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DE NOVO OR EARLY CONVERSION TO EVEROLIMUS AND LONG-TERM CANCER OUTCOMES IN KIDNEY TRANSPLANT RECIPIENTS: A TRIAL-BASED LINKAGE STUDY

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Abbreviations

ANZDATA – Australian and New Zealand Dialysis and Transplant Registry

BCC – basal cell carcinoma

CI – confidence interval

CNI – calcineurin inhibitor

CNI-WD – calcineurin inhibitor withdrawal

ESKD – end-stage kidney disease

HR – hazard ratio

MPA – mycophenolic acid

MTORI – mammalian target or rapamycin inhibition

NMSC – non-melanoma skin cancer

RCT – randomised controlled trial

RD-CNI – reduced dose calcineurin inhibitor

SCC – squamous cell carcinoma

SHR – sub hazard ratio

Abstract

Choice of immunosuppression may modify the risk of cancer after kidney transplantation, however long-term data are lacking. Using the Australian and New Zealand Dialysis and Transplant Registry, we compared the nine-year risk of incident cancer, non-melanoma skin cancer (NMSC) and death attributed to cancer among participants, from Australia and New Zealand, in four randomised-controlled trials which compared de novo or early switch to an everolimus-containing regimen with calcineurin-inhibitor-based triple therapy. An adjusted Cox-model with random-effects was used to determine such risks. Two hundred-seventy-nine patients (192 everolimus, 87 control) were followed for a median of 9-years (IQR 6.7, 11.2). Compared with control, everolimus-use was not associated with a reduction in the risk of incident cancer, NMSC or cancer-related death. [Unadjusted hazard-ratios (95% confidence interval) 0.86 (0.49-1.48), 0.58 (0.30-1.12) and 1.18 (0.32-4.38) respectively]. Subgroup analyses showed a 56% reduction for NMSC in patients randomized to everolimus + reduced-dose calcineurin-inhibitor vs. control [unadjusted HR 0.44 (0.21-0.92)], which remained significant after adjusting for age, gender and smoking (adjusted HR 0.45 (0.21-0.96)]. Although de novo or early switch to everolimus did not alter the 9-year risk of incident cancer or cancer-related death, everolimus with reduced-dose calcineurin-inhibitor strategy may reduce the long-term risk of NMSC.

Introduction

Cancer after kidney transplantation is a significant cause of morbidity and mortality. Compared with the general population, kidney transplant recipients are at three-fold greater risk of developing any cancer,[1] while the risk of non-melanoma skin cancer (NMSC) is increased in the order of 65-250 times.[2] In Australia, cancer-related deaths surpassed cardiovascular death as the leading cause of death with a functioning graft beyond the first year after transplantation between 2011 and 2015.[3]

Along with the traditional risk factors for cancer of older age, male gender, smoking and ultra-violet radiation,[4] immunosuppression burden is a potential modifiable risk factor. It is well accepted that overall cancer risk is related to the intensity of immunosuppression.[2,5] However, there is also increasing evidence of differential effects of classes of immunosuppression on cancer development after kidney transplantation.[6,7] Mammalian target of rapamycin inhibitors (mTORI) have shown anti-oncogenic properties in both animal and human studies.[6-9] There have been only three randomised controlled trials (RCT) of mTORI in kidney transplant recipients that were powered to assess cancer outcomes in the context of NMSC.[6,7,10] Compared with calcineurin-inhibitor (CNI)-based immunosuppression, two studies[6,7] found a significant reduction in the incidence of new cutaneous squamous cell carcinoma (SCC) in patients who converted from CNI to sirolimus. In contrast, another RCT[10] failed to demonstrate a benefit with respect to new SCC occurrence in the sirolimus arm. All three trials were limited in follow-up to 24-months and had high discontinuation rates, with up to half of the patients discontinuing sirolimus during the trial. Everolimus is a derivative of sirolimus and the only other mTORI approved for use in kidney transplantation with some differences in pharmacokinetic properties.[11,12] Although often considered comparative in efficacy and adverse events profile, direct head-to-head trials are lacking. Therefore, whether everolimus exerts similar anti-oncogenic effects such as those seen with sirolimus is uncertain and the longer-term effects of mTORI on cancer-related outcomes beyond 5-years are unknown.

Our study aimed to compare the incidence of all cancers between recipients who received everolimus-based immunosuppression with recipients maintained on standard triple therapy of CNI, mycophenolic acid (MPA) and corticosteroids. We linked participant

outcomes to an established registry with the aim to investigate the longer-term effects of everolimus on hard cancer-related outcomes.

Methods

Study eligibility

We included all randomised, prospective studies involving Australian and New Zealand participants that compared standard triple therapy (cyclosporine or tacrolimus, MPA and corticosteroids) to de novo or conversion from CNI to everolimus within six-months after kidney transplantation. (Figure 1). One study was excluded as patients were converted to everolimus after six months.[13]

Data collection and items

The sponsor provided all de-identified individual patient information including date of birth, gender, transplantation date and centre. Individual participant data were acquired using deterministic linkage to the Australian and New Zealand Dialysis and Transplantation (ANZDATA) registry. From the registry, we extracted data on recipient factors including age at transplantation, ethnicity, cause of end-stage kidney disease (ESKD), smoking history, time on dialysis and co-morbidities present at time of transplantation (coronary artery disease, peripheral vascular disease, diabetes mellitus, cerebrovascular disease, chronic lung disease and prior solid organ or skin cancers); and transplant factors of donor type, donor demographics (age, sex, the cause of death), human leukocyte antigen mismatch, maximum cytotoxic antibodies and induction therapy.

Risk of bias assessment in individual studies

All studies were assessed for risk of bias using the Cochrane Risk of Bias assessment tool[14] by two authors (TY and SJC or WL) independently. Disagreements were resolved by discussion. The risk of bias tool assesses the domains: random sequence generation; allocation concealment; blinding of participants and study personnel; blinding of outcome assessment; incomplete outcome data; selective reporting and other threats to validity such as the role of industry-sponsorship and whether trials published after 2005 reported trial registration in the primary trial report.

Study outcomes

We defined incident cancer as any malignant solid-organ cancer, melanoma or NMSC. ANZDATA classified cancers according to histology (e.g. adenocarcinoma, melanoma, SCC, lymphoproliferative disease, multiple myeloma, transitional cell carcinoma or other) and site (breast, kidney, trachea, bronchus and lung, skin etc.) as adapted from the International Classification of Disease for Oncology, first edition.[15] NMSC was defined as any invasive cutaneous SCC, basal cell carcinoma (BCC) or Merkel cell carcinoma. Cancer-related death was defined as any death to malignant disease.

