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Immunosuppression with mammalian target of rapamycin inhibitor and incidence of post-transplant cancer in kidney transplant recipients

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ORIGINAL ARTICLE

ABSTRACT

Background. Evidence is limited regarding the effect of *de novo* therapy with mammalian target of rapamycin (mTOR) inhibitors on cancer risk after kidney transplantation.

Methods. Collaborative Transplant Study data from 78 146 adult recipients of first deceased-donor kidney transplants (1999–2013) were analysed (4279 mTOR inhibitor, 73 867 no mTOR inhibitor) using standard methods. Propensity score matching was performed for analysis of basal cell and squamous cell skin cancer.

Results. Standardized incidence ratios (SIR) versus a matched non-transplant population showed reduced tumour incidence in recipients with *de novo* mTOR inhibitor therapy compared with no mTOR inhibitor for non-melanoma skin cancer (NMSC) (SIR 5.1 versus 6.1; P = 0.019) but not non-NMSC cancers (SIR 1.6 versus 1.7; P = 0.35). Within propensity score-matched groups (n = 4265), multivariable Cox regression analysis showed a trend to reduced NMSC with mTOR inhibition [hazard ratio (HR) 0.77; P = 0.063] but not for all non-NMSC tumours (HR 0.94; P = 0.59). A significant effect for mTOR inhibition was observed for basal cell carcinoma of the skin (HR 0.56; P = 0.004) but not squamous cell carcinoma (HR 0.87; P = 0.54).

Conclusions. *De novo* mTOR inhibition was associated with a substantially and significantly reduced risk of basal cell carcinoma of the skin after kidney transplantation. A significant reduction of the incidence of other cancers was not found.

Keywords: kidney transplantation, malignancy, mammalian target of rapamycin, non-melanoma skin cancer

INTRODUCTION

The risk of cancer is 2- to 3-fold higher in kidney transplant recipients than in the general population [1–3]. The most striking relative increases compared with a normal non-transplant population are found for Kaposi sarcoma, non-Hodgkin lymphoma and non-melanoma skin cancer [1, 2, 4]. Chronic immunosuppression therapy, designed to ensure global suppression of innate and adaptive immunity, suppresses immunosurveillance, creates a permissive environment for oncogenic viruses and may itself be oncogenic [5, 6].

During the last decade, encouraging data has emerged that immunosuppression based on inhibition of mammalian target of rapamycin (mTOR) may lower the risk of post-transplant cancer. Downstream effects of the mTOR signalling pathway modulate ribosome biosynthesis and thus the generation of proteins that are essential for cell growth, cell cycle progression and cell metabolism [7, 8], making it central to the regulation of normal cell function. Genetic mutations or other genetic alterations frequently lead to constitutional activation of mTOR signalling in neoplastic cells, and dysregulation of the mTOR pathway is a common finding in both solid tumours and haematological malignancies [7, 8]. In particular, the tumour suppressor gene phosphatase and tensin homologue deleted on chromosome 10 (PTEN), which inhibits activity of the upstream mTOR potentiator molecule AKT, is often mutated or inhibited in malignant conditions, contributing to mTOR activation. Thus, targeting the mTOR pathway to suppress tumour-promoting processes such as angiogenesis is a potentially promising approach for the control of malignancy. In transplant recipients, this would complement the reduced alloimmune reactivity for which mTOR inhibitors were developed. The mTOR inhibitor everolimus is licensed for the treatment of advanced renal cell carcinoma in the general population, and has shown promising results in randomized trials when used adjunctively or alone to improve progression-free survival in other types of malignancy [9–12]. Mathew *et al.* observed a reduced rate of skin cancer in a retrospective analysis of the initial mTOR inhibitor efficacy trials [13]. Two randomized studies in patients with evidence of skin tumours before

transplantation showed that switch from calcineurin inhibitor (CNI) therapy to sirolimus reduces the risk of recurrence of non-melanoma skin cancer (NMSC) after kidney transplantation [14, 15]. The trials enrolled 86 and 120 patients, respectively, all of whom were at high risk for events due to current or previous NMSC. Despite the relatively small populations, the results were unequivocal but do not necessarily apply to patients without prior NMSC. Campistol *et al.* performed a retrospective analysis of tumour occurrence in a multicentre randomized efficacy trial of conversion from cyclosporine plus mTOR inhibitor to mTOR inhibitor maintenance without cyclosporine, and noticed lower numbers of skin and non-skin tumours in the cyclosporine withdrawal arm when multiple tumours occurring in individual patients were included [16]. In the CONVERT trial, in which patients were converted from CNI to mTOR inhibitor maintenance therapy at 6–120 months post-transplant to test the efficacy of CNI-free maintenance immunosuppression, retrospective analysis showed a lower total tumour incidence post-conversion due to a significant reduction in skin cancers [17, 18]. The literature also includes cases of Kaposi sarcoma regression following switch from CNI therapy to an mTOR inhibitor [19, 20], but no randomized trials have been carried out.

