variables, or Wilcoxon rank-sum test, if noticeably skewed.

The effect of SRL on the incidence of malignancies was modeled using Cox regression with time-dependent covariates indicating if and when patients were converted to SRL, censoring for death. Similarly, Cox regression models were used for assessing the effect of malignancy and conversion to SRL on survival post-HT with both malignancy and conversion to SRL treated as time-dependent covariates. For the multivariate analyses, age, sex, combined organ transplantation, EBV, and cytomegalovirus (CMV) infections (both treated as time-dependent covariates), type of secondary IS (MMF vs. azathioprine), and type of induction therapy (OKT-3 vs. ATG) were included in the Cox regression models. In those patients who developed skin cancer, subsequent primary occurrence of NMSC was modeled using the Anderson-Gill model for multiple events of the same type. In this model, a patient who had a subsequent primary occurrence of NMSC remained in the risk set. To graphically demonstrate differences, we described incidence curves from Cox models that were conditional on patients being converted to SRL (and therefore, cancer-free survival) at or before 6 months post-HT, or maintained indefinitely on CNI (never converted to SRL). For the Anderson-Gill model, the time from first skin cancer occurrence was used as the time scale. All significance tests were 2-tailed and conducted at the 5%

significance level. Data were analyzed with SAS 9.4 (SAS Institute, Cary, North Carolina) and R 3.3 (R Foundation, Vienna, Austria) software.

RESULTS

BASELINE CHARACTERISTICS. Baseline demographic and clinical characteristics of the study participants are presented in Table 1. Baseline time was considered as the time of the follow-up visit at 12 months after HT for both the CNI and SRL groups. We identified 307 HT recipients who converted to SRL and 216 patients who remained on CNI IS therapy. At baseline, SRL patients were slightly older by 3.7 years, were more likely to receive combined heart and liver transplantation (SRL: 8.5% vs. CNI: 4.6%) but less frequently underwent heart and lung transplantation (SRL: 0.65% vs. CNI: 7.9%), and received ATG more frequently (SRL: 79% vs. CNI: 57.5%) and OKT3 less frequently (SRL: 21.2% vs. CNI: 42.1%) than patients on CNI-based IS. A trend toward a more frequent use of MMF (SRL: 71.3% vs. CNI: 63.4%) instead of azathioprine (SRL: 28.7% vs. CNI: 36.6%) was noted in the SRL group ($p = 0.066$). The rates of EBV and CMV viremia were similar between the SRL and CNI groups at baseline. Moreover, there was no significant difference in rates of acute cellular, antibody-mediated rejections, or hemodynamically significant rejection (defined as any proven rejection that was associated with allograft dysfunction and hemodynamic instability) between the 2 groups.

TABLE 1 Continued

	All Group (N = 523)	CNI Alone (n = 216)	SRL Converters (n = 307)	p Value (CNI vs. SRL)
Statins	450 (86.0)	179 (82.9)	271 (88.3)	0.080
Fibrates	24 (4.8)	6 (3.0)	18 (5.9)	0.142
Aspirin	77 (14.7)	26 (12.0)	51 (16.6)	0.146
Plavix	2 (0.38)	1 (0.46)	1 (0.33)	0.802
Anticoagulation	34 (6.5)	10 (4.7)	24 (7.8)	0.149
Diuretics	334 (63.9)	130 (60.2)	204 (66.5)	0.142
CCB	268 (53.1)	101 (51.0)	167 (54.4)	0.457
BB	58 (11.5)	17 (8.6)	41 (13.4)	0.101
ACE inhibitors	149 (29.5)	60 (30.3)	89 (29.0)	0.752
ISHLT CAV grade				0.576
0	322 (67.4)	112 (64.0)	210 (69.3)	
1	148 (31.0)	60 (34.3)	88 (29.0)	
2	4 (0.84)	1 (0.57)	3 (0.99)	
3	4 (0.84)	2 (1.14)	2 (0.66)	
Induction therapy				<0.0001
OKT-3	156 (29.8)	91 (42.1)	65 (21.2)	
ATG	367 (70.2)	125 (57.9)	242 (78.8)	
CMV viremia	125 (24.8)	49 (24.8)	76 (24.8)	0.998
CMV mismatch	142 (27.6)	52 (24.9)	90 (29.4)	0.259
EBV viremia	27 (5.4)	14 (7.3)	13 (4.3)	0.147
EBV mismatch	29 (5.6)	10 (4.8)	19 (6.2)	0.483
Cellular rejection, ≥2 R	107 (21.2)	50 (24.6)	57 (18.9)	0.121
Cellular rejection, 3R	21 (4.2)	9 (4.4)	12 (4.0)	0.800
AMR	41 (7.8)	12 (5.6)	29 (9.5)	0.103
HSR*	32 (6.3)	16 (7.8)	16 (5.2)	0.229

Values are as mean ± SD, n (%), or median (quartile 1 to quartile 3). *Hemodynamically significant rejection (HSR) was defined as an episode of acute graft rejection resulting in significant allograft dysfunction and hemodynamic instability.

ACE = angiotensin-converting enzyme; AMR = antibody-mediated rejection; ATG = antithymocyte globulin; AZA = azathioprine; BB = beta blocker; BMI = body mass index; BMT = bone marrow transplantation; CCB = calcium-channel blocker; CAV = cardiac allograft vasculopathy; CHD = congenital heart disease; CMV = cytomegalovirus; CNI = calcineurin inhibitor; DCM = dilated cardiomyopathy; EBV = Epstein-Barr virus; eGFR = estimated glomerular filtration rate; HCM = hypertrophic cardiomyopathy; HDL = high-density lipoprotein; HT = heart transplantation; ICM = idiopathic cardiomyopathy; IS = immunosuppression; ISHLT = International Society for Heart and Lung Transplantation; IVUS = intravascular ultrasound; LDL = low-density lipoprotein; LVEF = left ventricular ejection fraction; MMF = mycophenolate mofetil; OKT3 = muromonab-CD3; SRL = sirolimus.