Discontinuation rates were defined as the proportion of patients who discontinued either everolimus in the intervention arm or a CNI in the control arm at one and two years after transplantation.

Statistical Analyses

The primary analysis was based on intention-to-treat assignment. For descriptive statistics, we reported normally distributed variables as means +/- standard deviation (SD) and skewed continuous variables as medians and interquartile range (IQR). For categorical variables, we reported numbers and proportions (%) and used the Pearson's Chi-square test or Fisher's exact test to test for an association between two categorical variables.

The median follow-up was calculated using the reverse Kaplan-Meier method.[16] For time-to-first incident cancer and NMSC, we censored participants at the date-of-death or the date of the last follow-up. We analysed all individual-patient-data using an one-step meta-analysis approach to account for the clustering of patients between studies.[17] An adjusted hazard ratio (HR) and 95% confidence interval (CI) was calculated for all outcomes using a Cox proportional hazards model with random effects (shared frailty model) to account for statistical heterogeneity .[18] For each outcome, variables with a p-value>0.1 were screened out at the univariable stage. Backward elimination was used to drive the final model with treatment arm and risk factors for cancer such as age, gender, smoking and duration of dialysis included in the models a priori. We explored effect modification between the treatment arm and other covariates in the final model. An interaction was considered significant at a level of $p<0.01$. To account for the competing events of non-cancer deaths,

we repeated the analysis for all primary outcomes by performing an adjusted competing risk analysis using the method by Fine and Gray to provide a more conservative estimate of the effect of everolimus on cancer post-transplantation.[19] We performed a pre-specified subgroup analysis to evaluate the effect of different everolimus treatment algorithms; in combination with reduced-dose CNI and as a replacement for CNI (CNI-withdrawal). We also performed an on-treatment analysis in patients who remained on therapy after two-years and five-years for all cancer and NMSC outcomes. As a sensitivity analysis, we performed a fixed-effect meta-analysis (stratified Cox proportional hazards model) for all primary outcomes.[20] All analyses were performed on Stata, version 14.1 (StataCorp LP, College Station TX). We considered a p-value of <0.05 as statistically significant.

Results

Study and participant characteristics

In total, 192 participants were randomised to everolimus (131 to RD-CNI and 61 to CNI-WD) and 87 were randomised to the control (Table 1, Figure 1). All controls received either cyclosporine or tacrolimus and MPA. Most patients (93.2%) continued on corticosteroid maintenance (Table-1). The baseline characteristics of all participants are presented in Table 2 stratified by randomisation. The median age amongst participants in the everolimus arm was 47 years (SD 12.3) compared with 50 years (SD 11.6) in the control group with a predominance of male patients (67% vs. 70%). Glomerulonephritis (51% vs 50%) was the most frequent cause of end-stage kidney disease in both arms (Table-2) and approximately half of the patients in each arm received a deceased donor transplant (54% vs 51%). Overall, participants had low-to-moderate cancer risk, with a predominance of Caucasian race (87% vs. 83%) as the main risk factor for NMSC. Most patients had never smoked (55% vs 56%) or were former smokers (36% in both arms) and only 8 (4.2%) patients in the everolimus arm compared with 1 (1.2%) patient in the control arm had a history of any cancer prior to transplantation (Table 2).

Risk of bias

The overall risk of bias was low for three studies[21-23] and unclear for one study[24] as this study did not provide information on the method of random sequence generation, allocation concealment and blinding of outcome assessment. All four trials were industry-

sponsored, however the level of involvement by the sponsor in the data collection, data analyses and preparation of the manuscript were unclear; most studies did not provide information on these processes. For manuscripts published after 2005, one study provided information on trial registration [22], and one did not.[23]

The risk of bias for random sequence generation and allocation concealment was low or unclear (Figure 2). Three studies adequately described a robust method of randomisation and allocation concealment, [21-23] and one study did not provide adequate information.[24] None of the studies were blinded to either the participants or study personnel and thus all were judged to have a high risk of performance bias. In evaluating the outcome of biopsy-proven acute rejection, only one trial[21] described blinding of the outcome assessor to the treatment allocation of participants, thus the risk of detection bias for the remaining trials were unclear. The study protocol was only available for two studies [22,23] with both studies reporting the pre-specified outcomes.

Primary analyses

Incidence of any cancer (including NMSC)

The median patient follow-up time was 9 years (IQR 7.9, 9.9) for everolimus and 7 years (IQR 4.4, 9.2) for control. During follow-up, 72 patients developed an incident cancer, 51 (26.6%) in the everolimus group and 21 (24.1%) in the control group (p=0.6) (Table 3). Cumulative incidence of cancer by treatment group is shown in Figure 4. Compared with control, the unadjusted hazard ratio (HR) for everolimus was 0.86 (95% CI 0.49–1.48, p=0.6). After adjusting for age, gender and smoking, the HR for everolimus was 0.96 (95% CI 0.57 – 1.60, p=0.9). Increasing age (age>50 years=adjusted HR 3.0, 95% CI 1.6-4.8, p<0.001) and previous or current smoking (adjusted HR=1.8, 95%CI 1.1–2.9, p=0.01) were associated with increased risk of cancer.

Incidence of non-melanoma skin cancer

Thirty (15.6%) everolimus patients developed NMSC (16 SCC, 14 BCC) compared with 17 (19.5%) in controls (8 SCC, 9 BCC p=0.4)(Table 3). The cumulative incidence of NMSC by treatment group is shown in Figure 5. Compared with control, everolimus treatment was associated with a non-significant reduction in the risk of NMSC (unadjusted HR=0.58, 95%

CI=0.30–1.12, p=0.1). After adjusting for age, gender and smoking status, the HR for everolimus was 0.62 (95% CI 0.33–1.17, p=0.1). Only age greater than 50 years (unadjusted HR 3.61, 95% CI 1.92–6.77, p<0.001) was significantly associated with the development of NMSC.

Cancer-related deaths

A total of 39 participants died during the follow-up period (Table-3). Cancer was the most common cause of death (33%) followed by cardiovascular disease (28%). The proportion of patients who died from cancer was similar in both arms (everolimus=4.7%, control=3.4%; p=1.0). Compared with control, everolimus was not associated with an increased risk of death (unadjusted HR=1.18, 95% CI 0.32–4.38, p=0.8). After adjusting for age, gender and smoking, the HR for everolimus was 1.74 (95% CI 0.44 – 6.83 p=0.4). Increasing age (adjusted HR 1.08, 95% CI 1.01–1.15, p=0.02) and smokers (adjusted HR=5.29, 95% CI 1.15–24.38, p=0.03) were significantly associated with cancer-related death.