These findings raise the question of whether immunosuppression based on an mTOR inhibitor from time of transplant could reduce the toll of post-transplant cancer. However, it is difficult to investigate an effect of mTOR inhibition on the risk of *de novo* malignancy in a trial setting. Despite higher cancer rates than in the general population, the absolute tumour incidence is low and analyses of post-transplant tumour incidence have therefore commonly relied on retrospective evaluation of trials conducted for other reasons or registry data [1–3].

Since the introduction of mTOR inhibitors in 1999, the Collaborative Transplant Study (CTS) has collected data on more than 4000 kidney transplant patients who received either everolimus or sirolimus as part of the intention-to-treat immunosuppressive protocol and in whom information on *de novo* post-transplant malignancies was documented. Tumour rates in these patients were compared with those in patients who were transplanted and followed up during the same time period but did not receive an mTOR inhibitor in the intention-to-treat regimen.

MATERIALS AND METHODS

All adult recipients (≥18 years) of a first deceased-donor kidney transplant performed between 1 January 1999 and 31 December 2013 who were registered with the CTS (www.ctstransplant.org) and in whom data on *de novo* post-transplant malignancies were documented were eligible for inclusion in the analysis. The analysis excluded 1698 patients with evidence of pre-existing tumours prior to transplantation. Also excluded were recipients of combined organ transplants, including simultaneous pancreas-kidney transplants. The intention-to-treat immunosuppressive protocol was recorded at the time of initial registration with the CTS, shortly after transplantation. Data on graft and patient survival and development of post-transplant malignant tumours were requested from all transplant centres participating in the CTS. Data were provided at months 3, 6 and 12 post-transplant, and annually thereafter. Because underreporting of malignancies has been recognized as a potential problem, each participating centre was asked annually for written confirmation regarding the completeness and accuracy of data provided by the centre on malignancies, specifically addressing the reporting of NMSC and differentiation of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Only those centres that provided written confirmation on the completeness of tumour reporting were included in the current analysis. All tumours diagnosed in a patient after transplantation were considered for incidence computations i.e. not only the first malignancy. However, recurrence or additional appearance of the same type of tumour after an initial diagnosis of a given tumour was not counted separately for incidence computation. Malignancies were coded according to standard ICD 10 classification (C00–C96). All data were anonymized.

Standardized incidence ratios (SIR) were calculated for the rate of malignant tumours post-transplant compared with a non-transplant reference population stratified by age, gender and geographical region, derived from data reported by good quality registries to the World Health Organization (WHO) [21]. SIR values are an estimate of the occurrence of cancer in a given study population relative to what might be expected if the population were a 'normal' reference population. SIR for NMSC were based on registries that reported data on NMSC (without differentiation of BCC or SCC) to the WHO reference centre [21].

Because reference data on BCC and SCC of the skin are not available in the WHO reference base, propensity score matching was chosen for the comparison of patients registered with the CTS who were treated with an mTOR inhibitor or not, with respect to the occurrence of BCC and SCC. Propensity score matching using the method described by Sekhon [22] was also applied because mTOR inhibitor use varied over time and in different geographical regions. The rationale for propensity score matching is explained by Dehejia and Wahba as follows: 'It is well recognized that the estimate of a causal effect obtained by comparing a treatment group with a nonexperimental comparison group could be biased because of problems such as self-selection or some systematic judgment by the researcher in selecting units to be assigned to the treatment. Matching involves pairing treatment and comparison units that are similar in terms of their observable characteristics. When the relevant differences between any two units are captured in the observable (pretreatment) covariates, which occurs when outcomes are independent of assignment to treatment conditional on pretreatment covariates, matching methods can yield an unbiased estimate of the treatment impact' [23]. We used standard statistical software whereby we required a perfect match for geographical region, recipient gender, recipient race and categorized recipient age (age groups 18–39, 40–49, 50–59, 60–69, ≥70 years), and applied Mahalanobis distance-matching, choosing the closest available match for the following confounders: year of transplant, recipient age (years), donor age (years) and original underlying disease