DISTRIBUTION OF MALIGNANCIES DURING FOLLOW-UP. The distribution of the different types of malignancy during follow-up, including the absolute event number and rate per 100 person-years, are presented in Table 2. Most malignancies were NMSCs (n = 169; 92 in the CNI and 77 in the SRL groups). Among patients with this type of malignancy, 317 subsequent primary occurrences of NMSC were reported (n = 198 in the CNI and n = 119 in the SRL groups). Squamous cell carcinoma of the skin accounted for most of the NMSCs (n = 123; 70 in the CNI and n = 53 in the SRL groups) and subsequent primary occurrences of NMSCs (n = 276; n = 179 in the CNI and n = 97 in the SRL groups). As shown in Table 2, there were 92 overall de novo non-NMSC events, most of which occurred while patients were on CNI therapy and less frequently while on SRL therapy (65 cases vs. 27 cases in the CNI and SRL groups, respectively). PTLD was the most frequent malignancy within this group, with most cases reported in the CNI group (n = 22) compared with only 2 cases that were reported in the SRL group. Among the non-PTLD malignancies, those involving the gastrointestinal (n = 15), genitourinary (n = 15), and pulmonary (n = 13) tracts were the most frequent.

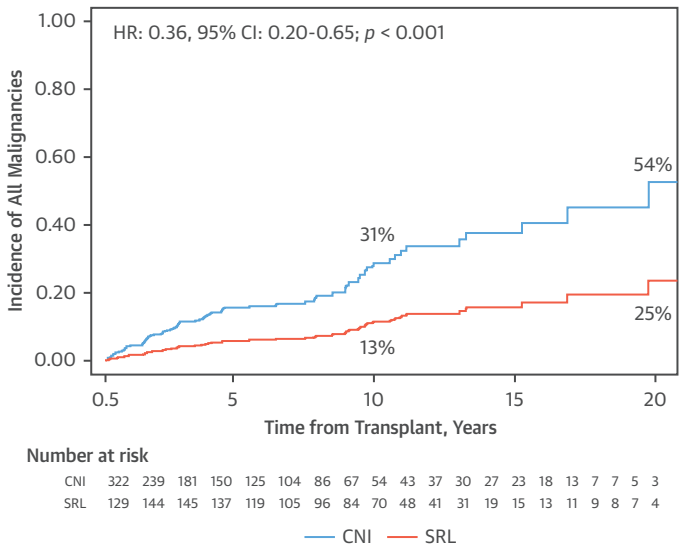
EFFECTS OF IS REGIMENS ON MALIGNANCY AND SKIN CANCER OUTCOMES. We identified 92 cases of overall de novo non-NMSC malignancies, 24 cases of PTLD, 68 cases of non-PTLD, and 169 cases of NMSC. During follow-up, overall non-NMSC malignancies occurred at a rate of 3.6 per 100 person-years for patients maintained on CNI and 2.2 per 100 person-years for patients converted to SRL. The estimated incidence of non-NMSC at 10 years post-HT was 31% (range 23% to 39%) of patients maintained on CNI and 13% (range 6% to 19%) of patients converted to SRL (unadjusted hazard ratio [HR]: 0.36; 95% confidence interval [CI]: 0.20 to 0.65 for SRL compared with CNI; $p < 0.001$) (Figure 1). Multivariate Cox

TABLE 2 Distribution of Cancer Events While on CNI or SRL Therapy

	Overall	While on CNI	While on SRL
Overall non-NMSC malignancy	92 (3.0)	65 (3.6)	27 (2.2)
PTLD	24 (0.6)	22 (1.0)	2 (0.1)
Non-PTLD	68 (1.8)	43 (2.0)	25 (1.4)
Gastrointestinal	15 (0.4)	11 (0.5)	4 (0.2)
Lung	13 (0.3)	7 (0.3)	6 (0.3)
Genitourinary	15 (0.4)	8 (0.4)	7 (0.4)
Hematology	6 (0.2)	4 (0.2)	2 (0.1)
Melanoma	11 (0.3)	8 (0.4)	3 (0.2)
Breast	4 (0.1)	2 (0.1)	2 (0.1)
Other	4 (0.1)	3 (0.1)	1 (0.1)
First NMSC	169 (5.3)	92 (4.9)	77 (5.9)
SCC	123 (2.9)	70 (3.7)	53 (4.0)
BCC	46 (2.4)	22 (1.2)	24 (1.8)
Total no. of subsequent primary occurrences of NMSC	317 (35.3)	198 (63.6)	119 (20.3)
SCC	276 (30.7)	179 (57.5)	97 (16.5)
BCC	41 (4.6)	19 (6.1)	22 (3.7)

Values are number (event count, calculated as event rate per 100 person-years).
BCC = basal cell carcinoma; NMSC = non-melanoma skin cancer; PTLD = post-transplant lymphoproliferative disorder; SCC = squamous cell carcinoma; other abbreviations as in Table 1.

FIGURE 1 Cumulative Incidences of Overall De Novo Non-NMSC Malignancies With CNI Versus SRL Therapy



The estimates were derived using a Cox regression model with a model term for sirolimus (SRL) entered as a time-dependent predictor and a model term for age at time of transplantation, and are described for a patient age 50 years (the average age). The incidence estimates are conditional on patients being alive without a malignancy and on the respective immunosuppression at 6 months post-transplantation. CI = confidence interval; CNI = calcineurin inhibitor; HR = hazard ratio; NMSC = non-melanoma skin cancer.

regression models after adjustment for age, sex, combined organ transplantation, type of induction and secondary IS therapies, as well as CMV and EBV infections (both treated as time-dependent covariates), demonstrated a significantly lower incidence of overall de novo non-NMSCs in the SRL group (adjusted HR: 0.34; 95% CI: 0.18 to 0.62; $p < 0.001$) similar to the unadjusted incidence (Table 3). The incidence of PTLD was significantly lower in the SRL group (unadjusted HR: 0.14; 95% CI 0.03 to 0.63; $p = 0.01$) (Figure 2A), and, after multivariate adjustment, SRL remained similarly associated with lower risk of PTLD (adjusted HR: 0.13; 95% CI: 0.03 to 0.59; $p = 0.009$) (Table 3). There was a trend toward a decreased incidence of non-PTLD malignancies among patients treated with SRL compared with those maintained on CNI IS therapy (unadjusted HR: 0.65; 95% CI: 0.38 to 1.10; $p = 0.09$, and adjusted HR: 0.62; 95% CI: 0.34 to 1.12; $p = 0.11$) (Figure 2B, Table 3). The incidence of the first NMSC after HT was similar in the SRL and CNI groups (unadjusted HR: 1.17; 95% CI: 0.85 to 1.60; $p = 0.34$; adjusted HR: 0.92; 95% CI: 0.66 to 1.28; $p = 0.62$) (Figure 3A, Table 4). However, SRL conversion was associated with a significantly decreased risk of subsequent primary occurrences of NMSC compared with CNI therapy (unadjusted HR: 0.44; 95% CI: 0.27 to 0.71; $p < 0.001$; adjusted HR: 0.44; 95% CI: 0.28 to 0.69; $p < 0.001$) (Figure 3B, Table 4).

