Epidemiogic aspects of skin cancer in organ-transplant recipients

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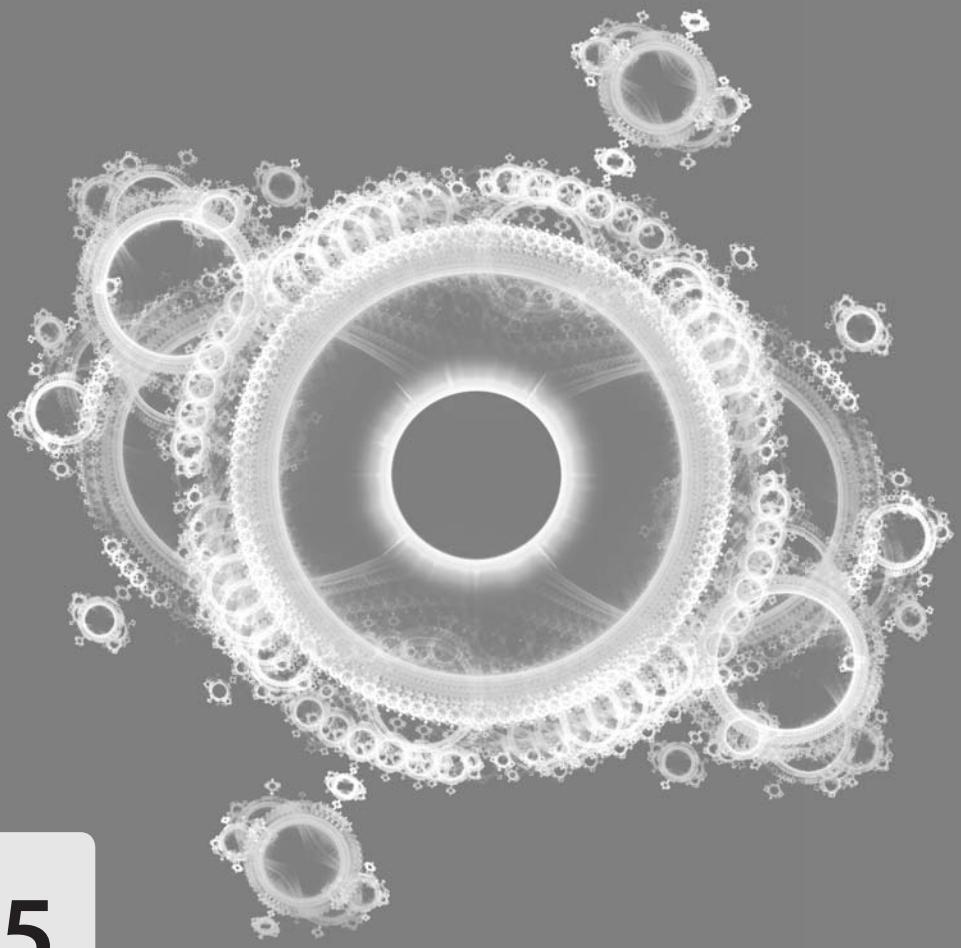

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Increased risk of squamous-cell carcinoma in simultaneous pancreas kidney transplant recipients compared with kidney transplant recipients

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Abstract

The purpose of this study was to ascertain the risk of non-melanocytic skin cancer (NMSC) in simultaneous pancreas kidney transplant recipients (SPKTRs) compared to kidney transplant recipients (KTRs) in relation to other potential risk factors of skin cancer. In a cohort study, 208 SPKTRs were compared with 1,111 KTRs who were transplanted during the same time period. The effects of age, sex, country of origin, time period after transplantation, HLA matching, immunosuppressive regimen and rejection treatments on the risk of NMSC were investigated in multivariable Cox's proportional hazard models. In SPKTRs the incidence of NMSC increased from 19 to 36%, respectively 10 and 15 years after transplantation which was significantly higher compared with that in KTRs (6 and 10%, respectively). After adjustment for age and sex, SPKTRs had a 6.2 (3.0-12.8) increased risk of squamous-cell carcinoma (SCC) compared to KTRs. An additional adjustment for maintenance immunosuppression decreased the hazard ratio to 3.1 (1.3-7.2) which indicates partial confounding by the immunosuppressive regimen. Adjustment for induction and rejection therapy or HLA mismatching did not change the hazard ratio significantly. SPKTRs have an increased risk of SCC compared with KTRs, despite partial confounding by the immunosuppressive regimen.

Introduction

Organ-transplant recipients are at increased risk for post-transplant neoplasms (Hardie *et al*, 1980; Hartevelt *et al*, 1990). Non-melanocytic skin cancers (NMSCs), especially squamous-cell carcinomas (SCC), are the most common malignancies and can cause substantial morbidity and even mortality (Hartevelt *et al*, 1990; Bouwes Bavinck *et al*, 1996; Naldi *et al*, 2000; Jensen *et al*, 2000; Euvrard *et al*, 2003; Otley *et al*, 2005b; Moloney *et al*, 2006).

Increasing age, male sex, and fair complexion are the most important host-related risk factors for skin cancer, and exposure to sunlight, smoking and infection with human papillomaviruses are the most important environmental risk factors (De Hertog *et al*, 2001; Kasiske *et al*, 2004; Bouwes Bavinck and Feltkamp, 2004; Bouwes Bavinck *et al*, 2008). Among organ-transplant recipients, immunosuppressive therapy forms an additional important risk factor (Hartevelt *et al*, 1990; Bouwes Bavinck *et al*, 2007). Both the duration and type of immunosuppression may play a role. Azathioprine (Aza) has been reported to induce selective UVA photosensitivity, which may result in a cascade of reactions in the skin, ranging from the induction of oxidative stress and mutagenic DNA lesions to the development of skin cancer (O'Donovan *et al*, 2005; Ulrich and Stockfleth, 2007; Cooke *et al*, 2007; Montaner *et al*, 2007). Cyclosporine A (CsA) can decrease DNA repair and impair UV-induced apoptosis, which also increases the risk of skin cancer (Yarosh *et al*, 2005). Poor HLA matching has been reported to be associated with an increased risk of NMSC (Bouwes Bavinck *et al*, 1991).

Among kidney-transplant recipients (KTRs) living in a temperate climate, the prevalence of NMSC at 10 years after transplantation varied between 10 and 27% and at 20 years between 40 and 60% (Hartevelt *et al*, 1990; Bordea *et al*, 2004; Moloney *et al*, 2006). In Australia, the incidence is even higher (Hardie *et al*, 1980; Bouwes Bavinck *et al*, 1996; Ramsay *et al*, 2002). Heart-transplant recipients seem to have a higher incidence of NMSC compared with KTRs, although this may be a consequence of older age at transplantation in this group (Mihalov *et al*, 1996; Naldi *et al*, 2000; Fortina *et al*, 2000). Less research has been conducted in patients receiving a liver transplant. After a follow-up period of 10 years, an incidence between 13 and 26% has been found in Dutch and Spanish liver-transplant recipients, respectively (Haagsma *et al*, 2001; Herrero *et al*, 2005). There are no studies that followed up lung-transplant recipients or simultaneous pancreas kidney transplant recipients (SPKTRs) for a longer period.

Since 1986, simultaneous pancreas kidney transplantations (SPKTs) are being performed in the Netherlands. At present, more than 200 patients received an SPKT at

the Leiden University Medical Center (LUMC). The main objective of this study was to calculate the cumulative incidence of skin cancer in SPKTRs compared with the incidence in KTRs who were transplanted in the same center during the same time period.

We hypothesized that the risk of skin cancer in SPKTRs would be higher compared with that in KTRs, because SPKTRs are exposed to a more potent immunosuppressive regimen and are not HLA-matched in contrast to KTRs.

Results

Baseline characteristics of the KTR and SPKTR

The baseline characteristics of the KTRs and SPKTRs are depicted in Table 1. The majority of the patients originated from the Netherlands. In the KTR group, there were significantly more patients originating from Mediterranean countries or from countries that are associated with a darker skin type (Table 1). Sex distribution did not differ significantly between the two groups, but the SPKTRs were on an average 7.4 years younger at first transplantation than were the KTRs ($P < 0.001$). The median follow-up time of the SPKTRs was shorter ($P = 0.014$), because, during the first few years, the number of SPKTs was still limited (Table 1). After adjustment for age, sex and immunosuppressive therapy, overall survival was significantly shorter for SKPTRs compared with KTRs, with an adjusted hazard ratio of 2.1 (1.5-3.1).

Cumulative incidence of skin cancer in the SPKTR compared with that in the KTR

The baseline characteristics of the KTRs and SPKTRs in relation to the development of SCC and basal-cell carcinoma (BCC) as first events are depicted in Table 2, and potential risk factors for NMSC, SCC and BCC are presented for KTRs and SPKTRs, separately, in Supplementary Figure S1 and Supplementary Tables S1a and S1b. Two of the KTRs had developed an SCC and a BCC and four only a BCC before transplantation. These skin cancers were not considered in the analyses. None of the SPKTRs had developed an SCC or a BCC before transplantation. The time period after transplantation was significantly associated with the occurrence of SCC and BCC ($P < 0.001$), but sex was not associated with skin cancer (Table 2). In the Cox's proportional hazard model, increasing age at transplantation was a risk factor for both types of skin cancer (Supplementary Tables S1a and S1b).

During the follow-up period until June 2007, a total of 109 skin cancers (73 SCCs and 36 BCCs) were diagnosed in 26 (12.5%) out of 208 SPKTRs (Table 2). During the

Table 1 Baseline characteristics of 1111 kidney transplant recipients and 208 simultaneous pancreas kidney transplant recipients.