(polycystic disease, diabetic nephropathy and other). The propensity score was calculated using a general linear model. Figure 1 illustrates the effect of propensity score matching on the stratification of confounders. The quality of propensity matching is further detailed in Supplementary data, Table S1. Following propensity matching, multivariable Cox regression analysis was performed in which the following confounders were considered: year of transplant, geographical region, recipient race, gender and age, donor age, and original disease leading to end-stage renal failure. P-values <0.05 were considered significant. All statistical analyses were conducted in R version 3.1.2 [24], including 'The Matching package for R' [22].

RESULTS

In total, 78 146 transplants were eligible for inclusion in the analysis. Documentation was provided by 224 kidney transplant centres (see Acknowledgments). The intention-to-treat immunosuppressive protocol included an mTOR inhibitor in 4279 cases (5.5%) and no mTOR inhibitor in the remaining 73 867 cases (94.5%). The total number of patient years was 329 500, with a mean follow-up of 4.2 years. For comparison, of the 1698 patients who were excluded from further analysis due to the existence of a pretransplant tumour, 4.6% were assigned to intention-to-treat immunosuppression including an mTOR inhibitor.

The distribution of patient and transplant characteristics varied between the two treatment cohorts (Table 1). Use of mTOR inhibitor therapy changed over the period of the study: only 3% of patients transplanted in 1999 received an mTOR inhibitor, increasing to a peak of 9% by 2002 but decreasing again to about 4% in more recent years. As might be expected, the type of immunosuppression differed substantially between groups, with lower use of CNIs and antiproliferative agents (azathioprine or mycophenolic acid) in the cohort given an mTOR inhibitor.

SIR for malignancies, taking account of recipient age, gender and geographical region, were calculated for the main ICD tumour groups in patients with or without intention-to-treat mTOR inhibitor treatment. This approach allows the inclusion of all patients and gives an estimation of the extent to which the tumour incidence in patients assigned to treatment with or without TOR inhibitor differed from the expected incidence in a non-transplant normal background population matched for age, gender and geographical origin, thereby generating a normalized estimation of the tumour-reducing capacity of mTOR inhibitor treatment. In total, 6923 patients with 8202 tumours (including ICDs not listed in Table 2 and also multiple but different types of tumours in a single patient) were documented in the 78 146 patients of the study population. A significantly lower tumour incidence in relation to the expected background incidence for patients treated on an intention-to-treat basis with an mTOR inhibitor versus no mTOR inhibitor was observed only for NMSC (C44) (SIR 5.1 versus 6.1 without mTOR inhibition; P = 0.019). When all other cancers excluding NMSC were assessed, a significant impact of treatment with mTOR inhibitor was not evident (SIR 1.6 with an mTOR inhibitor versus 1.7 without an mTOR inhibitor; P = 0.35) (Table 2).

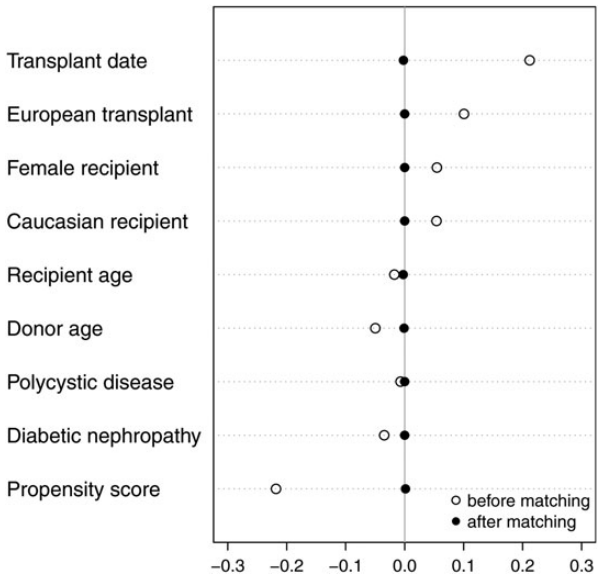

FIGURE 1: Dot plot of standardized mean differences (Cohen's d) for covariates before and after propensity score matching. In total, 4265 of 4279 mTOR inhibitor-treated patients were matched 1:1 with patients from the population of 73 867 recipients without mTOR inhibitor treatment. The plot illustrates that matching effectively eliminated the demographic differences between patients with or without mTOR inhibitor treatment.