OTHER RISK FACTORS OF MALIGNANCY AND SKIN CANCER. The results of the multivariate regression analysis for overall non-NMSC events and for the first and subsequent primary occurrences of NMSC events are presented in Tables 3 and 4, respectively. We identified age at the time of HT (adjusted HR: 1.03; 95% CI: 1.01 to 1.05; $p = 0.002$), combined organ transplantation (adjusted HR: 1.69; 95% CI: 1.03 to 2.78; $p = 0.04$), and EBV viremia (adjusted HR: 2.03; 95% CI: 1.02 to 4.01; $p = 0.04$) as significant predictors for overall de novo non-NMSC malignancy risk post-HT. EBV viremia was the only independent predictor of increased PTLD risk (adjusted HR: 7.46; 95% CI: 2.9 to 19.2; $p < 0.001$), whereas age and combined organ transplantation were the only significant predictors for non-PTLD malignancy risk (adjusted HR: 1.04; 95% CI: 1.02 to 1.07; $p = 0.001$; and adjusted HR 2.0; 95% CI: 1.15 to 3.48; $p = 0.01$, respectively) (Table 3). Age remained the only significant predictor of increased risk of the first NMSC post-HT (adjusted HR: 1.06; 95% CI: 1.04 to 1.07; $p < 0.001$) and subsequent primary occurrences of NMSC (adjusted HR: 1.05; 95% CI: 1.02 to 1.07; $p < 0.001$) (Table 4). Neither the type of secondary IS (MMF vs. azathioprine) nor

TABLE 3 Univariate and Multivariate Cox Regression Models Examining the Effect of Conversion to SRL Versus CNI on the Incidence of Overall Non-NMSC, PTLD, and Non-PTLD Malignancies

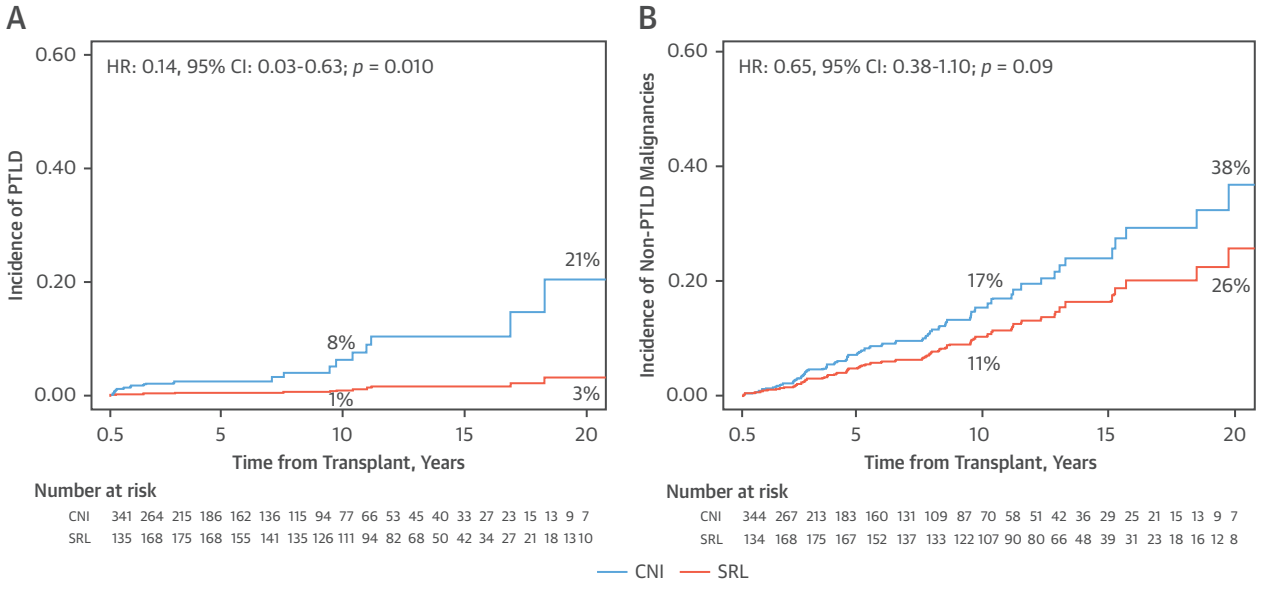
	Overall Non-NMSC Malignancies		PTLD		Non-PTLD	
	HR* (95% CI)	p Value	HR* (95% CI)	p Value	HR* (95% CI)	p Value
SRL conversion						
Univariate	0.36 (0.20–0.65)	<0.001	0.14 (0.03–0.63)	0.010	0.65 (0.38–1.10)	0.090
Multivariate	0.34 (0.18–0.62)	<0.001	0.13 (0.03–0.59)	0.009	0.62 (0.34–1.12)	0.110
Age at transplantation (per yr)						
Univariate	1.02 (1.01–1.04)	0.010	0.98 (0.95–1.01)	0.101	1.04 (1.02–1.06)	<0.001
Multivariate	1.03 (1.01–1.05)	0.002	0.99 (0.96–1.02)	0.482	1.04 (1.02–1.07)	0.001
Sex (M vs. F)						
Univariate	1.31 (0.81–2.11)	0.271	1.03 (0.43–2.50)	0.941	1.40 (0.78–2.50)	0.254
Multivariate	1.18 (0.70–1.99)	0.529	1.81 (0.61–5.33)	0.285	0.94 (0.51–1.74)	0.850
Combined organ transplantation						
Univariate	1.66 (1.05–2.63)	0.029	1.22 (0.48–3.08)	0.676	1.88 (1.11–3.17)	0.019
Multivariate	1.69 (1.03–2.78)	0.039	0.85 (0.27–2.67)	0.781	2.00 (1.15–3.48)	0.014
EBV infection						
Univariate	1.58 (0.81–3.08)	0.183	6.10 (2.56–14.57)	<0.001	0.84 (0.33–2.10)	0.704
Multivariate	2.03 (1.02–4.01)	0.042	7.46 (2.90–19.21)	<0.001	0.91 (0.36–2.34)	0.849
CMV infection						
Univariate	1.00 (0.62–1.60)	0.989	0.82 (0.32–2.07)	0.675	0.94 (0.54–1.61)	0.810
Multivariate	0.99 (0.60–1.62)	0.960	0.92 (0.35–2.45)	0.870	0.86 (0.49–1.51)	0.611
Secondary IS (MMF vs. AZA)						
Univariate	0.65 (0.42–0.99)	0.049	0.48 (0.20–1.15)	0.100	0.75 (0.45–1.23)	0.248
Multivariate	0.74 (0.46–1.19)	0.214	0.54 (0.20–1.43)	0.213	0.79 (0.46–1.36)	0.401
Induction therapy (OKT3 vs ATG)						
Univariate	1.53 (0.99–2.38)	0.056	1.63 (0.70–3.78)	0.260	1.30 (0.77–2.18)	0.325
Multivariate	1.23 (0.74–2.06)	0.422	1.25 (0.44–3.53)	0.675	1.06 (0.59–1.93)	0.837