	KTR*	SPKTR*	P value
No of patients	**1111**	**208**	
Country of origin			
Netherlands	973 (87.6)	203 (97.6)	
Mediterranean	58 (5.2)	3 (1.4)	P < 0.001
Suriname, Africa, Asia	80 (7.2)	2 (1.0)	
Male: N (%)	690 (62.1)	126 (60.6)	P = 0.677
Age at transplant (years)			
Median	48.6	40.5	P < 0.001
25% - 75%	37.8 – 58.5	34.8 – 46.0	
Follow-up (years)			
Median	6.9	6.4	P = 0.014
25% - 75%	3.6 – 12.1	3.5 – 10.1	
HLA mismatches			
0	178 (16.1)	1 (0.5)	
1-3	774 (70.2)	52 (25.0)	P < 0.001
4-6	151 (13.7)	155 (74.5)	
Unknown	8	0	
Death: N (%)	363 (33.0)	63 (30.4)	P = 0.475**
Unknown	10	1	

*KTR = kidney transplant recipient, SPKTR = simultaneous pancreas kidney transplant recipient.
** After adjustment for age, sex and immunosuppressive therapy overall survival was significantly shorter for SKPTR compared to KTR with an adjusted hazard ratio of 2.1 (1.5;3.1).

same follow-up period, 68 (6.1%) out of 1,111 KTRs developed altogether 223 skin cancers (102 SCCs and 121 BCCs). The overall SCC:BCC ratio in the KTR was 0.79. This ratio gradually increased with increasing time after transplantation with ratios of 0.67, 0.55, 0.71, and 1.0 during the first 2, 2-7, 8-12, and 13-17 years after transplantation, respectively. The overall SCC:BCC ratio in the SPKTR was 1.1. The ratios were 0, 1.1, and 1.4 during the periods between 2-7, 8-12, and 13-17 years after transplantation, respectively.

The cumulative incidences of NMSC, SCC and BCC in SPKTRs are compared with those in KTRs in Figure 1 and Supplementary Figure S2.

Table 2 Baseline characteristics according to the presence of non-melanocytic skin cancer and risk factors of skin cancer in kidney transplant recipients and simultaneous pancreas kidney transplant recipients.

	KTR*			SPKTR*			
Type of skin cancer	**No skin cancer (censored)**	**SCC* as first event**	**BCC* as first event**	**P-value**	**No skin cancer (censored)**	**SCC* as first event**	**BCC* as first event**	**P-value**
No. of patients: N (%)	1043 (94.0)	28 (2.5)	40 (3.6)		182 (87.5)	14 (6.7)	12 (5.8)	
No. of patients with SCC	0	28	10		0	14	4	
No. of patients with BCC	0	8	40		0	5	12	
SCC per pat with NMSC								
Mean (Min-Max)	0	2.5 (1-7)	0.8 (0-7)		0	4.9 (1-14)	0.3 (0-1)	
BCC per pat with NMSC								
Mean (Min-Max)	0	0.5 (0-5)	2.7 (1-20)		0	0.4 (0-2)	2.5 (1-12)	
Country of origin								
Netherlands	907 (87.0)	28 (100)	38 (95.0)		177 (97.3)	14 (100)	12 (100)	
Mediterranean	57 (5.5)	0	1 (2.5)	Pscc = 0.124	3 (1.6)	0	0	Pscc = 0.821
Africa, Asia	79 (7.5)	0	1 (2.5)	Pbcc = 0.323	2 (1.1)	0	0	Pbcc = 0.844
Male: N (%)	647 (62.0)	20 (71.4)	23 (57.5)	Pscc = 0.311 Pbcc = 0.562	109 (59.9)	9 (64.3)	8 (66.7)	Pscc = 0.746 Pbcc = 0.642
Age at transplant (years)								
Median	48.4	51.9	49.6	Pscc = 0.147	40.6	42.5	37.4	Pscc = 0.933
25% - 75%	37.7 – 58.1	40.9 – 61.7	44.8 – 60.2	Pbcc = 0.226	34.8 – 46.0	33.6 – 47.8	33.3 – 48.1	Pbcc = 0.566
Follow-up (years)								
Median	6.6	13.0	11.6	Pscc < 0.001	5.6	14.0	10.3	Pscc < 0.001
25% - 75%	3.5 – 11.6	9.5 – 19.3	7.8 – 16.8	Pbcc < 0.001	3.1 – 8.7	12.4 – 15.8	8.5 – 14.9	Pbcc < 0.001
HLA mismatches								
0	165 (15.9)	4 (14.3)	9 (22.5)		0	1 (7.1)	0	
1-3	728 (70.3)	23 (82.1)	23 (57.5)	Pscc = 0.263	46 (25.3)	4 (28.6)	2 (16.7)	Pscc = 0.391#
4-6	142 (13.7)	1 (3.6)	8 (20.0)	Pbcc = 0.221	136 (74.7)	9 (64.3)	10 (83.3)	Pbcc = 0.503
Unknown	8	0	0		0	0	0	

ATG or OKT3 as induction or rejection treatment								
No	760 (72.9)	21 (75.0)	30 (75.0)	Pscc = 0.802	44 (24.2)	2 (14.3)	1 (8.3)	Pscc = 0.400
Yes	283 (27.1)	7 (25.0)	10 (25.0)	Pbcc = 0.766	138 (75.8)	12 (85.7)	11 (91.7)	Pbcc = 0.208
Type of maintenance immunosuppression**								
P + Aza	70 (6.7)	9 (32.1)	5 (12.5)		0	0	0	
P + CsA	429 (41.3)	15 (53.6)	23 (57.5)		0	0	0	
P + MMF	95 (9.2)	2 (7.1)	2 (5.0)		0	0	0	
P + Tac	38 (3.7)	0	0	Pscc < 0.001	0	0	0	Pscc < 0.001
P + Aza + CsA	5 (0.5)	0	1 (2.5)	Pbcc = 0.064	64 (35.2)	14 (100)	5 (41.7)	Pbcc = 0.218
P + MMF + CsA	275 (26.5)	2 (7.1)	6 (15.0)		81 (44.5)	0	7 (58.3)	
P + MMF + Tac	126 (12.1)	0	3 (7.5)		37 (20.3)	0	0	
Unknown	5	0	0		0	0	0	
Level of immunosuppression***								
Low	419 (40.2)	20 (71.4)	25 (62.5)		0	0	0	
Moderate	561 (53.8)	7 (25.0)	11 (27.5)	Pscc = 0.004	44 (24.2)	2 (14.3)	1 (8.3)	Pscc = 0.627
High	63 (6.0)	1 (3.6)	4 (10.0)	Pbcc = 0.005	111 (61.0)	9 (64.3)	8 (66.7)	Pbcc = 0.362
Very high	0	0	0		27 (14.8)	3 (21.4)	3 (25.0)	

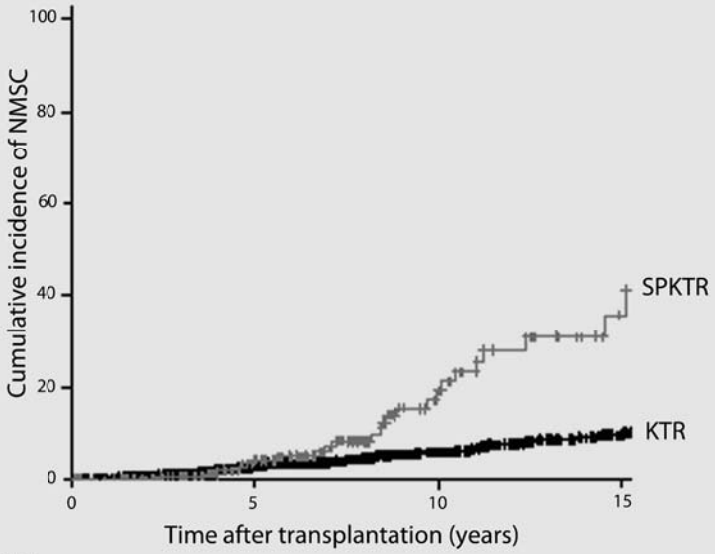
*KTR = kidney transplant recipient, SPKTR = simultaneous pancreas kidney transplant recipient, SCC = squamous-cell carcinoma, BCC = basal-cell carcinoma.

#For calculation of this p-value the numbers for 0 – 3 mismatches were combined.

**P = prednisolone; Aza = azathioprine; CsA = cyclosporine A; MMF =mycofenolatemofetil; Tac =tacrolimus.

*** The level of immunosuppression is defined in the methods.

Figure 1 Cumulative incidence of skin cancer in 208 simultaneous pancreas kidney transplant recipients (SPKTR) compared to 1111 kidney transplant recipients (KTR). The numbers of SPKTR and KTR at risk in relation to the years after transplantation are indicated in the Table.



Number af risk				
SPKTR	208	121	42	12
KTR	1105	691	356	147

Possible risk factors for skin cancer

To identify the possible factors that could explain the increased risk of skin cancer among SPKTRs compared with KTRs, we analyzed the influence of age, sex, country of origin, HLA matching, maintenance immunosuppressive regimen, induction and rejection treatments, and level of immunosuppression on the risk of skin cancer within the SPKTRs and KTRs (Supplementary Figure S1 and Supplementary Tables S1a and S1b).

HLA matching and skin cancer

No HLA matching is carried out in SPKTRs. Therefore, the number of mismatches was much higher among the SPKTRs than in KTRs (Table 1). HLA mismatching, however,

was not significantly associated with SCC or BCC in either the KTRs or the SPKTRs (Table 2, Supplementary Figure S1f and Supplementary Tables S1a and S1b).

Immunosuppressive regimens and skin cancer

The immunosuppressive regimens differed strongly between SPKTRs and KTRs, and changed considerably during the years (Table 3). SPKTRs always received triple therapy, whereas this regimen was introduced much later in KTRs (Table 3).

In both KTRs and SPKTRs, immunosuppressive regimens were associated with the development of SCC but not of BCC (Table 2, Supplementary Figure S1e and Supplementary Tables S1a and S1b). For the main analyses, the immunosuppressive regimens were categorized into three basic treatment groups: Aza in any combination, mycophenolatemofetil (MMF) in any combination, or CsA or tacrolimus (Tac) without Aza or MMF.

In the KTR group, immunosuppression with MMF compared with that with Aza was associated with a significantly decreased risk of SCC (Supplementary Figure S1e, SCC). The hazard ratio adjusted for age and sex was 0.15 (0.04-0.59) (Supplementary Table S1a). Additional adjustments for the simultaneous use of CsA; for triple versus duo therapy or for the number of HLA mismatches did not change this hazard ratio significantly. In the KTR group, immunosuppression with CsA was also associated with a significantly decreased risk of SCC compared with Aza (Supplementary Figure S1e, SCC). The hazard ratio adjusted for age and sex was 0.35 (0.15-0.84) (Supplementary Table S1a).