Table 1. Demographics of study patients, *n* (%)

Characteristic	Without mTORi *n* = 73 867	With mTORi *n* = 4279
Geographical region		
Europe	55 132 (75%)	3006 (70%)
North America	4000 (5%)	469 (11%)
Australia/New Zealand	4745 (6%)	186 (4%)
Other	9990 (14%)	618 (14%)
Recipient sex		
Female	28 079 (38%)	1513 (35%)
Male	45 788 (62%)	2766 (65%)
Transplant year		
1999–2005	39 656 (54%)	2838 (66%)
2006–2013	34 211 (46%)	1441 (34%)
Recipient age (years)		
18–39	16 903 (23%)	892 (21%)
40–49	16 383 (22%)	948 (22%)
50–59	20 402 (28%)	1331 (31%)
≥60	20 179 (27%)	1108 (26%)
Prophylactic Ab induction therapy		
No Ab induction	41 465 (56%)	2021 (47%)
ATG	6991 (9%)	498 (12%)
Anti-IL2R	23 351 (32%)	1690 (39%)
Other	2060 (3%)	70 (2%)
Initial immunosuppression		
Calcineurin inhibitors	70 490 (95%)	3200 (75%)
Antiproliferatives	67 139 (91%)	1496 (35%)
Steroids	68 481 (93%)	3843 (90%)

All characteristics are significantly different between the mTOR inhibitor-treated patients and the control group without mTOR inhibitor therapy (P < 0.001).
Ab, antibody; ATG, antithymocyte globulin; IL2R, interleukin-2 receptor; mTORi, mammalian target of rapamycin inhibitor.

Table 2. Incidence per 100 000 person years (Inc) and standardized incidence ratios (SIR) taking account of age, gender and geographical region

Cancer (ICD 10)	Without mTORi *n* = 73 867				With mTORi *n* = 4279				P
	Cases	Inc	SIR	95% CI	Cases	Inc	SIR	95% CI	
Lip, oral cavity and pharynx (C00–14)	136	41	1.4	1.2–1.7	10	53	1.6	0.8–2.9	0.76
Digestive organs (C15–26)	593	179	1.0	0.9–1.1	33	175	1.0	0.7–1.3	0.70
Colorectum (C18–21)	270	82	0.9	0.8–1.0	14	74	0.8	0.4–1.3	0.70
Respiratory and intrathor. organs (C30–39)	504	152	1.2	1.1–1.3	34	180	1.4	1.0–1.9	0.52
Lung (C33)	452	136	1.3	1.1–1.4	30	159	1.4	1.0–2.0	0.51
Melanoma of skin (C43)	161	49	2.4	2.1–2.8	10	53	2.4	1.1–4.4	0.97
Non-melanoma skin (C44)	3231	1007	6.1	5.9–6.3	163	883	5.1	4.3–5.9	**0.019**
Mesothelial and soft tissue (C45–49)	147	44	5.2	4.4–6.1	15	79	8.7	4.9–14.3	0.057
Kaposi sarcoma (C46)	113	34	35.9	29.6–43.2	10	53	43.7	21.0–80.4	0.55
Breast, female patients (C50)	224	177	1.0	0.8–1.1	9	132	0.7	0.3–1.3	0.31
Female genital organs (C51–58)	169	133	1.4	1.2–1.7	6	88	1.0	0.3–2.1	0.32
Male genital organs (C60–63)	441	218	1.5	1.4–1.7	24	200	1.4	0.9–2.0	0.58
Prostate (C61)	405	200	1.5	1.4–1.7	23	191	1.4	0.9–2.1	0.72
Urinary tract (C64–68)	662	200	2.9	2.7–3.2	31	164	2.3	1.5–3.2	0.14
Kidney (C64–66)	513	155	6.9	6.3–7.5	27	143	6.0	4.0–8.7	0.49
Eye, brain, central nervous system (C69–72)	65	20	1.5	1.1–1.9	4	21	1.5	0.4–4.0	0.94
Thyroid, endocrine glands (C73–75)	82	25	4.3	3.4–5.3	3	16	2.5	0.5–7.4	0.37
Lymphoid, haematopoietic tissue (C81–96)	540	163	3.5	3.2–3.8	30	159	3.3	2.2–4.6	0.74
Non-Hodgkin lymphoma (C82–85)	437	132	6.1	5.5–6.6	26	138	5.9	3.9–8.7	0.93
All cancers without non-melanoma skin	3756	1158	1.7	1.6–1.7	211	1137	1.6	1.3–1.8	0.35

P-values for significance between SIR with or without mammalian target of rapamycin inhibitor (mTORi). Significant P value is shown in bold.