Discontinuation of study drug

Retention of original therapy at one and two years post-study entry were significantly different between the groups. At one-year post-study entry, 49 (29.5%) patients in everolimus arm had discontinued study drug compared with 4 (4.65%) control patients (p < 0.001). At two-year post-study entry, 66 (41%) patients had discontinued everolimus versus 9 (10.5%) in the control group (p<0.001) (Table-3).

Subgroup Analyses

The effect of everolimus with reduced-dose CNI or CNI-withdrawal versus control on cancer outcomes

In subgroup analyses, neither RD-CNI or CNI-WD influenced overall cancer-risk after transplantation compared with control (RD-CNI: adjusted HR 0.98, 95% CI 0.56–1.72, p=0.9 CNI-WD: adjusted HR 1.31, 95% CI 0.69–2.49, p=0.4) (Figure 4). However, everolimus+RD-CNI was associated with a reduced risk of NMSC of around 56% versus control (unadjusted HR 0.44, 95% CI 0.21–0.92, p=0.02) (Figure 6). The reduction in NMSC remained significant after adjusting for age, male gender and smoking (HR 0.45, 95% CI 0.21–0.96, p=0.04). The

reduction in the risk of NMSC was not seen in the CNI-WD group in the unadjusted (HR 1.12, 95% CI 0.49–2.57, p=0.8) or adjusted analysis (HR 0.98, 95% CI 0.42–2.30, p=1.0) (Figure 4).

On-treatment analysis

We performed exploratory analyses for patients who remained on-treatment after two and five years for incident cancer and NMSC. Treatment estimates were similar to our ITT primary analysis, thus only the adjusted analyses are presented here. Two-years post-randomisation, 103 (57%) everolimus vs. 78 (90%) controls remained on-treatment. One-hundred and seventy-three (62%) patients were included in the two-year analysis. For the outcome of incident cancer, the adjusted HR for everolimus was 0.87 (95% CI 0.48–1.61, p=0.7) versus control. There was a non-significant reduction in the risk of NMSC for the everolimus group (adjusted HR=0.61, 95% CI 0.29 – 1.27, p=0.2). We further compared the risk of cancer in two time-periods post-randomisation: 0 to 3 years (where the on-treatment group had at least 2 years of everolimus exposure) vs. > 3 years. We found no difference in the risk of incident cancer during these two time periods. For incident cancer occurring between 0-3 years post-randomisation, the adjusted HR for everolimus was 0.69 (0.27-1.71, p = 0.4). For incident cancer that occurred greater than 3 years post-randomisation, the adjusted HR was similar; 0.68 (0.26-1.78, p = 0.4) for everolimus compared with control.

Five years after randomisation, 78 (41%) everolimus vs 73 (84%) controls remained on-treatment. One-hundred and forty-six (52%) were included in the analysis. For the outcome of any cancer, the adjusted HR for everolimus was 0.66 (0.30-1.45, p=0.3). For the outcome of NMSC, the adjusted HR for the everolimus group was 0.57 (0.23-1.40, p=0.2).

Competing risk analysis

To account for non-cancer death as a competing risk for cancer incidence, we performed competing risk analyses for all primary outcomes. The treatment estimates for everolimus versus control were similar to the random effects model for both the unadjusted and adjusted models and thus only the adjusted HRs are presented here. For the outcome of incident cancer, the subdistribution hazard ratio (SHR) for everolimus was 0.92 (95% CI 0.56–1.52, p=0.7). For NMSC, the SHR for everolimus was 0.62 (95% CI 0.32– 1.21, p=0.1) and for cancer-death, the SHR for everolimus was 1.60 (95% CI 0.53–4.77, p=0.4).

Sensitivity analysis

As a sensitivity analysis, we repeated the analyses using a stratified Cox regression model. The treatment estimates did not substantially alter the direction or magnitude of effects. For the outcome of any cancer, the adjusted HR was 0.95 (95% CI 0.55–1.66) for any-everolimus, 0.65 (95% CI 0.33–1.28) for RD-CNI and 1.63 (95% CI 0.763.48) for CNI-WD. For NMSC, the adjusted HR was 0.56 (95% CI 0.27–1.17) for any-everolimus, 0.35 (95% CI 0.38-2.83) for RD-CNI and 1.03 (95% CI 0.38-2.83) for CNI-WD. For cancer-related death, the adjusted HR was 1.47 (95% CI 0.37– 5.79) for any-everolimus, 1.66 (95% CI 0.34–8.17) for RD-CNI and 2.65 (95% CI 0.23–30.63) for CNI-WD.

Discussion

In this registry-linked study of 279 trial participants from Australia and New Zealand, de novo or early introduction of everolimus was not associated with a reduction in the risk of incident cancer or cancer-related mortality after kidney transplantation. However, recipients randomised to everolimus and reduced exposure CNI experienced a 56% reduction in NMSC compared to CNI-based triple therapy, suggesting that the combination of everolimus and RD-CNI may reduce the risk of NMSC after transplantation. The major cause of death over the follow-up period was attributed to cancer, a result mirrored by the latest 2016 ANZDATA report.[3] Thus, whilst cancer has become the dominant cause of death beyond the first year after kidney transplantation in Australia and New Zealand, we could find no evidence that this was reduced by everolimus use. Our study also demonstrated a high rate of discontinuation, especially in the first two-years of commencing everolimus, reflecting previous experience with mTORI.[13,26]

MTORIs have long been investigated as an alternative maintenance immunosuppression in kidney transplant recipients. One key rationale for its use in preference to CNI has been the potential to reduce cancer risk. In a large, uncontrolled registry study of over 33,000 deceased donor kidney transplant recipients, mTORI significantly reduced the risk of developing any cancer and NMSC.[27] Subsequent meta-analyses focussed on estimating the relationship between mTORI exposure and overall cancer[28] and NMSC risk,[26,29] with conflicting results. The earliest meta-analysis of mTORI demonstrated no significant

difference in the risk of cancer, however all trials had less than two-years of follow-up and did not report on NMSC outcomes.[30] A more recent meta-analysis of conversion from CNI to everolimus did not demonstrate any benefit with respect to overall cancer risk.[28] Two larger meta-analyses of mTORI with aggregate data[26] and individual-patient-data[29] showed a reduction in NMSC by approximately 50%. These findings are similar to our results in the RD-CNI arm, where we found a similar reduction in NMSC risk. The individual-patient-data meta-analysis by Knoll et al. is the only meta-analysis to have shown a benefit of sirolimus on a reduction in overall cancer risk inclusive of solid-organ and NMSC. However, an increased risk of all-cause death was also seen in the sirolimus group.[29] Overall, trials included in these meta-analyses were clinically heterogeneous, differed vastly in terms of treatment regimens and included patients with widely varying risks of cancer. Therefore, it remains uncertain as to how clinicians should incorporate the results of these meta-analyses into their decision making. Our study attempted to further explore the clinical heterogeneity and its impact on cancer risk. While our study did not demonstrate a benefit of everolimus regimens overall in terms of cancer and NMSC risk, a reduction in NMSC risk was detected for everolimus + RD-CNI, though not for CNI-withdrawals. The reduction of NMSC risk in the everolimus and reduced-dose CNI regimen has been previously shown in a small study based on a single trial which was also captured within our meta-analysis.[31] The benefit of this regimen is now further supported by the addition of individual-patient-data from three other RCTs.