In the SPKTR group, immunosuppression with MMF compared with that with Aza was also associated with a decreased risk of SCC (Supplementary Figure S1e, SCC). The hazard ratio could not be calculated, however, because all SCC cases were immunosuppressed with Aza in any combination and none with MMF in any combination (Table 2 and Supplementary Table S1b). SPKTRs who had maintenance therapy with MMF in any combination seemed to have an increased risk of BCC compared with patients who were using maintenance therapy with Aza in any combination, although statistical significance was not reached, and this increased risk was not observed in KTRs (Supplementary Figure S1e, BCC). As almost all SPKTRs were immunosuppressed with CsA, either in combination with prednisolone and Aza or with prednisolone and MMF (Table 2), the risk of SCC associated with the use of CsA could not be calculated in the SPKTR group.

Table 3 Immunosuppressive therapy of 1111 kidney transplant recipients and 208 simultaneous pancreas kidney transplant recipients according to time periods of transplantation, corresponding with major changes in immunosuppressive regimen.

	KTR*				SPKTR*			
Transplantation period	**1986 - 1995**	**1996 – 2001**	**2002 - 2005**	**P-value**	**1986 - 1995**	**1996 – 2001**	**2002 - 2005**	**P-value**
No of patients*	530	289	292		72	77	59	
SCC total (1st event)	34 (25)	3 (2)	1 (1)		16 (13)	2 (1)	0 (0)	
BCC total (1st event)	33 (28)	10 (8)	5 (4)		10 (5)	7 (7)	0 (0)	
ATG or OKT3 as induction or rejection treatment								
No	327 (61.7)	223 (77.2)	261 (89.4)	P < 0.001	11 (15.3)	22 (28.6)	14 (23.7)	P = 0.148
Yes	203 (38.3)	66 (22.8)	31 (10.6)		61 (84.7)	55 (71.4)	45 (76.3)	
Type of maintenance immunosuppression**	N (%)	N (%)	N (%)		N (%)	N (%)	N (%)	
P + Aza	83 (15.7)	1 (0.3)	0		0	0	0	
P + CsA	374 (70.8)	86 (29.9)	7 (2.4)		0	0	0	
P + MMF	37 (7.0)	41 (14.2)	21 (7.2)		0	0	0	
P + Tac	1 (0.2)	27 (9.4)	10 (3.4)	P < 0.001	0	0	0	P < 0.001
P + Aza + CsA	6 (1.1)	0	0		72 (100)	8 (10.4)	3 (5.1)	
P + MMF + CsA	26 (4.9)	99 (34.3)	158 (54.5)		0	69 (89.6)	19 (32.2)	
P + MMF + Tac	1 (0.2)	34 (11.8)	94 (32.4)		0	0	37 (62.7)	
Unknown	2	1	2		0	0	0	

Level of immunosuppression***							
Low	307 (57.9)	124 (42.9)	33 (11.3)		0	0	0	
Moderate	211 (39.8)	132 (45.7)	236 (80.8)	P < 0.001	11 (15.3)	22 (28.6)	14 (23.7)	P = 0.179
High	12 (2.3)	33 (11.4)	23 (6.9)		45 (62.5)	47 (61.0)	36 (61.0)	
Very high	0	0	0		16 (22.2)	8 (10.4)	9 (15.3)	

*KTR = kidney transplant recipient; SPKTR = simultaneous pancreas kidney transplant recipient; SCC = squamous-cell carcinoma, BCC = basal-cell carcinoma..
**P = prednisolone; Aza = azathioprine; CsA = cyclosporine A; MMF =mycofenolatemofetil; Tac =tacrolimus.
*** The level of immunosuppression is defined in the methods.

Induction and rejection treatments and level of immunosuppression in relation to skin cancer

Among SPKTRs, induction or rejection treatments with antithymocyte globulin (ATG) or muromonab (OKT3) were not associated with an increased risk of NMSC, SCC or BCC (Supplementary Figure S1h-j). The hazard ratios adjusted for age, sex and immunosuppressive therapy for induction and rejection treatments to develop NMSC were 0.91 (0.38-2.2) and 1.5 (0.42-5.4), respectively. For SCC, the adjusted hazard ratios were 0.92 (0.29-3.0) and 1.3 (0.15-10.1), respectively, and for BCC they were 0.68 (0.18-2.6) and 2.4 (0.49-12.1), respectively.

Owing of insufficient numbers of induction treatments among KTRs in this subgroup, we could only calculate the hazard ratios for rejection treatments. The adjusted hazard ratios were 0.75 (0.42-1.4), 0.63 (0.25-1.6), and 0.83 (0.38-1.8) for NMSC, SCC and BCC, respectively.

As the biological effects of ATG and/or OKT3 are supposed to be similar before and after the transplantation, induction and rejection treatments with ATG and/or OKT3 were combined. Treatment with ATG and/or OKT3 at any time was not significantly associated with the development of NMSC, SCC or BCC in this study (Supplementary Figure S1j and Supplementary Tables S1a and S1b).

Triple therapy and treatment with ATG and/or OKT3 are the most important factors determining the level of immunosuppression. By combining these treatment modalities, we estimated a "general" level of immunosuppression. Using this estimation, the level of immunosuppression was not consistently associated with NMSC, SCC or BCC (Supplementary Figure S1k and Supplementary Tables S1a and S1b). In the SPKTR, we also calculated the median daily

doses of prednisone, Aza, MMF, CsA and Tac, none of which were associated with skin cancer (data not shown).

SPKTRs have an increased risk of SCC compared with KTRs, which can be partly explained by confounding by an immunosuppressive regimen

Non-stratified Kaplan-Meier analyses and analyses stratified for potentially confounding factors are shown in Supplementary Figure S2 and non-adjusted and adjusted hazard ratios of developing NMSC, SCC or BCC in SPKTRs compared with those in KTRs are presented in Table 4.

Table 4 Risk of skin cancer in simultaneous pancreas kidney transplant recipients compared to kidney transplant recipients with adjustment for potentially confounding factors using Cox proportional hazard analyses.

Adjustments	Non melanocytic skin cancer	Squamous-cell carcinoma as first event	Basal-cell carcinoma as first event
No adjustment	*3.0 (1.9;4.8)*	*4.2 (2.2;8.1)*	*2.5 (1.3;4.9)*
Age	4.0 (2.4;6.5)	6.3 (3.1;13.0)	3.1 (1.5;6.1)
Sex	3.0 (1.9;4.8)	4.1 (2.1;8.0)	2.5 (1.3;4.9)
Age and sex	*4.0 (2.4;6.5)*	*6.2 (3.0;12.8)*	*3.1 (1.5;6.2)*
Age, sex and country of origin*	3.8 (2.3;6.2)	5.7 (2.8;11.8)	3.0 (1.5;6.0)
Age, sex and HLA mismatching**	3.3 (1.7;6.3)	8.3 (3.4;20.2)	1.7 (0.72;4.0)
Age, sex and maintenance immunosuppression***	3.0 (1.7;5.5)	3.1 (1.3;7.2)	3.1 (1.4;6.9)
Age, sex and ATG or OKT3 as induction or rejection treatment	3.9 (2.3;6.7)	6.3 (2.9;13.9)	2.9 (1.4;6.2)
Age, sex and level of immunosuppression****	2.4 (1.0;5.9)	6.5 (1.7;25.3)	1.3 (0.43;4.0)
Age, sex, HLA mismatching and maintenance immunosuppression	2.5 (1.2;5.1)	3.8 (1.4;10.2)	1.8 (0.68;4.5)

*Netherlands and neighbor countries; Mediterranean countries; or Suriname, Africa or Asia.
**No; 1-3; or 4-6 HLA A, B and DR mismatches.
***Aza in any combination; MMF in any combination; or CsA or Tac without Aza or MMF,
****Low, moderate, high or very high immunosuppression as explained in the methods.

The Kaplan-Meier analyses show an increased risk of SCC in SPKTRs compared with that in KTRs in almost all strata (Supplementary Figure S2). Supplementary Figure S2d shows that SPKTRs were much younger at transplantation than were KTRs. Adjustment for age, therefore, increased the hazard ratio for the association between transplanted organ and SCC (Table 4). Supplementary Figure S2f shows that risk of SCC was much lower in the group of patients who were immunosuppressed with MMF in any combination. Adjustment for maintenance immunosuppression decreased the hazard ratio for the association between transplanted organ and SCC, which was adjusted for age and sex from 6.2 (3.0-12.8) to 3.1 (1.3-7.2), which suggests a partial confounding by maintenance immunosuppression (Table 4). Adjustment for other potentially confounding factors did not reduce the hazard ratios for SCC notably (Table 4).

The risk of BCC in SPKTR compared with that in KTR was reduced after adjustment for HLA mismatching and for the level of immunosuppression, and, when all relevant potentially confounding factors were introduced into the Cox's proportional hazard model, the increased risk of BCC largely disappeared (Table 4).

Discussion

This study showed, after adjustment for age and sex, a 6.2-fold (95% CI: 3.0-12.8) increased risk of SCC in SPKTRs than in KTRs who were transplanted in the same center during the same time period. After an additional adjustment for maintenance immunosuppression, this risk decreased to 3.1 (1.3-7.2). The risk of BCC was not statistically significantly increased in SPKTRs after adjustment for potentially confounding factors.

Maintenance immunosuppressive therapy with MMF in any combination had led to a significantly decreased risk of SCC compared with maintenance immunosuppressive therapy with Aza. SPKTRs were more often immunosuppressed with Aza than were KTRs. Adjustment for this factor, indeed, reduced the risk of SCC in SPKTRs compared with that in KTRs, suggesting that the increased risk of SCC in SPKTR can be partly explained by confounding by the type of maintenance immunosuppressive therapy. There remained, however, a statistically significant three-fold increased risk of SCC in SPKTR, for which we looked for other potential explanations.

Apart from an obligate history of diabetes in the SPKTRs and differences in maintenance immunosuppression, other differences discerning SPKTRs from KTRs are more frequent induction and rejection therapies, and the absence of HLA matching

in SPKTRs. Moreover, these factors could potentially explain the increased risk of SCC in SPKTRs compared with that in KTRs.