Propensity score-matching analysis was undertaken to substantiate this finding via a comparison of matched patients with or without mTOR inhibitor treatment within the CTS population and to allow differentiation of BCC and SCC. Because a large number of patients without mTOR inhibitor treatment were available for matching ($n = 73\,867$), a match could be obtained for 4265 of the 4279 patients receiving an mTOR inhibitor (99.7%). Within the propensity score-matched groups, multivariable Cox regression analysis considering the confounders listed in the Materials and methods section was performed to compare the 5-year cumulative risk of malignancy between patients with or without mTOR inhibitor therapy. Consistent with the SIR analysis, administration of an mTOR inhibitor was associated with a reduced risk for NMSC, although the difference did not reach statistical significance [hazard ratio (HR) 0.77; 95% confidence interval (CI) 0.59–1.01; P = 0.063] (Figure 2). For all other tumours, there was no significant difference in incidence between patients treated with or without mTOR inhibitor (HR 0.94; 95% CI 0.74–1.18; P = 0.59) (Figure 2). When an additional analysis was performed in which patients with BCC or SCC of the skin were differentiated, it became evident that the antitumour effect of mTOR inhibitor therapy was restricted to BCC. Multivariable Cox regression analysis showed that mTOR inhibitor therapy was associated with a markedly reduced risk of post-transplant BCC of the skin (HR 0.56; 95% CI 0.38–0.83; P = 0.004) whereas the incidence of SCC was not significantly reduced (HR 0.87; 95% CI 0.56–1.36; P = 0.54) (Figure 3). Our analysis did not show a significantly lower rate of Kaposi sarcoma or melanoma in patients given an mTOR inhibitor but it should be borne in mind that the overall incidence of these tumours is low, which limits the likelihood of detecting significant differences.

Since the tumour protective action of *de novo* mTOR inhibitor treatment was restricted to BCC of the skin, we attempted to estimate the clinical impact of tumour prevention by analysing the effect of tumour occurrence on patient survival after tumour diagnosis. Recipients who developed BCC of the skin showed a high subsequent patient survival rate that did not differ significantly from that of patients without any cancer (5-year patient survival after diagnosis of BCC was 85.8%, as compared with 85.6% in patients standardized for age and length of follow-up who had not developed any cancer). As shown in Figure 4, recipients who developed SCC of the skin (the incidence of which was not significantly affected by mTOR inhibitor treatment) had a markedly lower patient survival rate after diagnosis than those developing BCC. The difference was accounted for by death due to malignancy: in patients with SCC, 35% of all deaths were due to cancer whereas in patients with BCC the corresponding fraction was 14% (P < 0.001).

Since switch to mTOR inhibitor treatment has been associated with improved kidney graft function [25–29], patients are sometimes converted at several weeks or months post-transplant from a regimen free of mTOR inhibition to a maintenance regimen that includes an mTOR inhibitor. We examined whether such conversion during the first post-transplant year was associated with a reduced tumour incidence after the first post-transplant year. To exclude bias, patients who were registered as having developed a tumour during the first post-transplant year were excluded from this part of the analysis. Propensity score matching and Cox regression analysis of 1651 patients switched to mTOR inhibitor and 1651 controls showed no significant effect of switching to treatment with an mTOR inhibitor on the occurrence of skin cancer during Years 2–6 (NMSC: HR 0.93, P = 0.69; BCC: HR 1.02, P = 0.93; SCC: HR 0.81, P = 0.49).