*Hazard ratios (HRs) comparing patients receiving SRL relative to patients receiving CNI were derived using a Cox model with a time-dependent covariate for conversion to SRL and after adjustment for age, sex, combined organ transplantation, EBV and CMV infections (both treated as time-dependent covariates), and secondary and induction immunosuppressive therapies.
CI = confidence interval; other abbreviations as in Tables 1 and 2.

the type of induction therapy (ATG vs. OKT3) were associated with the risk of overall non-NMSC, PTLD, non-PTLD, or NMSC events in this HT cohort. In addition, we found no impact of rejection burden on the incidence of non-NMSC or NMSC. Specifically, patients with at least 1 treated cellular rejection or antibody-mediated rejection event, as well as those with a higher number of treated rejection events during the first year post-HT had similar rates of malignancy both in the univariate and multivariate models (data not shown).

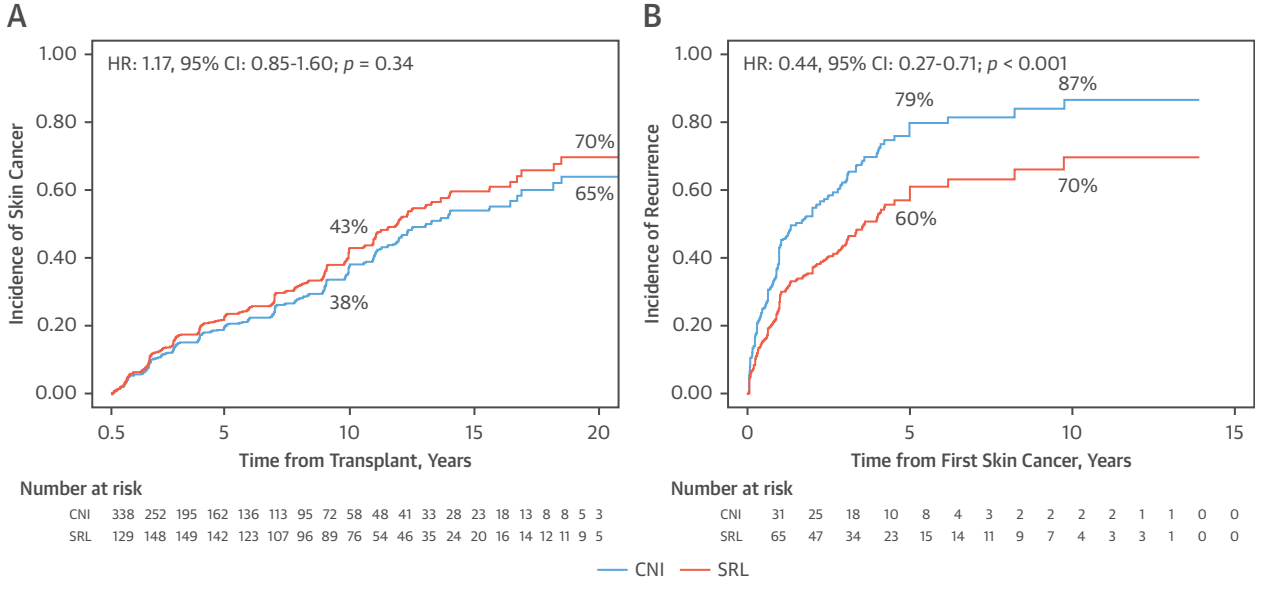
EFFECTS OF MALIGNANCY AND IS REGIMEN ON SURVIVAL. For overall de novo malignancies, the rate of death increased from 3.6 per 100 person-years in patients without malignancy to 13.5 per 100 person-years in patients with malignancy. Using a Cox model with malignancy treated as a time-dependent covariate, estimated survival at 10 years was markedly decreased to 36% (range 24% to 55%) from 70% (range 65% to 75%) among patients with and without malignancy, respectively (unadjusted HR for mortality: 3.85; 95% CI: 2.71 to 5.48; $p < 0.0001$) (Figure 4A). After adjustment for age, sex, combined organ transplantation, and type of induction therapy, non-NMSC malignancy remained significantly associated with a higher risk of mortality (adjusted HR: 3.93; 95% CI: 2.74 to 5.62; $p < 0.0001$) (Table 5). In addition to non-NMSC malignancies, in this multivariate regression analysis, we identified age at the time of HT (HR: 1.02; 95% CI: 1.01 to 1.03; $p < 0.0001$), female sex (HR: 1.45; 95% CI: 1.04 to 2.04; $p = 0.026$), and OKT3 induction therapy (HR: 1.47; 95% CI: 1.08 to 2.00; $p = 0.015$) as significant predictors of mortality after HT. When stratified according to malignancy type, both PTLD (adjusted HR: 2.43; 95% CI: 1.33 to 4.43; $p = 0.004$) and non-PTLD malignancy (adjusted HR: 4.10; 95% CI: 2.77 to 6.04; $p < 0.0001$) were associated with increased risk of mortality compared with those who did not have these types of malignancies (Figure 4B and 4C, Table 5). The survival rates were similar between patients who developed NMSC and those who did not develop NMSC (adjusted HR: 1.27; 95% CI: 0.87 to 1.84; $p = 0.21$) (Figure 4D, Table 5). Consistent with

FIGURE 2 Cumulative Incidences of PTLD and Non-PTLD Comparing Patients Converted to SRL With Those Maintained on CNI Immunosuppression



(A) Conversion to SRL was significantly associated with decreased rates of post-transplantation lymphproliferative disorder (PTLD) (p = 0.01), and **(B)** with a trend toward decreased rates of non-PTLD (p = 0.09) compared with continued CNI therapy. Other abbreviations as in Figure 1.