The incidence of NMSC in patients with type 1 diabetes has not been systematically studied (Zendehdel *et al*, 2003; Swerdlow *et al*, 2005). Only Zendehdel *et al* (2003) showed a modest, but statistically nonsignificant increase of NMSC, with a standardized incidence ratio of 1.9 (0.6-4.3) in patients who had type 1 diabetes mellitus for more than 15 years (Zendehdel *et al*, 2003). In organ-transplant recipients, diabetes was associated with a decreased risk of NMSC (Kasiske *et al*, 2004; Otley *et al*, 2005a). It is therefore not likely that type 1 diabetes may explain the increased risk of SCC among SPKTRs.

Induction treatments, impending graft rejection, and the subsequent rejection therapies were not associated with SCC or BCC in this study, although the follow-up periods may still have been too short to detect such an effect. Adjustment for induction and rejection treatments did not change the increased risk of SCC in SPKTRs, excluding also these factors as major causes for the increased risk of SCC in SPKTRs.
Although HLA matching has been reported to be associated with skin cancer in an earlier study (Bouwes Bavinck *et al*, 1991), we were not able to confirm this association in this study. Adjustment for HLA matching did not influence the risk of SCC among SPKTRs; hence, poor HLA matching could not explain the increased risk of SCC in SPKTRs. The risk of BCC in SPKTRs, compared with that in KTRs, however, decreased after adjustment for HLA matching, suggesting that poor HLA matching could partly explain the increased risk of BCC in SPKTRs.

Differences in the number of induction and graft rejection treatments, as well as HLA matching, did not provide a good explanation for the increased risk of SCC in SPKTRs compared with KTRs. However, other differences between the two groups might be responsible for this outcome. Compared with KTRs, in SPKTRs, a second transplanted organ is present. Induction of tolerance is an important goal of clinical organ transplantation (Kean *et al*, 2006; Kawai *et al*, 2008), and may also have undesirable side effects, such as an increased risk of skin cancer. We speculate that transplanted pancreas may induce tolerance against an additional set of allo-peptides in the HLA antigens of the donor. Although we are not aware of any published examples of this mechanism in humans who have received a double set of other organs (for example, heart and lung), a reduced rejection rate of the transplanted heart has been described in rats who received a heart in combination with a lung or spleen (Westra *et al*, 1991). An increased cross-reactive tolerance against SCC-associated antigens in the host could then lead to an increased risk of SCC in SPKTRs, which could potentially affect SCC more severely than BCC, as SCCs are more antigenic cancers than are BCCs (Muchemwa *et al*, 2006). Future studies should point out whether this hypothesis is true.

The overall SCC:BCC ratio in this study was 0.79, which is lower than the ratio of 1.6 in our earlier study (Hartevelt *et al*, 1990). After the introduction of maintenance therapy with MMF instead of Aza, a decreased risk of SCC was observed, while the risk of BCC was not decreased or even possibly increased. Therefore, this change in maintenance therapy may explain, at least partly, the lower SCC:BCC ratio. The length of the follow-up period may form another explanation, as BCCs tend to occur earlier after transplantation than SCCs, but after a latent period, the cumulative incidence of SCC increases more rapidly than that of BCC.

The high collinearity of the immunosuppressive regimen, as well as HLA matching with the type of organ transplanted and the relatively limited numbers of first events, is the most important limitation of this study. The high collinearity could easily result in overfitting in the model so that the association between transplanted organ and skin cancer could disappear. The limited numbers of first events provided insufficient power, limiting the number of reliable stratified analyses.

As the risk of developing skin cancers in transplant recipients is highly increased, excessive exposure to sunlight should be avoided and use of daily sunscreen should be advised. In addition, strict control in an outpatient clinic is important for diagnosing skin cancers at an early stage, facilitating the best treatment and preventing further complications.

Materials and methods

Patients

All 208 patients who received a SPKT at the LUMC between March 1986 and January 2006 were included in this cohort study and were compared with all 1,111 KTRs transplanted in the LUMC during the same time period. The study adhered to the Declaration of Helsinki Principles and the medical ethical committee of the LUMC had approved the study design.

Collection of data

Data recorded for all SPKTRs and KTRs included the country of origin, the dates of the transplantations, age at transplantation, sex, and the dates of death or last follow-up visit. During the first post-transplant years, all patients with functional grafts were seen in the Department of Nephrology, LUMC. Only 88 (6.7%) patients (4 SPKTRs and 84 KTRs) were later followed up in other centers in the Netherlands. In total, 11 (0.8%) patients (1 SPKTR and 10 KTRs) were lost to follow-up, mainly because they moved to another country.

The country of origin was used as a rough estimation of the skin type. Altogether, 1,176 patients originated from the Netherlands or countries with a comparable distribution of skin type. A total of 61 patients originated from Mediterranean countries (1 from France; 2 from Israel; 2 from Iran; 2 from Iraq; 1 from Italy; 20 from Morocco; 1 from Spain; 1 from Tunisia; 26 from Turkey; and 5 from (former) Yugoslavia) and 82 from countries with a dark skin type (29 from Africa; 9 from Indonesia; 5 from other parts of Asia; and 39 from Suriname or Dutch Antilles).
Patients with skin problems were also seen and followed up at the Department of Dermatology, LUMC. Skin biopsies were routinely carried out when skin cancers were suspected. Skin cancer data were collected from the computerized oncological registry of the LUMC, the database from the department of Pathology, and from the national histological database (PALGRA). Follow-up data were collected until June 2007.

Of 1,111 KTRs, 9 recipients (5 with malignant melanoma, 2 with Kaposi's sarcoma, 1 with sweat gland carcinoma and 1 with fibrosarcoma) were present, but no SPKTR who developed skin cancers other than NMSC after transplantation. These skin cancers are not further discussed.

Immunosuppressive regimens and HLA matching

Information about the initial and maintenance immunosuppressive therapy of all patients was obtained from the Eurotransplant database. Type of induction therapy and the number and type of rejection treatments were collected from the flow sheets in the medical charts of the department of nephrology.

For SPKTRs, the initial and maintenance immunosuppressive therapy between 1986 and 1995 consisted of prednisolone (P) (7.5-10 mg/day), Aza (50-100 mg/day) and CsA (200-300mg/day). Between 1996 and 2001, almost all new patients were treated with prednisolone (7.5-10 mg/day), MMF (2,000 mg/day) and CsA (200-300 mg/day). Since 2002 the immunosuppressive treatment of all new patients consisted of prednisolone (7.5-10 mg/day), MMF (1,000-1,500 mg/day) and Tac (6-10 mg/day). In most SPKTRs, maintenance therapy was identical to initial treatment.

For KTRs, immunosuppressive treatment initially consisted of duo therapy with prednisolone and Aza, but shortly after 1986, all new KTRs were immunosuppressed with prednisolone and CsA. After 1996, triple therapy also became the treatment of choice among KTRs, whereby, initially, most new KTRs were treated with prednisolone, MMF, and CsA, and later, most new KTRs were treated with prednisolone, MMF and Tac. The target blood levels for immunosuppressive drugs were the same for the KTR group as for the SPKTR group. Of 1,111 KTRs, in 667 (60%) recipients, maintenance

therapy was identical to initial therapy. Starting in 1996, in 39 patients, CsA was switched to MMF, and in 23 patients, MMF was added to prednisolone and CsA. Details of maintenance immunosuppressive regimens, categorized according to three time periods of transplantation, for all SPKTRs and KTRs are provided in Table 3.

A total of 112 of the 208 SPKTRs received induction therapy to prevent a rejection of the graft by administration of OKT3 (24 patients), ATG (63 patients), daclizumab (23 patients) or basiliximab (2 patients). With the exception of some rare patients, induction treatments with ATG and/or OKT3 were not given to KTRs who were transplanted in the LUMC. Starting in 2000, however, induction treatment with basiliximab became common practice among KTRs.

SPKTRs and KTRs, in whom acute graft rejections were observed, were almost always initially treated with methylprednisolone. When this therapy was not sufficient to prevent further rejection, a second and third rejection treatment with ATG and once more with methylprednisolone, respectively, was given. In exceptional cases, OKT3 was given when a fourth rejection treatment was needed.

To estimate the level of immunosuppression, we categorized the patients into four groups. Triple therapy instead of duo therapy and therapy with ATG or OKT3 as induction or rejection therapy were considered as factors increasing the level of immunosuppression. "Low " level of immunosuppression was defined as duo therapy without induction or rejection therapy with ATG or OKT3; "moderate" level of immunosuppression was defined as (a) triple therapy without induction or rejection therapy or (b) duo therapy with induction or rejection therapy with ATG or OKT3; "high" level of immunosuppression was defined as (a) triple therapy with induction or rejection therapy with ATG or OKT3, or (b) duo therapy with both induction and rejection therapy with ATG or OKT3; and "very high" level of immunosuppression was defined as triple therapy and both induction and rejection therapy with ATG or OKT3.

The degree of HLA mismatching for HLA-A, HLA-B, and HLA-DR antigens was assessed by counting the antigens present in the donor but absent in the recipient.

Statistical analyses

For analyses of SCCs and BCCs together, we used the term NMSC. We used all recipients with SCC (with or without BCC) and all recipients with BCC (with or without SCC) to calculate the cumulative incidence of SCCs and BCCs (Kaplan-Meier analyses). For all other analyses involving SCC and BCC, we used patients with SCCs or BCCs as first event to avoid patients with both SCCs and BCCs being used twice in our analyses. Performing our analyses on all recipients with SCC (with or without BCC) or

on all recipients with BCC (with or without SCC) did not lead to significantly different outcomes.

The initial and maintenance immunosuppressive therapies were categorized into three basic treatment groups: duo or triple therapy with Aza in any combination, duo or triple therapy with MMF in any combination, and duo therapy without Aza or MMF (i.e. a combination of prednisolone with CsA or prednisolone with Tac). If no data were available for the maintenance immunosuppressive therapy, the data of the initial immunosuppressive therapy were used. For all our analyses with immunosuppressive therapy, we used the subcategorization of maintenance therapy because the patients were, generally, exposed to this regimen for the most prolonged period of time.

Because ATG and OKT3 exert by far the highest immunosuppressive effect, induction and rejection treatments were dichotomized into those with and without ATG and/or OKT3. Because the biological effects of ATG and OKT3 are supposed to be similar before and after the transplantation, exposures to ATG and/or OKT3 as induction or rejection treatment were also combined for our analyses.