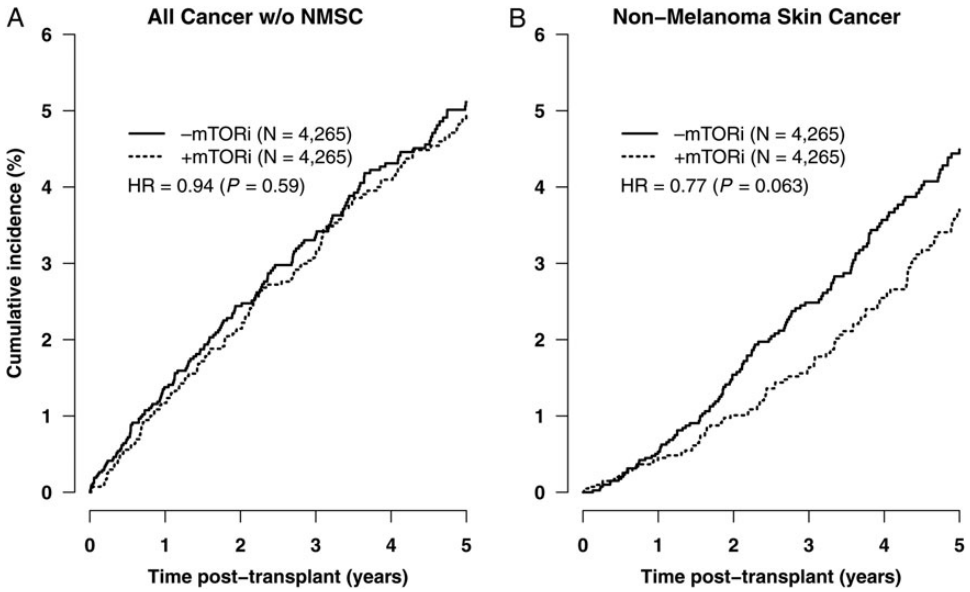

FIGURE 2: Influence of mammalian target of rapamycin inhibitor (mTORi) therapy on the cumulative incidence of (**A**) all cancers excluding non-melanoma skin cancer (NMSC) and (**B**) NMSC. Hazard ratio (HR) values were calculated for a propensity score-matched population using multivariable Cox regression.

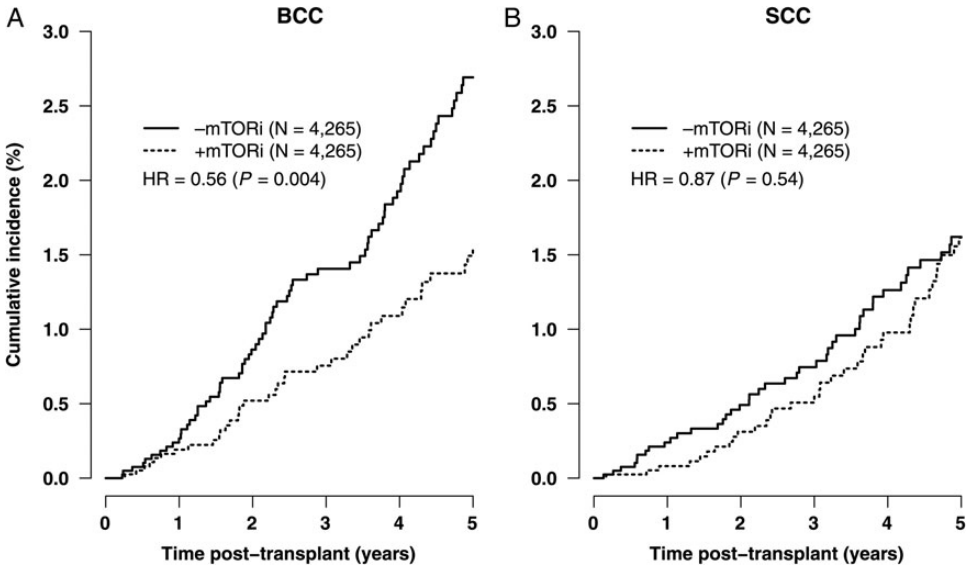

FIGURE 3: Influence of mammalian target of rapamycin inhibitor (mTORi) therapy on the cumulative incidence of non-melanoma skin cancer. (**A**) Basal cell carcinoma (BCC) and (**B**) squamous cell carcinoma (SCC). Hazard ratios (HR) were calculated for a propensity score-matched population using multivariable Cox regression.

DISCUSSION

This analysis of prospectively recorded data from a large series of kidney transplant patients showed a reduction in the risk of NMSC when an mTOR inhibitor was given from the time of transplant. No effect of mTOR inhibition on the incidence of tumours other than NMSC was observed.