FIGURE 3 Cumulative Incidences of First and Subsequent Primary Occurrences of NMSC After HT Comparing SRL With CNI Maintenance Therapy



(A) Conversion to SRL was associated with similar rates of incident (first ever) NMSC after transplantation (p = 0.34), but **(B)** significantly decreased rates of subsequent primary occurrences of NMSC (p < 0.001). HT = heart transplantation; other abbreviations as in Figures 1 and 2.

TABLE 4 Univariate and Multivariate Cox Regression Models Examining the Effect of Conversion to SRL Versus CNI on the Incidence of First and Subsequent Primary Occurrences of NMSC

	NMSC (First Occurrence)		NMSC (Subsequent Primary Occurrences)	
	HR* (95% CI)	p Value	HR* (95% CI)	p Value
SRL conversion				
Univariate	1.17 (0.85–1.60)	0.337	0.44 (0.27–0.71)	<0.001
Multivariate	0.92 (0.66–1.28)	0.615	0.44 (0.28–0.69)	<0.001
Age at transplantation (per yr)				
Univariate	1.06 (1.04–1.08)	<0.001	1.05 (1.03–1.08)	<0.001
Multivariate	1.06 (1.04–1.07)	<0.001	1.05 (1.02–1.07)	<0.001
Sex (M vs. F)				
Univariate	1.56 (1.09–2.23)	0.014	2.23 (1.07–4.64)	0.032
Multivariate	1.18 (0.82–1.70)	0.374	1.24 (0.63–2.45)	0.532
Combined organ transplantation				
Univariate	1.22 (0.86–1.74)	0.259	1.07 (0.60–1.88)	0.826
Multivariate	1.16 (0.81–1.66)	0.415	0.98 (0.62–1.57)	0.947
EBV infection				
Univariate	0.92 (0.54–1.56)	0.748	0.79 (0.38–1.62)	0.511
Multivariate	1.03 (0.59–1.77)	0.927	0.91 (0.39–2.15)	0.834
CMV infection				
Univariate	1.14 (0.82–1.57)	0.434	1.68 (1.06–2.67)	0.027
Multivariate	1.05 (0.75–1.46)	0.775	1.48 (0.99–2.21)	0.054
Secondary IS (MMF vs. AZA)				
Univariate	1.13 (0.82–1.54)	0.463	0.73 (0.45–1.20)	0.221
Multivariate	1.11 (0.79–1.55)	0.554	0.81 (0.54–1.23)	0.331
Induction therapy (OKT-3 vs. ATG)				
Univariate	0.71 (0.50–0.99)	0.045	1.26 (0.76–2.10)	0.376
Multivariate	0.84 (0.58–1.23)	0.370	0.97 (0.60–1.56)	0.897

*HRs comparing patients who received SRL with patients who received CNI were derived using a Cox model with a time-dependent covariate for conversion to SRL and after adjustment for age, sex, combined organ transplantation, EBV and CMV infections (both treated as time-dependent covariates), and secondary and induction immunosuppressive therapies.

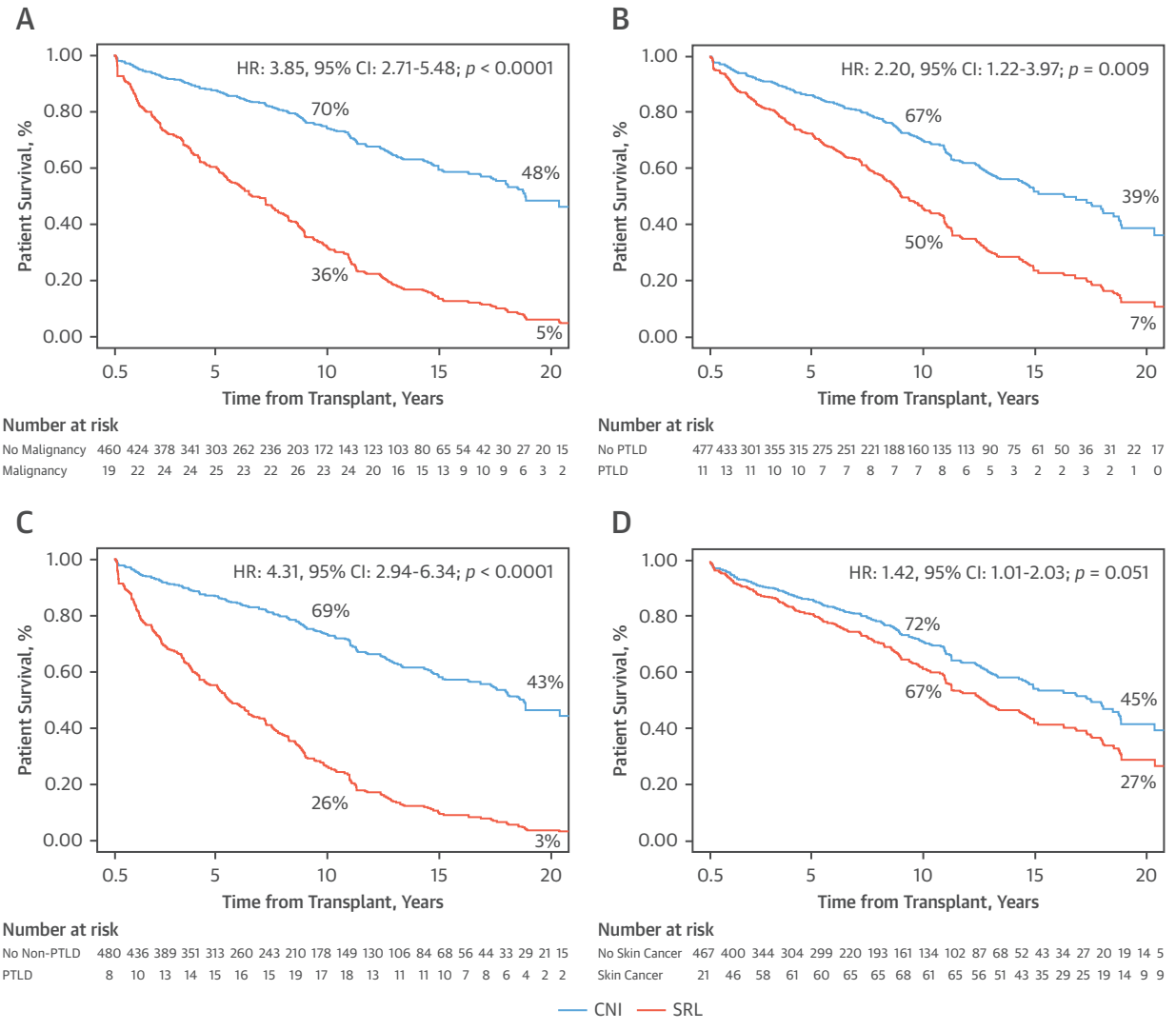
Abbreviations as in Tables 1 to 3.