Differences between patients with and without skin cancer were analyzed by Chi-square (categorical variables) and Student's T-tests (continuous variables). Kaplan-Meier survival analyses were used to estimate the cumulative incidence of skin cancer after transplantation. Cox's proportional hazard analyses were used to calculate hazard ratios for the development of skin cancer and to adjust for potentially confounding factors. As opening dates for both analyses, we used the date of the first transplantation; as closing dates, we used the date of diagnosis of the first SCC or BCC, the date of the patient's death, the date of last follow-up, the date that they were lost to follow-up, or, if the patients were still seen in an outpatient clinic, we used the date of the end of the study (1 June 2007). The patients were not censored from analyses at graft failure. Censoring patients from analyses because of failure of the first graft did not lead to significantly different outcomes. We assessed proportionality of hazards by plotting Schoenfeld residuals for relevant covariates and by introducing interactions of relevant covariates with time in the Cox's proportional hazard model. For all statistical analyses we used SPSS version 14.0.1 (SPSS Inc, Chicago, IL).

Analytic strategy to test for confounding

First, potential risk factors for NMSC, SCC and BCC were identified with Kaplan-Meier analyses stratified for SPKTR and KTR (Supplementary Figure S1) and in multivariable Cox's proportional hazard models (Supplementary Tables S1a and S1b). Subsequently, possible confounding of the association between transplanted organ and skin cancer was tested with Kaplan-Meier analyses stratified for the potential risk factors of interest

(Supplementary Figure S2) and in multivariable Cox's proportional hazard models (Table 4). The Cox's proportional hazard analyses were initially carried out without any adjustment and subsequently with adjustments for age and sex. The hazard ratios adjusted for age and sex were further adjusted for other potentially confounding factors (Table 4). Age and sex, HLA matching and maintenance immunosuppression had the most important modulating effect on the association between transplanted organ and skin cancer, and these factors were, therefore, included in the final model. Maintenance immunosuppression, use of ATG or OKT3, and level of immunosuppression could not be included in the model together because of collinearity and overfitting.

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Supplementary analyses

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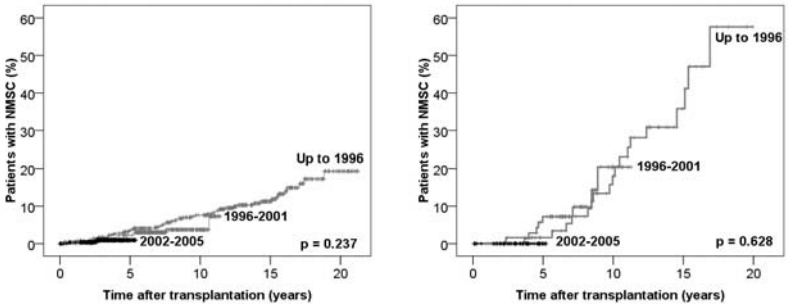
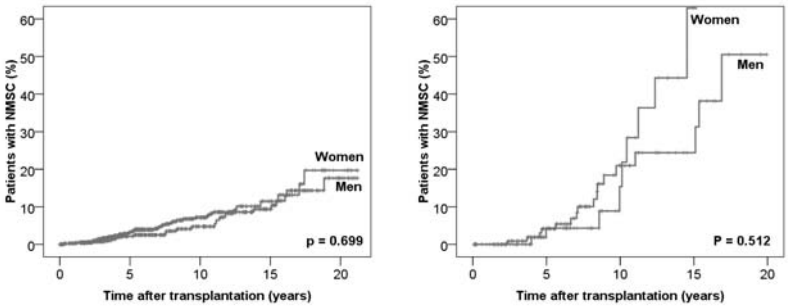
KTR **SPKTR**

A) Proportion of patients with NMSC by time after transplantation categorized by period of transplantation.



B) Proportion of patients with NMSC by time after transplantation categorized by sex.



C) Proportion of patients with NMSC by time after transplantation categorized by ethnicity.

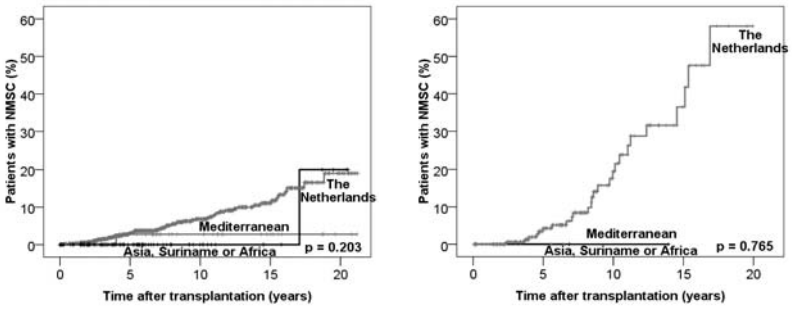
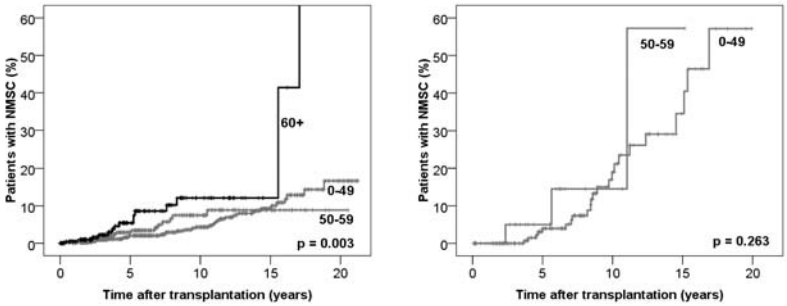


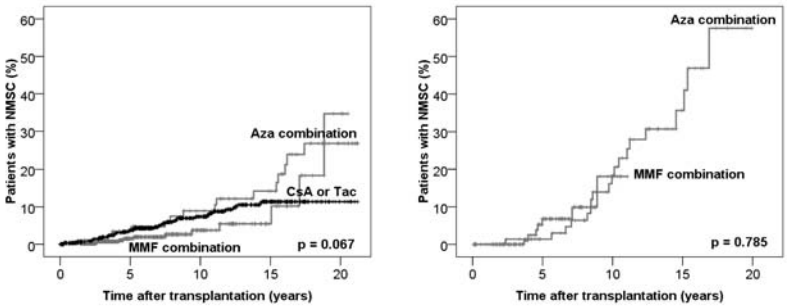
Fig S1 a-c: Risk factors for NMSC in KTR and SPKTR, page 1.

KTR SPKTR

D) Proportion of patients with NMSC by time after transplantation categorized by age.



E) Proportion of patients with NMSC by time after transplantation categorized by maintenance immunosuppressive regimens.



F) Proportion of patients with NMSC by time after transplantation categorized by number of mismatches.

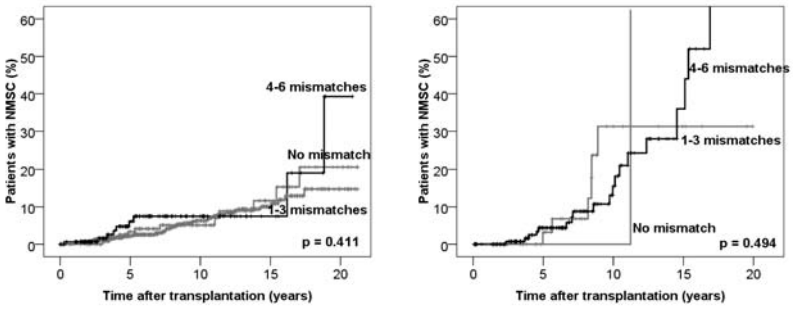
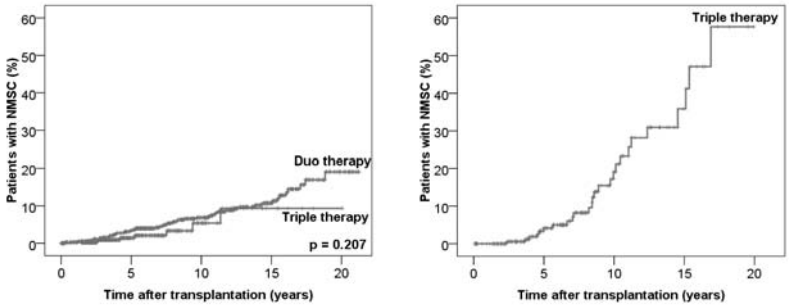


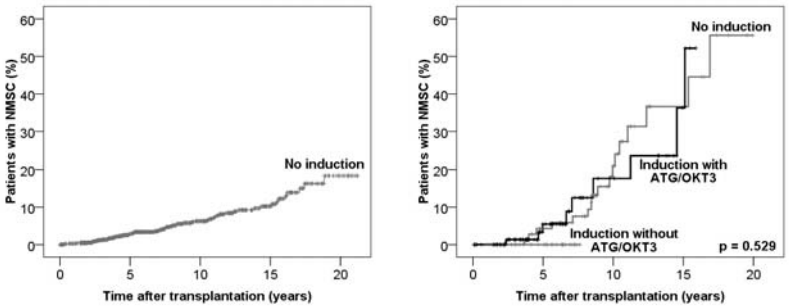
Fig S1 d-f: Risk factors for NMSC in KTR and SPKTR, page 2.

KTR **SPKTR**

G) Proportion of patients with NMSC by time after transplantation categorized by duo or triple therapy. In the SPKTR none of the patients received duo therapy.



H) Proportion of patients with NMSC by time after transplantation categorized by induction therapy. In de KTR none of the patients received induction therapy.



I) Proportion of patients with NMSC by time after transplantation categorized by number of rejections.