Skin cancers are the most frequently occurring post-transplant cancers. Of these, 95% are NMSC, largely SCC and BCC [30]. Our analysis showed that the effect of *de novo* mTOR inhibition on NMSC was limited to a reduction of BCC of the skin, an effect that was substantial and statistically significant, whereas no significant decrease was observed for the rate of SCC. BCC rarely metastasize and exert little effect on survival. Data from the CTS show that patient survival after diagnosis of BCC was virtually identical to that of patients without any tumour, whereas the 5-year patient survival rate for patients with SCC was significantly lower (Figure 4). If the anti-oncogenic effect of *de novo* mTOR inhibition is limited to BCC of the skin, it is unlikely to generate a clinically relevant change in survival. It should be borne in mind, however, that while BCC is not associated with increased mortality, it is an unwelcome

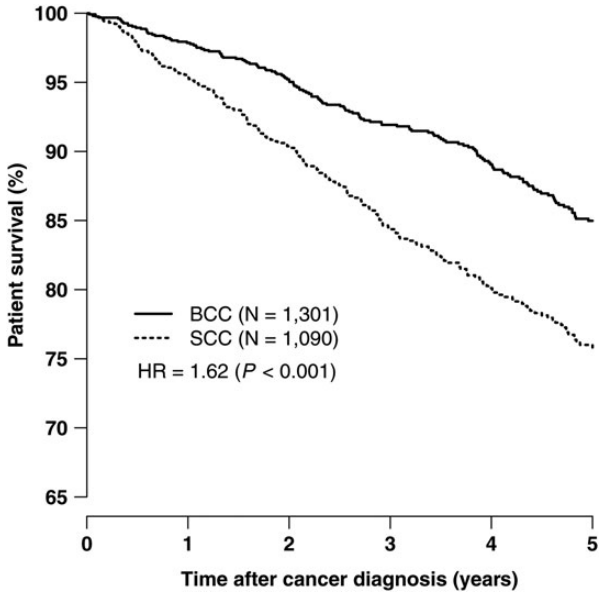

FIGURE 4: Patient survival after diagnosis of squamous cell carcinoma (SCC) or basal cell carcinoma (BCC) of the skin. Hazard ratio (HR) was calculated by multivariable Cox regression.

experience for the patient and incurs a significant management burden and expense.

Two other registry analyses have addressed the question of an anti-tumour effect of mTOR inhibition after kidney transplantation, although they did not assess rates of skin cancer. A recent publication based on US Scientific Registry for Transplant Recipients by Yanik *et al.* evaluated the incidence of cancer in a cohort of over 32 000 kidney transplants (1995–2009) [31]. Patients who were given sirolimus treatment at any point post-transplant were included. That analysis did not show a significantly lower incidence of cancer during sirolimus use (HR 0.88; 95% CI 0.70–1.11), but basal cell and squamous cell cancers were excluded from the analysis. The authors reported a 75% higher rate of prostate cancer in patients on mTOR inhibitor treatment, a finding that we could not confirm in our patient population, which included a far larger number of patients with prostate cancer (428 versus 108 cases), thereby reducing the likelihood of statistical error. A previous registry analysis published in 2006, also based on a US cohort of kidney transplant patients (1996–2001, $n = 33\,249$), by Kauffman *et al.* found a significantly lower rate of *de novo* malignancy when mTOR inhibition was given as discharge immunosuppression ($P < 0.001$), but again rates of skin cancer were not examined [32]. These two registry analyses, both based on US data, thus came to contradicting conclusions regarding the effect of mTOR inhibitor treatment on the incidence of non-skin cancer. Our present analysis covers a more recent transplant period (1999–2013) that partly overlaps with the study period covered by the analysis of Yanik *et al.* (1995–2009) [31], and our conclusion agrees with that reached by these authors [31]. A recent meta-analysis of 20 randomized controlled trials and two observational studies came to the conclusion that there is no significant evidence for a tumour-reducing effect of mTOR inhibitor treatment on cancers other than NMSC [33]. In that meta-analysis, the evidence for a reduction of NMSC was considered convincing, although there was no separate assessment of basal or squamous cell cancer due to a lack of data [33]. Thus, our current analysis confirms in a larger patient series that mTOR inhibitor treatment is associated with a reduced incidence of skin cancer after renal transplantation. It extends current knowledge by demonstrating that the tumour-reducing efficacy of *de novo* mTOR inhibitor treatment is restricted to BCC of the skin.