our previous results that showed increased survival probabilities following conversion to SRL among HT recipients who had at least 1 intravascular ultrasound study (14), we found that in this larger cohort of HT patients (including all HT patients, regardless of having an intravascular ultrasound study) that patients who were converted to SRL before 6 months and who were alive at 6 months had a survival probability of 82% (range: 76% to 88%) at 10 years and of 47% (range: 35% to 62%) at 20 years of follow-up compared with 57% (range: 50% to 64%) and 26% (range: 17% to 40%) in patients who were maintained on a CNI, respectively ($p < 0.0001$) (Figure 5A). After adjustment, conversion to SRL was associated with remarkably decreased risk of mortality following HT (adjusted HR: 0.48; 95% CI: 0.34 to 0.68; $p < 0.0001$) (Table 6). Conversion to SRL was also associated with increased estimated rates of malignancy-free survival (70% vs. 38%, and 40% vs. 17% at 10 and 20 years of follow-up for the SRL and CNI groups, respectively; $p < 0.0001$) (Figure 5B, Table 6).

DISCUSSION

The present study demonstrated that conversion to SRL as primary IS, with withdrawal of CNI therapy, was associated with a significantly decreased incidence of overall de novo non-NMSC malignancies, independently of other risk factors, among a large cohort of patients who underwent HT. Because of the lack of a large pool of HT recipients treated with mTOR inhibitors to date, this study provided the strongest evidence supporting the beneficial effects of SRL on development of de novo malignancies following HT. These favorable effects seen with long-term SRL use were largely driven by substantial risk reduction in PTLD risk, but also by a trend toward a lower incidence of other life-threating malignancies. Although the incidence of first NMSC post-HT did not differ considerably between the 2 groups, subsequent primary occurrences of NMSC were significantly lower in patients who received SRL compared with those who were maintained on CNI IS therapy.

FIGURE 4 Patient Survival Probabilities After HT Comparing Patients With and Without De Novo Malignancy



Survival curves were derived using a Cox regression model with malignancy treated as a time-dependent predictor and conditional on patients being alive at 6 months post-transplantation. **(A)** Overall non-NMSC de novo malignancies, **(B)** PTLD, **(C)** non-PTLD, and **(D)** NMSC. Abbreviations as in **Figures 1 to 3**.

Furthermore, survival post-HT was dramatically decreased after development of overall de novo non-NMSC malignancy, and the reduction in overall life-threatening malignancy risk after conversion to SRL was associated with improvement in late survival (**Central Illustration**). Our findings supported a SRL maintenance IS strategy after HT, when tolerable, for decreasing risk of malignancy and improving late survival post-HT beyond its cardiac-related benefits.

After an approach of additional improvement in cancer screening in HT patients, implementation of IS protocols that confer diminished carcinogenic properties is necessary to mitigate malignancy risk and improve late survival. Conventional IS medications, especially CNIs, have specific tumor-promoting activities (8,9). In contrast, SRL is an exceptional immunosuppressant due to its additive inhibitory effects on tumor growth, including antiproliferative, antimigratory, and antiangiogenic activities in various tissues, beyond its effects on the immune system. However, clinical data to support malignancy risk reduction with the use of SRL, or other mTOR inhibitors, in the HT population is lacking. Although data from kidney transplantation patients suggest

TABLE 5 Multivariate Cox Regression Model Examining the Effect of Different Types of Malignancies on Death After HT

	Overall Non-NMSC Malignancies		PTLD		Non-PTLD		NMSC	
	HR* (95% CI)	p Value	HR* (95% CI)	p Value	HR* (95% CI)	p Value	HR* (95% CI)	p Value
Malignancy	3.93 (2.74–5.62)	<0.0001	2.43 (1.33–4.43)	0.004	4.09 (2.77–6.04)	<0.0001	1.27 (0.87–1.84)	0.214
Age at transplantation (per yr)	1.02 (1.01–1.03)	<0.0001	1.02 (1.01–1.03)	<0.0001	1.02 (1.01–1.03)	0.002	1.02 (1.01–1.03)	0.003
Sex (M vs. F)	0.98 (0.69–1.38)	0.892	1.10 (0.78–1.56)	0.581	1.03 (0.73–1.46)	0.865	1.14 (0.81–1.62)	0.441
Combined organ Transplant	0.69 (0.49–0.96)	0.026	0.72 (0.52–0.99)	0.046	0.70 (0.50–0.97)	0.035	0.72 (0.52–1.00)	0.049
Induction therapy (OKT-3 vs. ATG)	1.47 (1.08–2.00)	0.015	1.52 (1.12–2.07)	0.008	1.50 (1.10–2.04)	0.01	1.55 (1.14–2.10)	0.005