The differences in NMSC risk between the RD-CNI and CNI-withdrawal subgroups is somewhat counter-intuitive, as studies have shown an increased risk of NMSC associated with a higher cyclosporine exposure.[32] One possible explanation may be related to the differential MPA exposure between the subgroups. All patients in the control arm received up to 1440 mg of MPA/day, while none of the patients in the RD-CNI group received MPA. In the CNI-withdrawal arm, less than half of the patients were maintained on MPA. We also tested MPA as a predictor for NMSC in a random effects and fixed effects Cox regression model. However, in both the unadjusted and adjusted models, MPA was not significantly associated with an increased risk of NMSC (Supplementary Table-1). Observational studies have shown a significant association between azathioprine and skin cancer in the kidney transplant population [33] and in the inflammatory bowel disease population[34], however, the

risk of cancer in patients on MPA is not well understood. One long-term linkage study found a similar incidence of cancer, 15-years after kidney transplantation between patients randomised to mycophenolate versus azathioprine.[35] Our findings are, however, exploratory in nature and therefore should be interpreted with caution. Further prospective studies are required to confirm these findings.

Our study found that one-third of deaths were attributable to cancer, with a similar proportion of cancer-deaths between everolimus and control patients. According to the ANZDATA registry, cancer is the leading cause of death, followed closely by cardiovascular death in Australian and New Zealand kidney transplant recipients.[5] Despite the apparent rising trend in cancer-related death, there is a paucity of studies reporting specifically on cancer-related death after kidney transplantation. Farrugia et al.[36] presented data from a population-based English cohort of kidney transplant recipients between 2001 and 2012 and found cancer to be equal to cardiovascular disease and infection-related death as the most common causes of death, after a median follow-up of 4.4 years. The effect of mTORI on cancer-related death is even less well understood. In a large registry cohort study, the risk of cancer-related death was found to be increased in the mTORI use group.[37] One possible reason for this finding was indication bias, where patients at increased risk of cancer were selectively switched to mTORI. Knoll et al[29] found a higher risk of all-cause mortality among sirolimus patients, but the proportion of cancer-related death was similar between the groups.[29] Based on the available evidence, cancer-related death is a significant issue amongst long-term kidney transplant recipients, and the use of mTORI has not been shown to modify this risk. It should be noted that the number of cancer-related deaths in our study was small, thus it may have been underpowered to detect an association. Given that cancer is the most common and feared complication among kidney transplant recipients[38], cancer-incidence and death should always be investigated as hard outcomes. For a trial to be adequately powered on such outcomes would require a prolonged period of follow-up and prohibitive costs. Therefore, linkage to existing databases and registries for individual patient outcomes remains an innovative and more practical solution to this problem.[5,31]

Our study again confirmed the high discontinuation rates amongst mTORI users, although we were not able to explore the reasons for this. Lim et al.[26] found that the pooled discontinuation rates at one-year after switch from CNI to mTORI was ~26%. Our study demonstrated similar discontinuation rates at one-year of 30%, increasing to over 40% at two-years post-transplantation. The high rates of discontinuation in our study may have contributed to the apparent non-significant results in our primary outcomes and although limited by power, the on-treatment analysis also failed to demonstrate any reduction in a cancer-risk for patients remaining on-treatment for at least two-years. The consistent direction of benefit amongst those remaining on everolimus suggests a larger study is required. Despite a high discontinuation rate, our study found that in recipients with no history of skin cancer, the risk of NMSC was reduced by half in participants randomised to everolimus and reduced-dose CNI. The poor tolerability of mTORIs to date may limit the widespread routine use of mTORI-based regimens and future trials should evaluate reasons for drug discontinuation so that strategies can be applied to improve medication tolerability and adherence.

Our study has several strengths and limitations. We had individual-patient-data for all patients with less than 5% missing baseline covariates. All cancer outcomes are prospectively reported to the ANZDATA registry and have been shown to be accurate and concordant with the New South Wales Cancer Registry.[15]All studies were randomised at study entry, minimising the risk of selection bias. All patients were successfully linked to the ANZDATA registry providing an invaluable insight into the long-term outcomes of patients after randomisation. To the authors' knowledge, all multicentre RCTs of everolimus in Australian and New Zealand kidney transplant recipients were captured in this study. However, smaller single-centre studies and unpublished research may have been missed, raising the potential for publication bias. Although time-to-first NMSC was one of our primary outcomes, the overall burden of NMSC may have been a more appropriate measure of the morbidity. As ANZDATA only records the first episode of NMSC, we were unable to assess this important clinical outcome. Medication use was documented at pre-specified time-points by ANZDATA, therefore it is possible that patients temporarily ceased the study drug and recommenced it at a later time. The rate of discontinuation of study drug within our study was, however, comparable to rates published in other studies.[6,10,22,28,32] Finally,

the majority of the participants were of Caucasian race, therefore the results may not be generalisable to other racial groups.

In conclusion, everolimus use was not associated with a reduction in the 9-year risk of incident cancer or cancer-related death compared with standard CNI-based therapy. Everolimus was associated with a high discontinuation rate, however despite this, we found a significant reduction of NMSC in the everolimus plus RD-CNI subgroup. This novel finding warrants a larger RCT, which is powered to assess the efficacy and safety of everolimus with RD-CNI treatment to establish its clinical application in kidney transplant recipients.

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Disclosure:

The authors of this manuscript have conflicts of interest to disclose as described by the American Journal of Transplantation. WL, JK, SC, RW, PO'C, GRR and SJC have served on the Australian Novartis Transplant Advisory Board and have received sponsorship (to institution) for trial conduct and/or international conference attendance. The other authors have no conflicts of interest to disclose.

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Figure Legends

Figure 1 – Study flow

Figure 2 – Risk of Bias of included studies: review authors' judgements about each risk of bias item presented as percentages across all included studies.