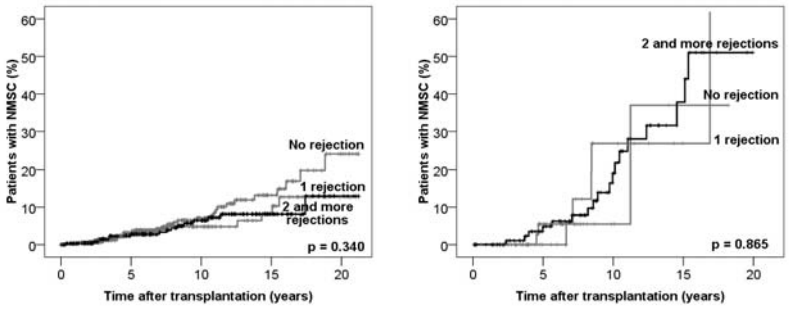
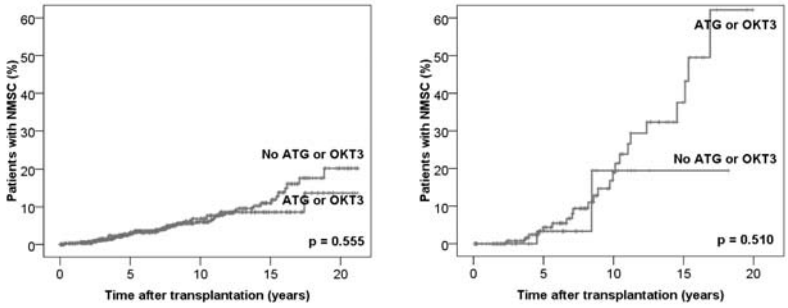


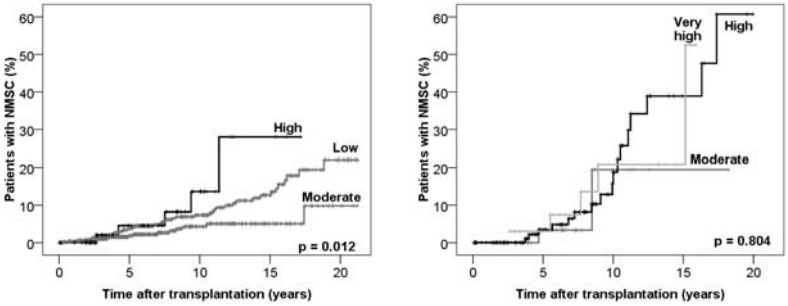
Fig S1 g-i: Risk factors for NMSC in KTR and SPKTR, page 3.

KTR SPKTR

J) Proportion of patients with NMSC by time after transplantation categorized by induction or rejection therapy with or without ATG or OKT3.



K) Proportion of patients with NMSC by time after transplantation categorized by level of immunosuppression*; low, moderate, high, very high. In the KTR none of the patients reached a very high level of immunosuppression. In the SPKTR none of the patients received a low level of immunosuppression.



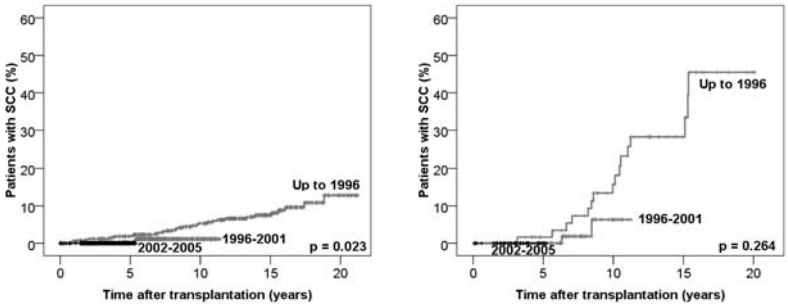
Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, NMSC = non melanoma skin cancer, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

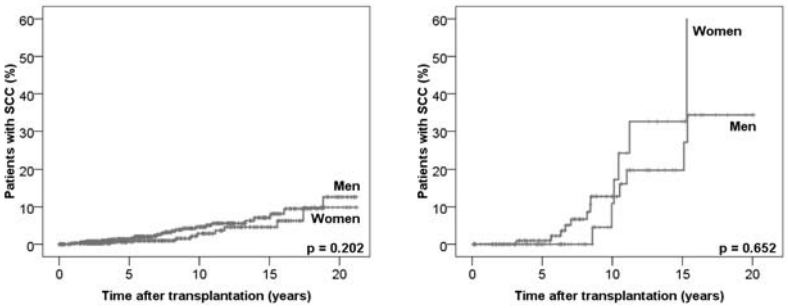
Fig S1 j-k: Risk factors for NMSC in KTR and SPKTR, page 4.

KTR SPKTR

A) Proportion of patients with SCC by time after transplantation categorized by period of transplantation.



B) Proportion of patients with SCC by time after transplantation categorized by sex.



C) Proportion of patients with SCC by time after transplantation categorized by ethnicity.

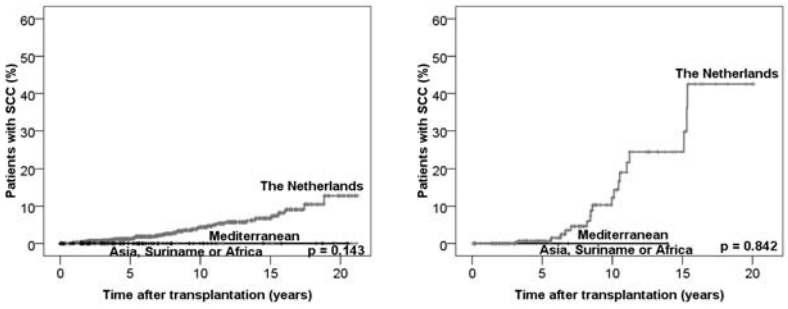
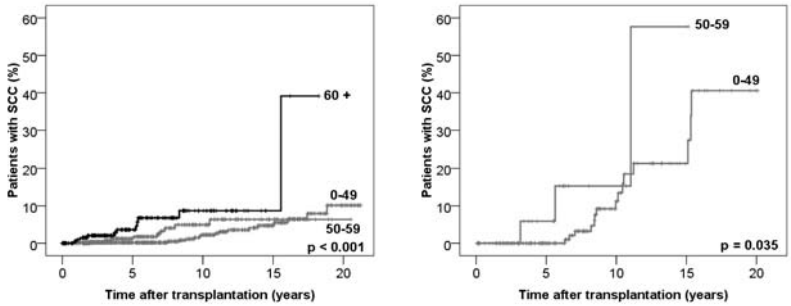


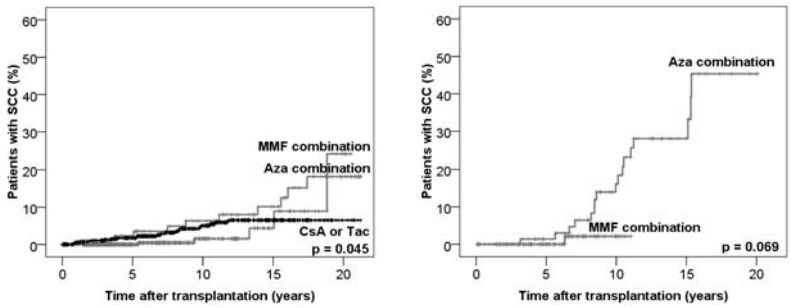
Fig S1 a-c: Risk factors for SCC in KTR and SPKTR, page 5.

KTR **SPKTR**

D) Proportion of patients with SCC by time after transplantation categorized by age.



E) Proportion of patients with SCC by time after transplantation categorized by maintenance immunosuppressive regimens.



F) Proportion of patients with SCC by time after transplantation categorized by number of mismatches.

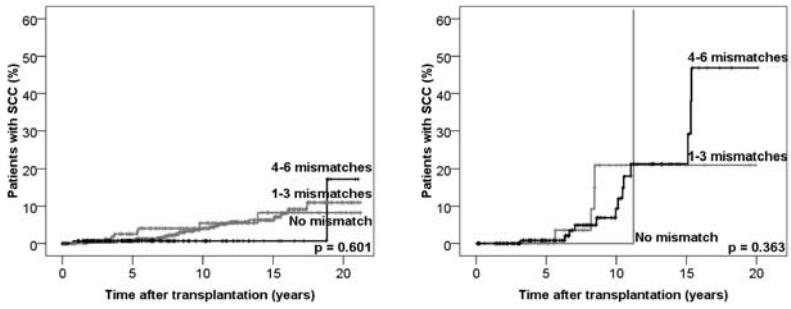
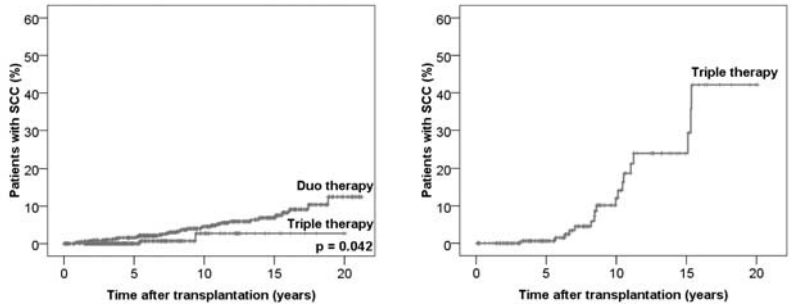


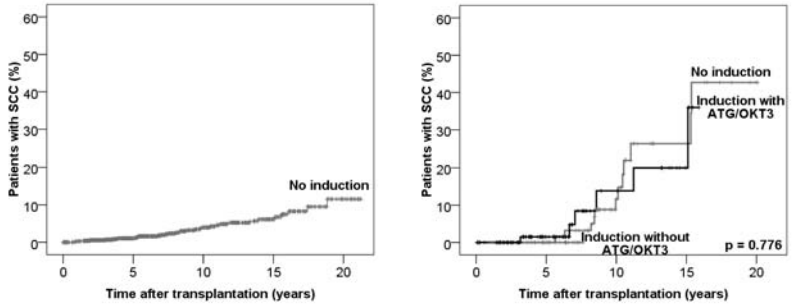
Fig S1 d-f: Risk factors for SCC in KTR and SPKTR, page 6.

KTR **SPKTR**

G) Proportion of patients with SCC by time after transplantation categorized by duo or triple therapy. In the SPKTR none of the patients received duo therapy.



H) Proportion of patients with SCC by time after transplantation categorized by induction therapy. In de KTR none of the patients received induction therapy.



I) Proportion of patients with SCC by time after transplantation categorized by number of rejections.