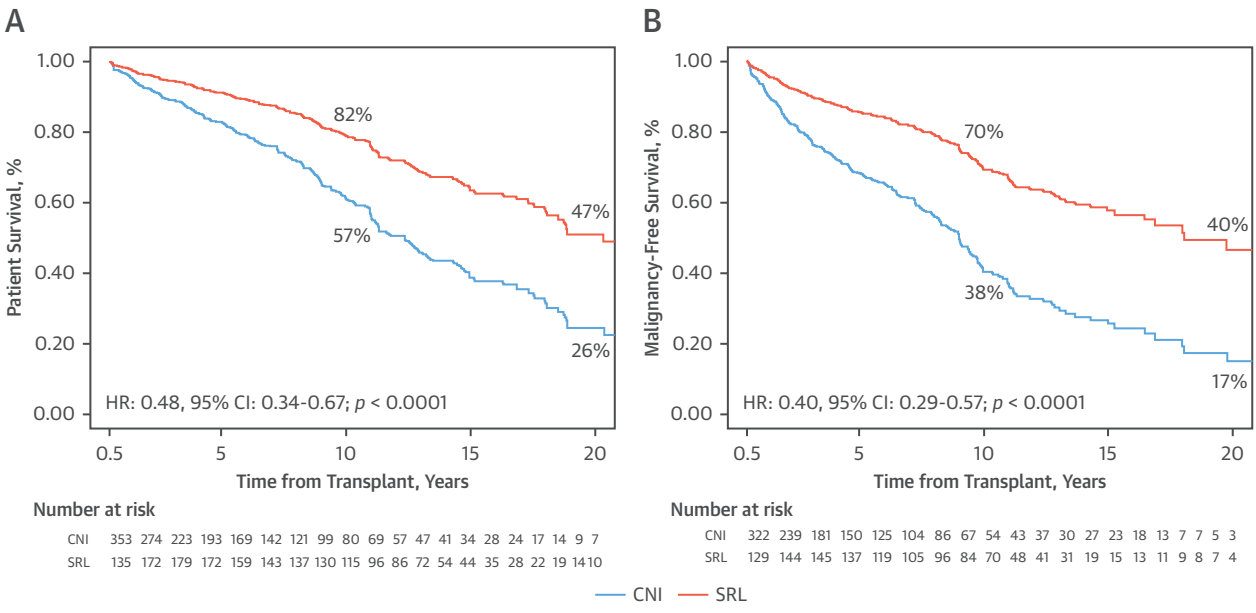
*HRs for incidences of death comparing patients with and without de novo malignancy post-HT were derived using a Cox model with a time-dependent covariate for malignancy after adjustment for age, sex, combined organ transplantation, and type of induction therapy.

Abbreviations as in Tables 1 to 3.

that mTOR inhibitors may be effective in reducing and treating some malignancies (15–17), their relevance to the prophylaxis of malignancies in HT setting is more uncertain. For instance, the exceedingly higher intensity of IS required after HT may affect the incidence and tumor types observed in this population.

In the HT population, a 5-year study that included 78 HT recipients randomized to receive tacrolimus plus MMF, tacrolimus and SRL, or SRL and MMF, found no difference in cancer incidence among the 3 groups (18). However, this study was limited by the younger age of the study participants and the small number of cancer events (n = 5) after 5 years of follow-up. A more recent cross-sectional analysis (13) showed that application of mTOR inhibitor therapy for >1 year after HT was correlated with lower cancer prevalence, especially of non-cutaneous malignancies. The mechanisms underlying the beneficial antineoplastic properties of SRL were corroborated by evidence in animal models that mTOR inhibition suppressed development and growth of tumors (9,10). SRL suppressed tumor growth factor-β and led to cell cycle arrest (19). SRL-dependent inhibition of cell proliferation attenuated oncogenic transformation as observed in experimental models of

FIGURE 5 Patient Survival and Malignancy-Free Survival Probabilities in HT Recipients Comparing Patients Converted to SRL With Those Maintained on CNI Therapy



(A) Both patient survival and **(B)** malignancy-free survival were significantly increased among patients who were converted to SRL compared with CNI maintenance therapy. Abbreviations as in Figures 1 to 3.

TABLE 6 Multivariate Cox Regression Model Examining the Effect of Conversion to SRL Compared With Continued CNI IS on the Incidences of Death and the Composite of Overall Non-NMSC Malignancies and Death

	Death		Overall Non-NMSC Malignancies or Death	
	HR* (95% CI)	p Value	HR* (95% CI)	p Value
Sirolimus conversion	0.48 (0.34–0.68)	<0.0001	0.39 (0.27–0.56)	<0.0001
Age at transplantation (per yr)	1.02 (1.01–1.04)	<0.0001	1.02 (1.01–1.04)	<0.0001
Sex (M vs. F)	1.12 (0.79–1.58)	0.517	1.32 (0.97–1.80)	0.076
Combined organ transplantation	0.73 (0.53–1.01)	0.056	0.91 (0.68–1.21)	0.507
Induction therapy (OKT-3 vs. ATG)	1.24 (0.90–1.71)	0.191	1.18 (0.89–1.58)	0.254

*HRs comparing patients who received SRL with patients who received CNI were derived using a Cox model with a time-dependent covariate for conversion to SRL and after adjustment for age, sex, combined organ transplantation, and type of induction therapy.
Abbreviations as in Tables 1 to 3.

primary and metastatic tumor growth (19). Furthermore, SRL exerted antiangiogenic activities, which led to decreased production of vascular endothelial growth factor and to a markedly inhibited response of vascular endothelial cells to stimulation by vascular endothelial growth factor (10). Besides its antiangiogenetic effects, SRL inhibits lymphangiogenesis and cell proliferation via inhibition of p70 S6K kinase, Janus kinase/signal transducer and activator of transcription proteins activity (20). These effects are related to the direct effects of SRL on vascular endothelial growth factor, p70 S6K, and Akt kinase as suggested by the regression of Kaposi sarcoma lesions, which overexpress these molecules by using a SRL conversion strategy (21). Another potential factor that contributes to the beneficial effects of SRL on tumor proliferation is the use of lower doses of CNIs when these are combined with SRL. Therefore, the complete withdrawal of CNI therapy according to our IS protocol may provide further explanation for the greater benefit on malignancy incidence seen in the present study.