The current study focused on patients in whom mTOR inhibition was included as part of the initial intention-to-treat regimen in order to provide the most clear-cut assessment of the effect of mTOR inhibitors. It should be noted that mTOR inhibitor-treated patients usually receive lower doses of concomitant CNI medication than recipients given no mTOR inhibitor. Dantal *et al.* were first to report a significant relationship between cyclosporine dose and skin cancer [34]. Using 1-year medication as an indicator, our data on a limited subset of patients show that mTOR inhibitor-treated patients received significantly lower maintenance doses of cyclosporine and tacrolimus ($P < 0.001$), but we found no evidence for a particularly low rate of NMSC in the 1079 patients who received a CNI-free *de novo* mTOR inhibitor regimen (data not shown). Published data regarding an effect of conversion to mTOR inhibitor on *de novo* skin cancer rates are contradictory. While Alberu *et al.* found a lower rate of skin cancer after conversion to mTOR inhibitor treatment in a prospective randomized trial [18], Hoogendijk-van den Akker *et al.* in another prospective randomized trial failed to show any effect [35]. Retrospective analysis of the randomized CNI withdrawal trial in mTOR inhibitor-treated patients reported by Campistol *et al.* [16] showed significantly lower rates of both skin and non-skin cancer in patients receiving mTOR inhibitor treatment in whom CNI was withdrawn. To what extent CNI withdrawal, mTOR inhibitor therapy or both was responsible for the reduced tumour incidence—particularly that of non-skin tumours given the proven tumour-promoting capacity of cyclosporine [36]—is unknown. This study is also difficult to compare with other analyses because recurrent and multiple tumours (a maximum of 27 tumours in one single patient) were included in the tumour count and patient age, the most important confounder, was not considered despite being critical for tumour analysis.

A possible limitation of our analysis is that patients considered to be at high risk for cancer might have been preferentially assigned to an mTOR inhibitor. This cannot be excluded but is unlikely to have applied in many cases since the anti-tumour potential of mTOR inhibition was largely unknown during much of the study period, particularly in the early years (1998–2004) when the majority of mTOR inhibitor-treated patients were registered. Transplant year was considered as a potential confounder in the multivariable analysis and patients and controls were matched for transplant year in the propensity score analysis. Moreover, patients analysed in this series who had no evidence of pretransplant malignancy were assigned to an mTOR inhibitor-containing immunosuppressive regimen at a similar rate (5.5%) to patients in whom a pretransplant tumour was diagnosed (4.6%). This suggests that our analysis is unlikely to have been influenced in any meaningful way by preferential selection for mTOR inhibitor treatment in patients

considered to be at increased tumour risk. Unlike some previous randomized studies that focused on disease recurrence in patients with a previous diagnosis of NMSC [14, 15], our analysis focused on *de novo* cancer in patients treated with an mTOR inhibitor as part of the intention-to-treat regimen who had no prior history of malignancy.

Like all registry studies, our analysis has the limitation that the accuracy of reporting is not equivalent to that in a prospective trial. However, since the 1980s the CTS study has placed particular emphasis on the accurate and complete reporting of tumour data during every clinical update, with special emphasis on tumours of the skin. All participating centres were aware of this and a centre's data were included in this analysis only if the accuracy and completeness of tumour records was confirmed in writing by the participating centre. Neither single-centre nor multicentre studies can absolutely guarantee that all skin tumours are recognized and recorded. The large number of patients analysed here ensures that small inaccuracies in reporting would not exert a major effect on the analysis of tumour incidence. A further limitation of our work is that daily or cumulative dosages of mTOR inhibitor and other immunosuppressive drugs could not be analysed because these data are not recorded in the CTS database.

In conclusion, analysis of this large international cohort of kidney transplant patients indicates that inclusion of an mTOR inhibitor in the *de novo* immunosuppressive regimen significantly reduces the risk of BCC of the skin after kidney transplantation. This analysis did not show a significant influence of mTOR inhibitor treatment on the incidence of other post-transplant cancers.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

AUTHOR CONTRIBUTIONS

G.O. and B.D. participated in research design, performance of the research, data analysis and in the writing of the article. C.S. participated in research design, performance of the research and in the writing of the article. C.U. participated in data analysis and in the writing of the article.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. The results presented in this paper have not been published previously in whole or part.

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