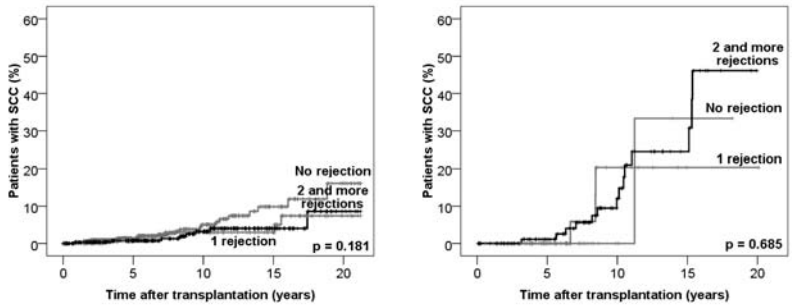
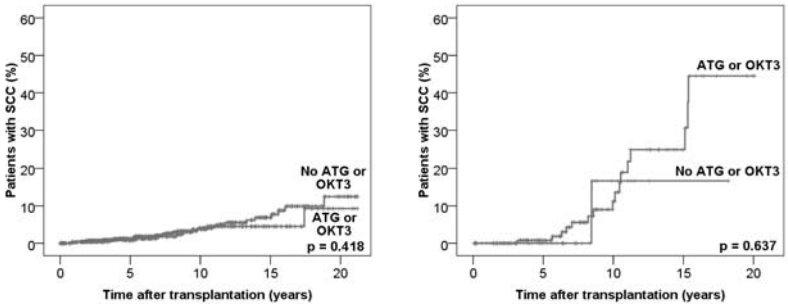


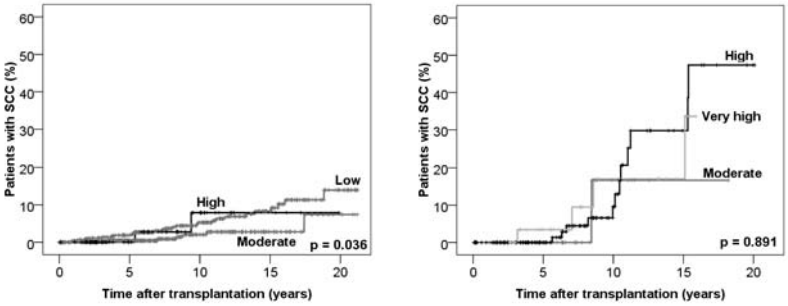
Fig S1 g-i: Risk factors for SCC in KTR and SPKTR, page 7.

KTR SPKTR

J) Proportion of patients with SCC by time after transplantation categorized by induction or rejection therapy with or without ATG or OKT3.



K) Proportion of patients with SCC by time after transplantation categorized by level of immunosuppression*; low, moderate, high, very high. In the KTR none of the patients reached a very high level of immunosuppression. In the SPKTR none of the patients received a low level of immunosuppression.



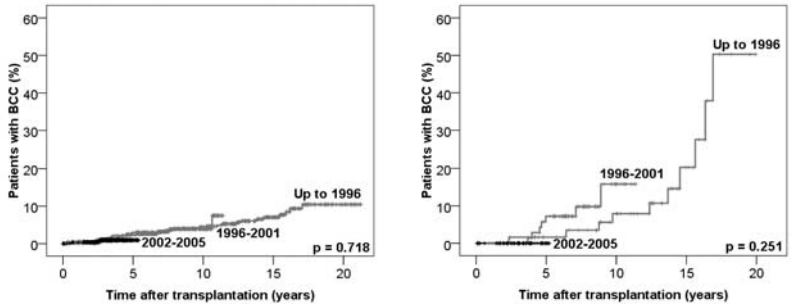
Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, SCC = squamous cell carcinoma, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

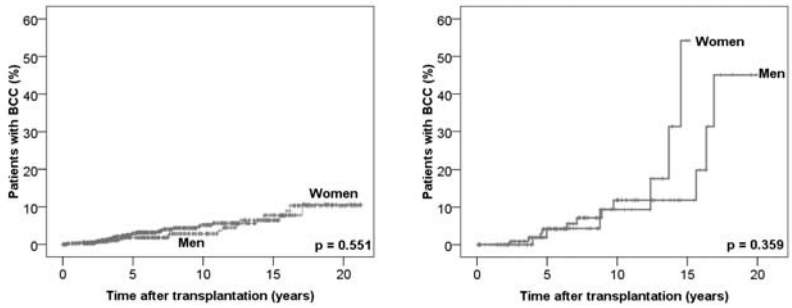
Fig S1 j-k: Risk factors for SCC in KTR and SPKTR, page 8.

KTR SPKTR

A) Proportion of patients with BCC by time after transplantation categorized by period of transplantation.



B) Proportion of patients with BCC by time after transplantation categorized by sex.



C) Proportion of patients with BCC by time after transplantation categorized by ethnicity.

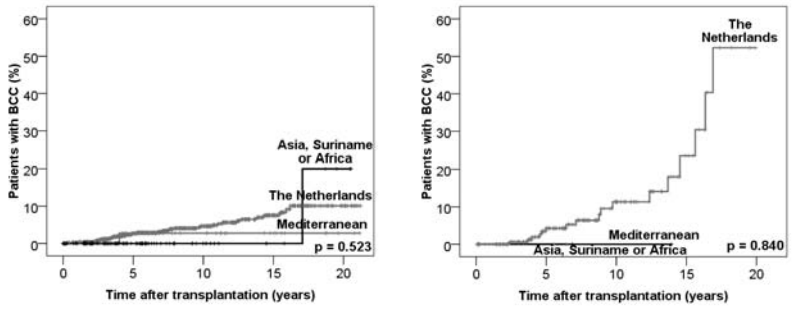
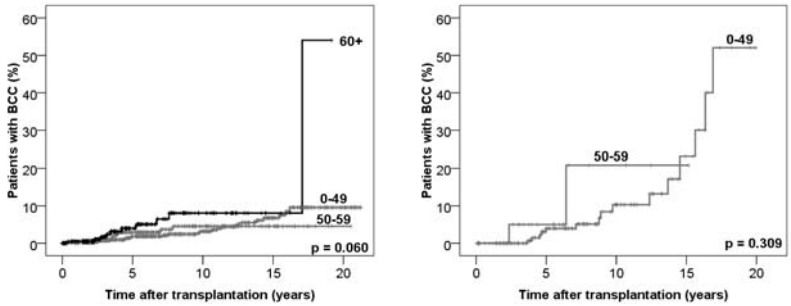


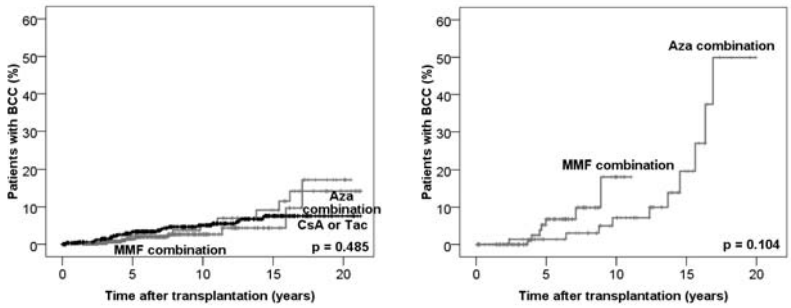
Fig S1 a-c: Risk factors for BCC in KTR and SPKTR, page 9.

KTR **SPKTR**

D) Proportion of patients with BCC by time after transplantation categorized by age.



E) Proportion of patients with BCC by time after transplantation categorized by maintenance immunosuppressive regimens.



F) Proportion of patients with BCC by time after transplantation categorized by number of mismatches.

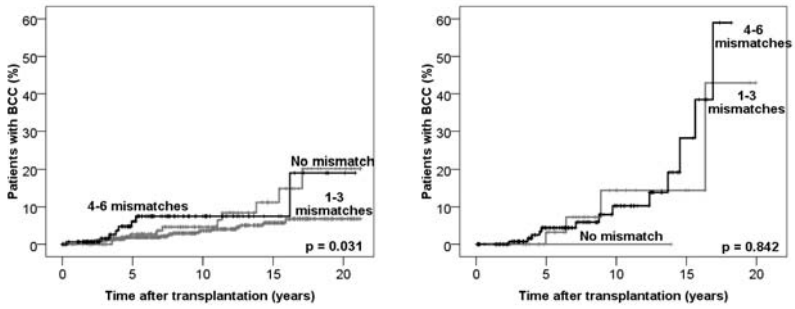
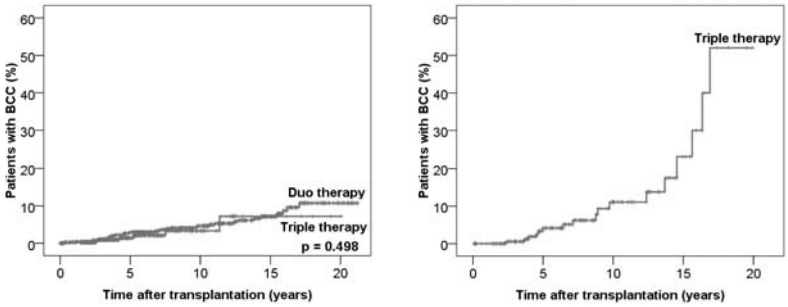


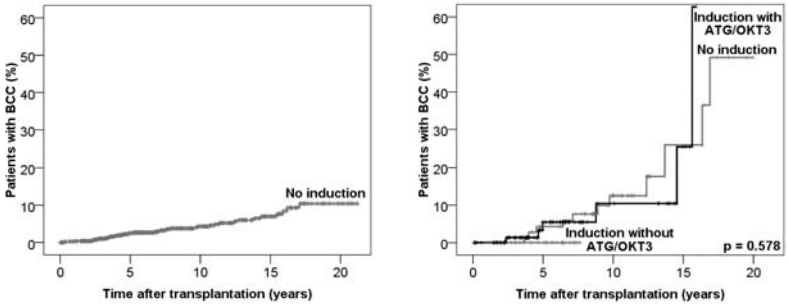
Fig S1 d-f: Risk factors for BCC in KTR and SPKTR, page 10.

KTR **SPKTR**

G) Proportion of patients with BCC by time after transplantation categorized by duo or triple therapy. In the SPKTR none of the patients received duo therapy.



H) Proportion of patients with BCC by time after transplantation categorized by induction therapy. In de KTR none of the patients received induction therapy.



I) Proportion of patients with BCC by time after transplantation categorized by number of rejections.