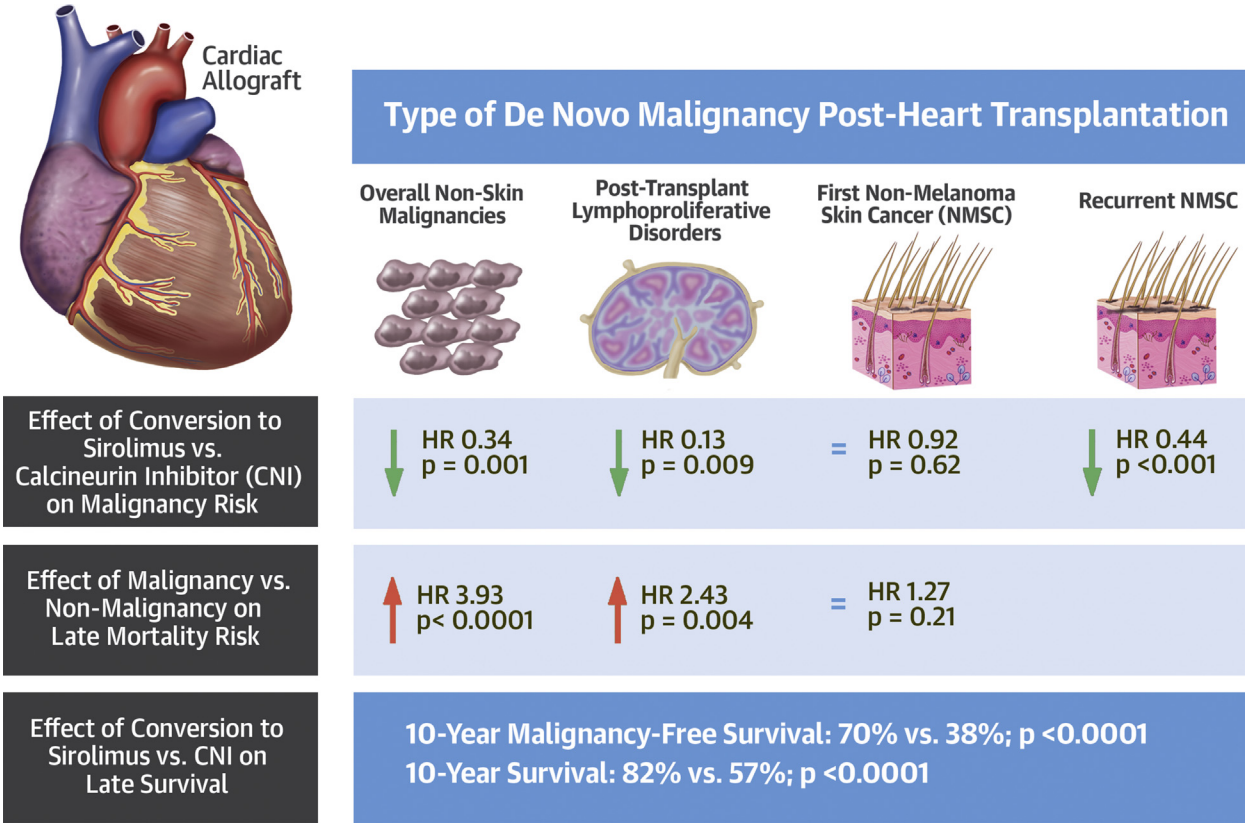
Despite the observed benefit with SRL-based IS strategy on overall de novo malignancies, our findings suggested more pronounced effects on PTLD incidence, which were independent of EBV infection and type of induction therapy. The mechanisms behind which SRL confers more protection against PTLD, compared with other solid cancers, are not fully understood. One study showed that the PI3K/Akt/mTOR pathway was constitutively active in EBV-positive B lymphomas from patients with PTLD, and that SRL combined with PI3K-δ inhibitor synergistically suppressed the proliferation of EBV-positive B lymphoma cells (22). This might provide a mechanistic explanation of the PTLD risk benefit seen in patients converted to SRL in our cohort. In support of our findings, everolimus was shown to have significant activity in non-Hodgkin lymphoma in the relapsed setting and in combination therapy (23,24).

This study provided further data regarding the risk of first and subsequent primary occurrences of NMSC events in HT recipients in association with conversion to SRL. Unlike the general population, in which basal cell carcinoma is more frequent than squamous cell carcinoma, solid organ transplantation recipients develop squamous cell skin cancer more frequently (6,25). In a patient-level meta-analysis of 5,876 kidney and kidney-pancreas transplantation recipients from 21 randomized trials, SRL was associated with a significantly decreased incidence of skin cancer (15). However, these findings were not confirmed by a subsequent study (26). Based on our observation, it appeared that substitution of CNI by SRL as primary IS had a more positive impact on susceptible patients with a history of NMSC post-HT, but future studies are needed to explore these differential effects of SRL on NMSC prophylaxis versus subsequent NMSC occurrences. Moreover, unlike other solid cancers and lymphoproliferative disorders, survival after HT was not affected by the development of NMSC despite the potentially more aggressive nature of these post-HT skin tumors. This was consistent with a previous study by Brewer et al. (27) that showed low mortality attributable to skin cancer after HT.

The main limitation of SRL use is drug intolerance. Approximately 15% of our patients did not tolerate SRL, and although patients may tolerate additional trial of conversion later during follow-up, >10% of patients could not tolerate the conversion process and remained on CNI therapy. Intolerance to SRL is largely due to sever gastrointestinal symptoms, mouth ulcers, and refractory edema that requires its discontinuation.

STUDY LIMITATIONS. There were several limitations inherent to the observational, retrospective, nonrandomized design of our study. As in any observational study, we could not exclude residual confounding associations, although we adjusted for

CENTRAL ILLUSTRATION **Sirolimus and Malignancy Risk After Heart Transplantation**



Asleh, R. et al. J Am Coll Cardiol. 2019;73(21):2676–88.

Long-term use of sirolimus after withdrawal of calcineurin inhibitor (CNI) among heart transplantation (HT) recipients who tolerate this conversion is an effective immunosuppressive strategy for risk reduction of overall de novo malignancy and subsequent improvement in late survival. HR = hazard ratio; NMSC = non-melanoma skin cancer.

the most clinically relevant covariates known to affect the risk of malignancy. In addition, doses of IS medications used for both induction and maintenance IS were not recorded for all patients. The main strengths of this study were the use of more efficient statistical models, sample size, the length of follow-up, and the standardized and commonly used process of conversion to SRL in our institution. Despite these promising observational findings, we acknowledge that in the absence of data from randomized controlled trials, our data should be interpreted cautiously.

CONCLUSIONS

This single-center cohort analysis with a mean follow-up of 10 years provided strong evidence of reduced incidence of de novo non-NMSC malignancies, PTLDs, and lower subsequent primary occurrences of NMSC in HT recipients who were converted to SRL-based IS with complete CNI withdrawal post-HT. Although validation of these findings in a prospective randomized controlled study are lacking, this analysis provided evidence that conversion to SRL-based regimens was an effective approach for mitigation of malignancy risk related to long-term IS therapy, providing late survival benefit with this IS strategy.

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PERSPECTIVES

COMPETENCY IN PATIENT CARE: Substitution of SRL for a CNI as a primary immunosuppressive therapy after HT is associated with a lower incidence of de novo malignancies, PTLD, and subsequent primary occurrences of NMSCs, and with improved late survival.

TRANSLATIONAL OUTLOOK: Randomized studies are necessary to confirm these findings and to understand the mechanisms by which SRL reduces the risk of malignancies after HT.

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KEY WORDS heart transplantation, immunosuppression, malignancy, sirolimus