Figure 3 – Cumulative incidence of any cancer according to immunosuppression groups (everolimus vs. control)

Figure 4 – Risk of any cancer and NMSC in kidney transplant recipients treated with everolimus compared with controls.

Figure 5 - The cumulative incidence of non-melanoma skin cancer according to immunosuppressive groups (everolimus vs. control)

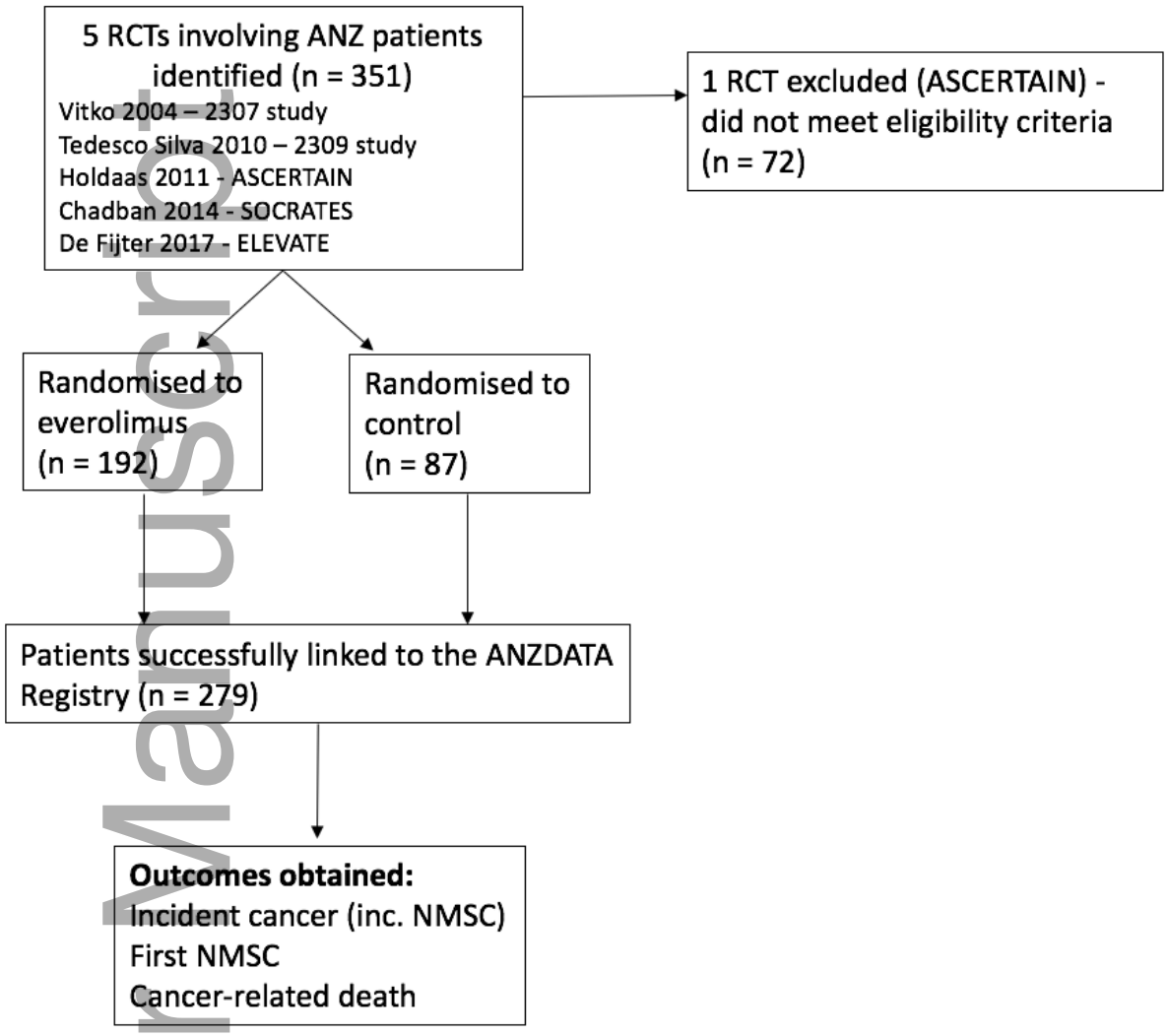
Figure 6 – The cumulative incidence of non-melanoma skin cancer by treatment subgroup (everolimus + reduced dose calcineurin inhibitor vs. control)

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1: Mycophenolic acid versus no mycophenolic acid and the risk of non-melanoma skin cancer

Figure 1 – Study flow



RCT = randomised controlled trials, ANZ – Australiana and New Zealand, *n* = numbers of participants, ANZDATA = Australian and New Zealand dialysis and transplantation registry, NMSC = non-melanoma skin cancer

Table 1: De novo or early conversion everolimus trials in Australian and New Zealand kidney transplant recipients

Trial	No.	Trial Type	Intervention 1	Intervention 2	Control
Vitko et al. 2004	46	*de novo*	Evl + RD-CNI (CsA) + CS (46)		
Tedesco Silva et al. 2010	95	*de novo*	Evl + RD-CNI (CsA) + CS (66)		CsA + MPA + CS (29)
Chadban et al. 2014	88	Week 2 conversion	Evl + RD-CNI (CsA) + CS-WD (19)	Evl + CNI-WD + CS (36)	CsA + MPA + CS (33)
de Fijter et al. 2017	50	Week 10 - 14 conversion		Evl+ CNI-WD+ MPA + CS (25)	CsA or Tac* + MPA + CS (25)
Total	279		131	61	87

No. = number of participants, CsA = cyclosporine; Evl = everolimus; RD = reduced dose; WD = withdrawal; MPA = mycophenolic acid; AZA = azathioprine; CS = corticosteroid; CNI = calcineurin-inhibitor; Tac = tacrolimus

*24/25 patients were on tacrolimus

The numbers in the brackets denotes no. of participants in that arm

Table 2 – Baseline characteristics of all trial participants

	Everolimus (n = 192)	**Control (n = 87)**
Demographics		
Age (mean, SD)	46.9 (12.3)	49.6 (11.6)
Male (n, %)	128 (66.7)	61 (70.1)
Caucasian (n, %)	167 (87)	72 (83)
Years on dialysis (median, IQR)	1.7 (0.7, 3.5)	1.5 (0.3, 4.3)
No. previous transplant (n, %)	1 (0.5)	2 (2.3)
Missing (n)	0	0
Cause of ESKD (n, %)		
Glomerulonephritis	98 (51)	43 (49.4)
IgA nephropathy	34 (18)	20 (23)
FSGS	22 (11)	12 (14)
Membranous GN	4 (2)	3 (3)
Other	38 (20)	8 (9)
Diabetic nephropathy	16 (6.3)	7 (8.1)
Renovascular/HTN	11 (5.7)	2 (2.3)
Polycystic	35 (18.2)	18 (20.7)
Other	36 (18.8)	17 (19.5)
Missing	0	0
Comorbidities (n, %)		
Any cancer	8 (4.2)	1 (1.2)
Non-melanoma skin cancer	5 (2.6)	1 (1.2)
Coronary artery disease	21 (10.9)	19 (21.9)
Diabetes mellitus	19 (9.9)	10 (11.5)
Smoking		
Current	18 (9.4)	7 (8.1)
Former	68 (35.6)	31 (35.6)
Never	105 (55.0)	49 (56.3)
Missing	1	0

Donor Characteristics		
Age (mean, SD)	47.3 (14.3)	45.7 (14.5)
Male (n, %)	86 (44.8)	38 (43.7)
Deceased donor	106 (54.2)	44 (50.6)
Missing	0	0
Immunology/Transplant		
No. of HLA mismatches (mean, SD)	3.4 (SD 1.6)	3.1 (SD 1.8)
Ischaemic time in hours (mean, SD)	8.3 (SD 6.3)	7.2 (SD 5.2)
Peak PRA > 25% (n, %)	12 (6.3)	11 (12.6)
Any Induction therapy (n %)	161 (83.9)	76 (87.4)
Missing (n)	2	0
Transplant era (n, %)		
2000–2008	157 (81.8)	51 (58.6)
2008 +	35 (18.2)	36 (41.4)
Missing	0	0

N = number of participants, SD = standard deviation, IQR = interquartile range, ESKD = end-stage kidney disease, HTN = hypertension, PRA = panel-reactive antibody. The control arm consisted of a calcineurin inhibitor + mycophenolic acid

Figure 2 – Risk of Bias of included studies: review authors' judgements about each risk of bias item presented as percentages across all included studies.

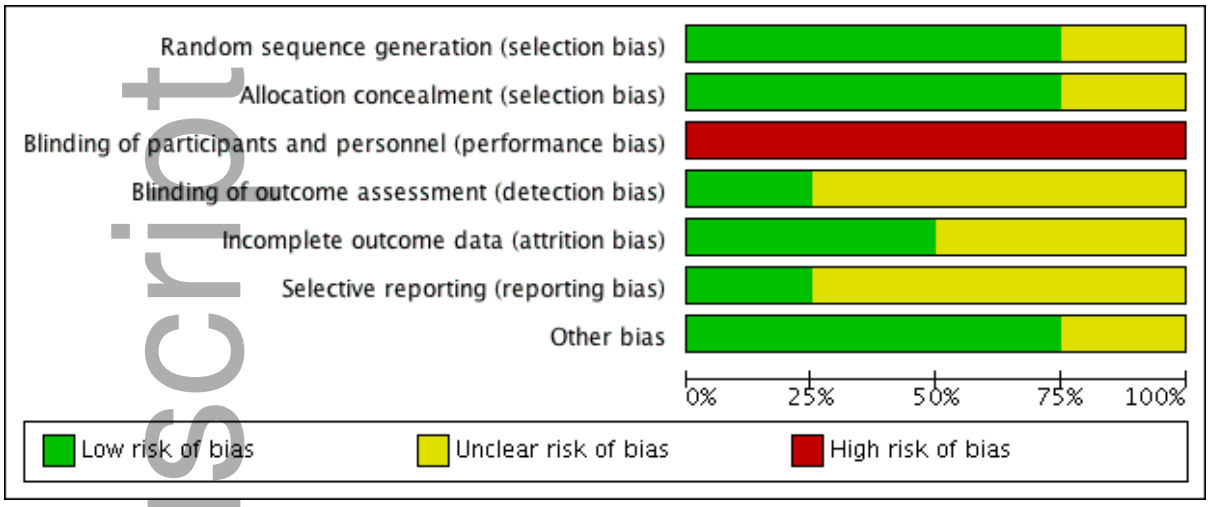


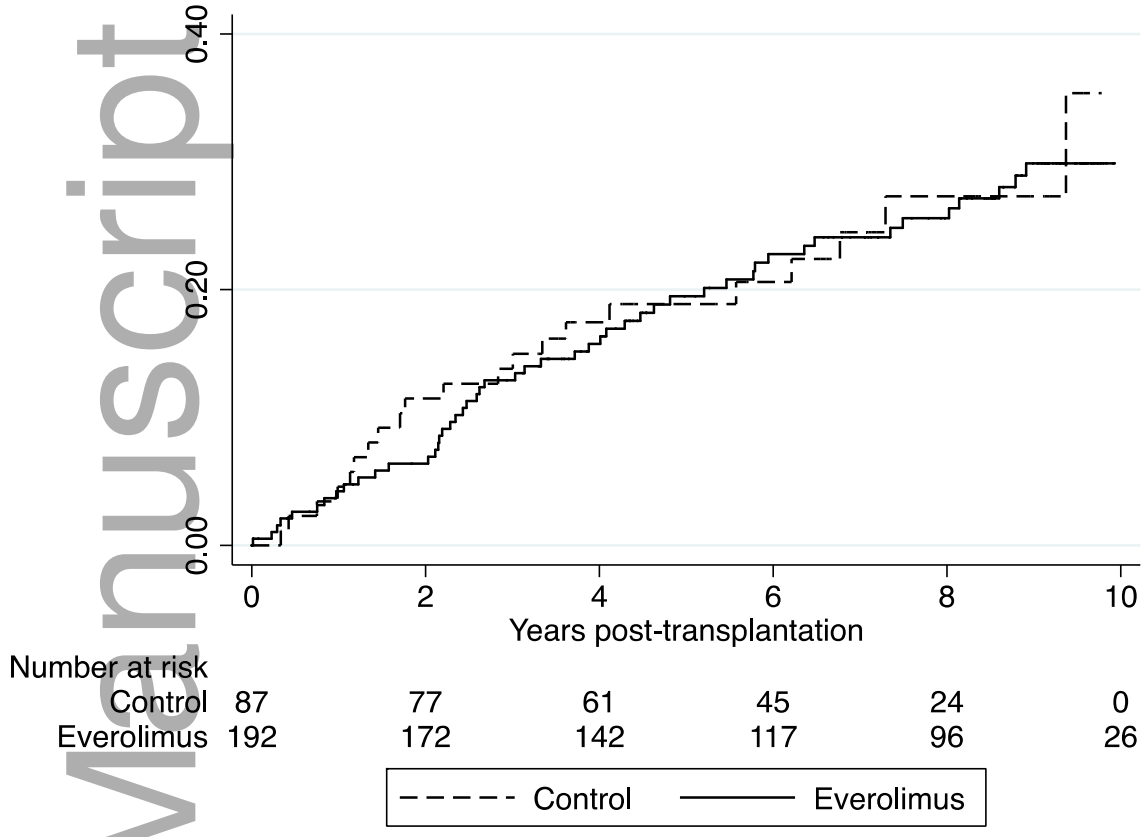
Table 3 – Cancer, death and discontinuation outcomes in de novo and conversion trials of everolimus in Australian and New Zealand.

Outcome	**Everolimus (n = 192)**	**Control (n = 87)**	***P* value**
First episode of any cancer (n, %)	51 (26.6)	21 (24.1)	0.6
NMSC	27 (14.1)	15 (17.2)	
SCC (non-skin)	5 (2.6)	1 (1.1)	
TCC	0 (0)	1 (1.1)	
Adenocarcinoma	8 (4.2)	0 (0)	
Melanoma	5 (2.6)	1 (1.1)	
Multiple myeloma	1 (0.5)	0 (0)	
Lymphoma	0 (0)	1 (1.1)	

Other	5 (2.6)	2 (2.3)	
First episode NMSC (n, %)	30 (15.6)	17 (19.5)	0.4
SCC	16 (8.3)	8 (9.2)	
BCC	14 (7.3)	9 (10.3)	
Deaths (n, %)	30 (15.6)	9 (10.3)	0.2
Cancer	9 (4.7)	3 (3.4)	
Cardiovascular	10 (5.2)	1 (1.1)	
Infection	5 (2.6)	3 (3.4)	
Other	6 (3.1)	2 (2.3)	
Discontinuation (n, %)			
Year 1	49 (29.5)	4 (4.7)	< 0.001
Year 2	66 (41)	9 (10.5)	< 0.001
Missing	23 (12.0)	1 (1.1)	

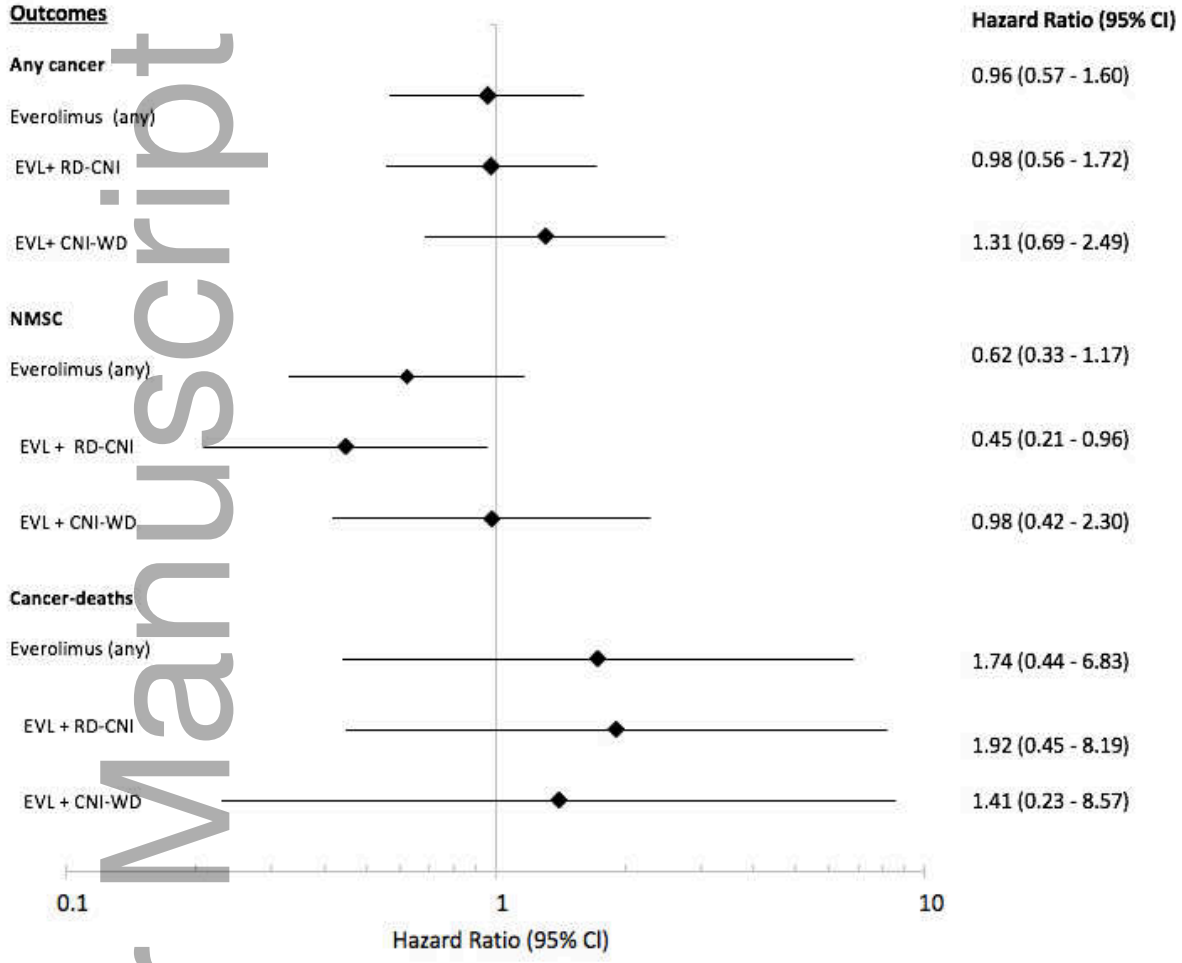
n = number, NMSC = non-melanoma skin cancer, SCC = squamous cell cancer, TCC = transitional cell cancer. The control group = a calcineurin inhibitor + mycophenolic acid.

Figure 3 – Cumulative incidence of any cancer according to immunosuppression groups (everolimus vs. control)



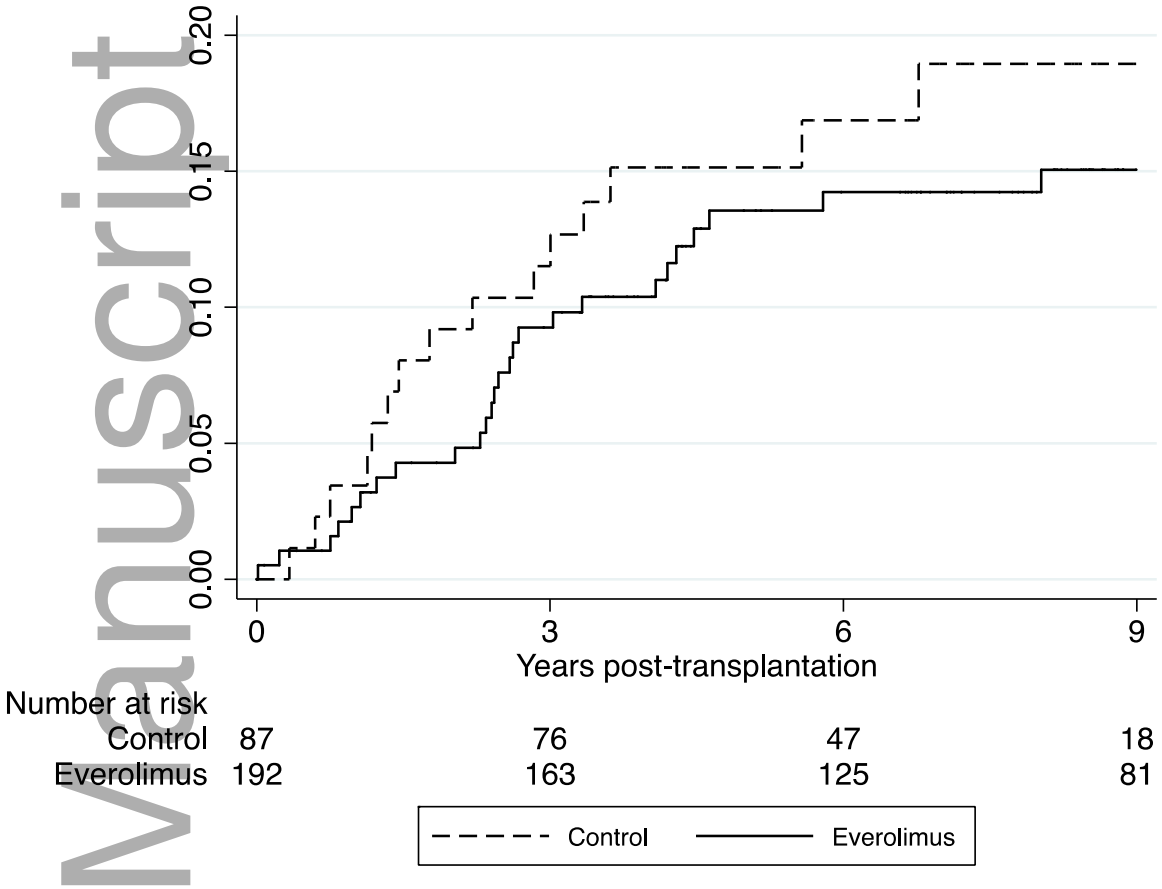
Log-rank test = 0.8

Figure 4 – Risk of any cancer and NMSC in kidney transplant recipients treated with everolimus compared with controls.



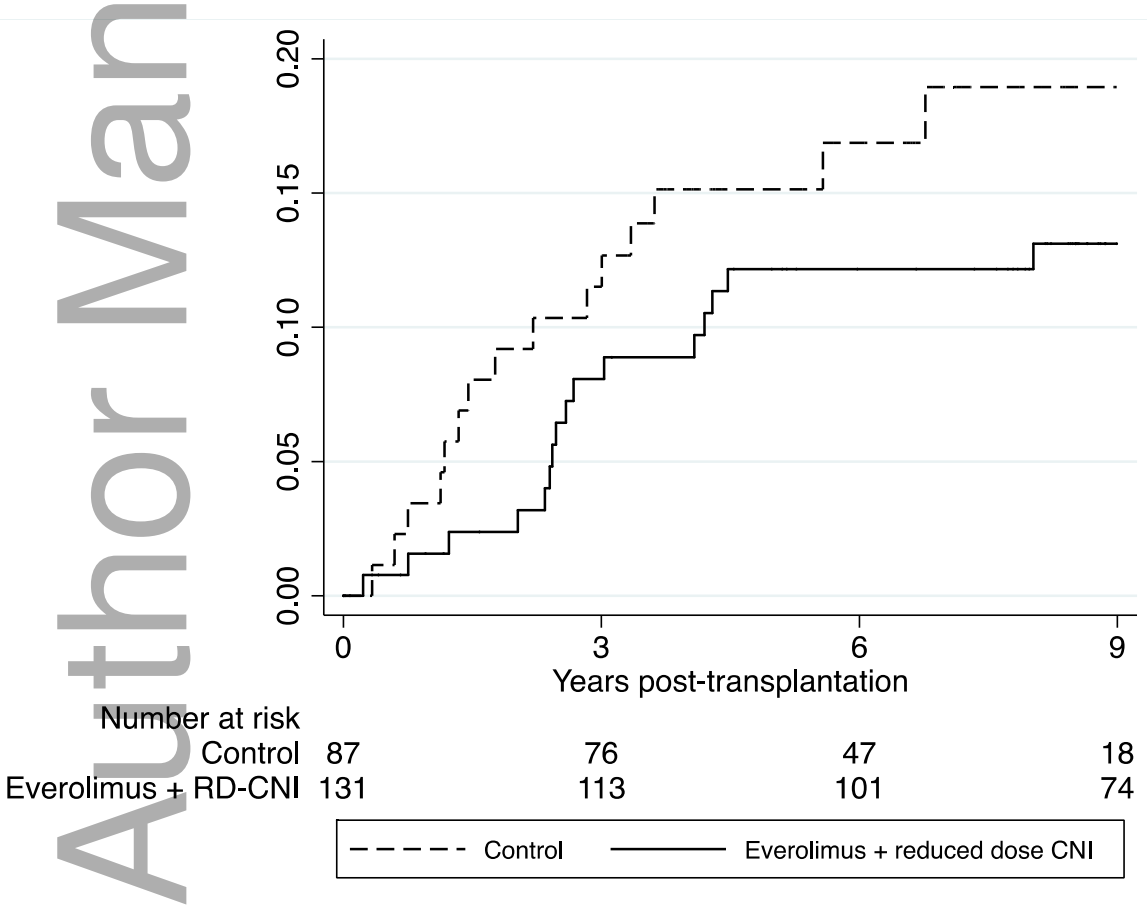
EVL = everolimus, RD-CNI = reduced dose calcineurin inhibitor, CNI-WD = calcineurin inhibitor withdrawal

Figure 5 - The cumulative incidence of non-melanoma skin cancer according to immunosuppressive groups (everolimus vs. control)



Log-rank test p = 0.1

Figure 6 – The cumulative incidence of non-melanoma skin cancer by treatment subgroup (everolimus + reduced dose calcineurin inhibitor vs. control)



Log-rank test p = 0.01