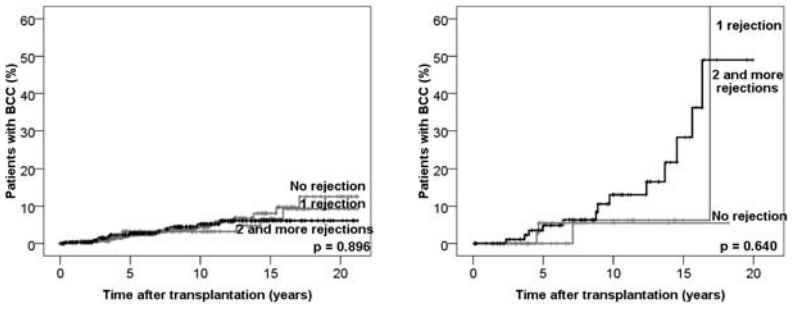
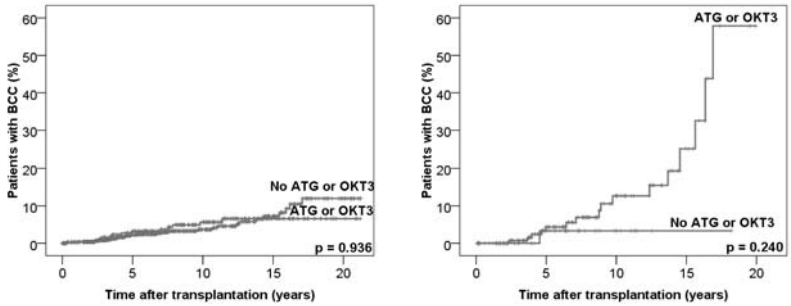


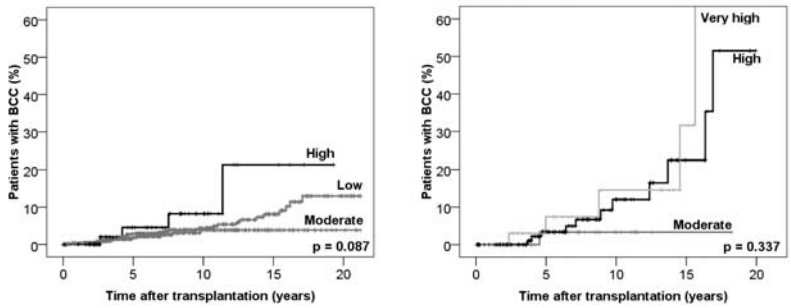
Fig S1 g-i: Risk factors for BCC in KTR and SPKTR, page 11.

KTR SPKTR

J) Proportion of patients with BCC by time after transplantation categorized by induction or rejection therapy with or without ATG or OKT3.



K) Proportion of patients with BCC by time after transplantation categorized by level of immunosuppression*; low, moderate, high, very high. In the KTR none of the patients reached a very high level of immunosuppression. In the SPKTR none of the patients received a low level of immunosuppression.

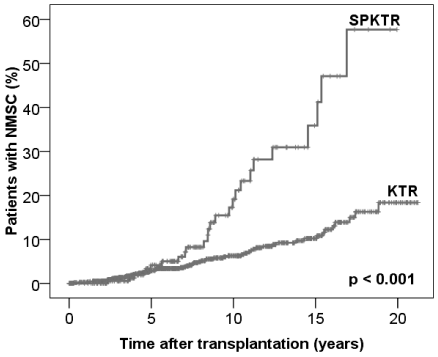


Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, BCC = basal cell carcinoma, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

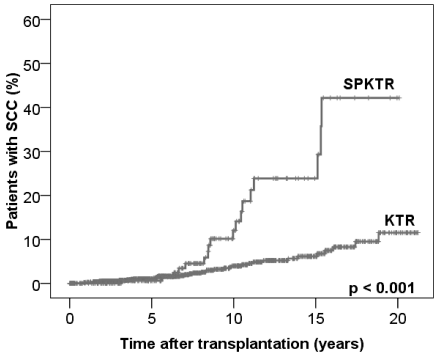
Fig S1 j-k: Risk factors for BCC in KTR and SPKTR, page 12.

Non Melanocytic Skin Cancer



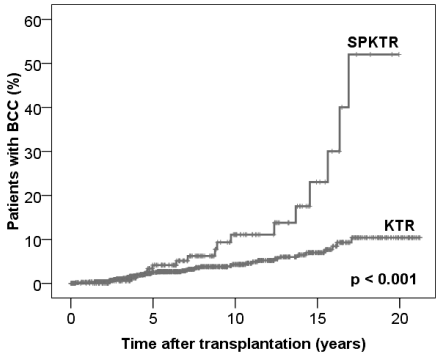
Yrs after transplantation	5	10	15
KTR	3%	6%	10%
SPKTR	4%	19%	36%

Squamous Cell Carcinoma



Yrs after transplantation	5	10	15
KTR	1%	4%	6%
SPKTR	1%	13%	25%

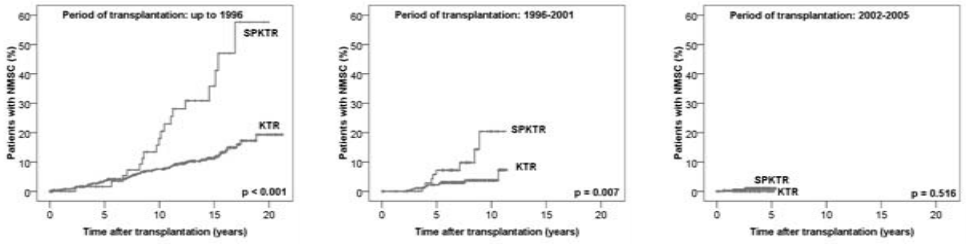
Basal Cell Carcinoma



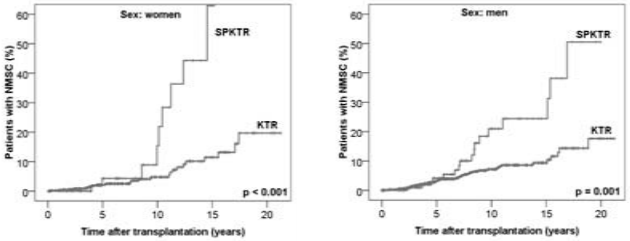
Yrs after transplantation	5	10	15
KTR	3%	4%	7%
SPKTR	4%	12%	25%

Fig S2: Risk of NMSC, SCC and BCC in SPKTR compared to KTR unstratified, page 0.

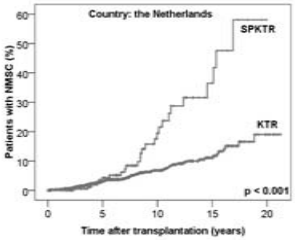
A) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by period of transplantation.



B) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by women and men.



C) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by country of origin.



For the Mediterranean and Asia, Suriname or Africa group no Kaplan-Meier curve could be calculated.

Fig S2 a-c: Risk of NMSC in SPKTR compared to KTR stratified for different factors, page 1.

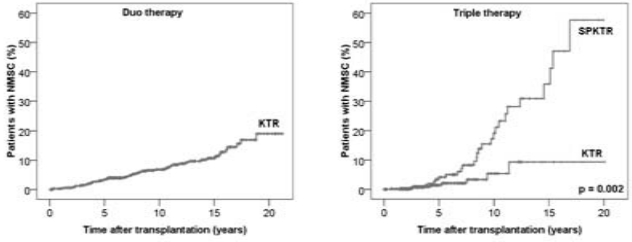
D) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by age period.

E) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by number of mismatches.

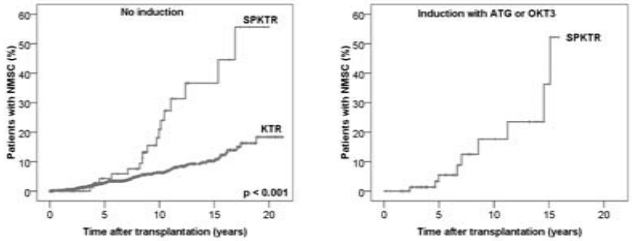
F) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by immunosuppressive therapy.

Fig S2 d-f: Risk of NMSC in SPKTR compared to KTR stratified for different factors, page 2.

G) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by duo or triple maintenance immunosuppressive therapy.



H) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by induction therapy.



For the group induction without ATG or OKT3, i.e. induction with methylprednisolone, daclizumab, basiliximab, no Kaplan-Meier curve could be calculated.

I) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by number of rejections.

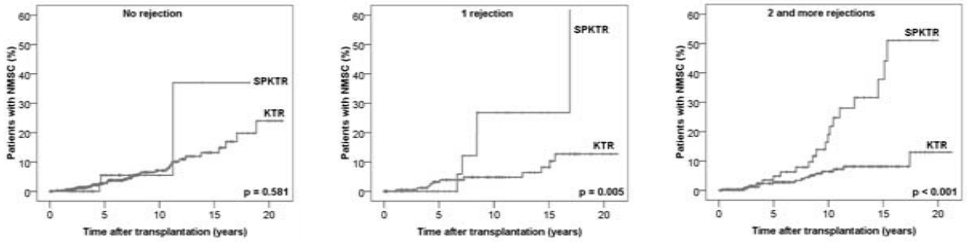


Fig S2 g-i: Risk of NMSC in SPKTR compared to KTR stratified for different factors, page 3.

J) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by induction or rejection therapy with or without OKT3 or ATG.

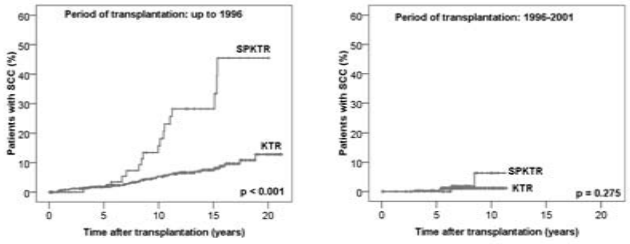
K) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by level of immunosuppression*.

Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, NMSC = non melanoma skin cancer, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

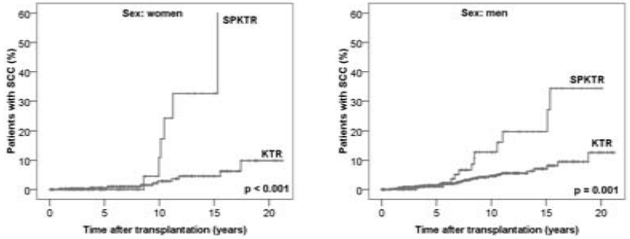
Fig S2 J-k: Risk of NMSC in SPKTR compared to KTR stratified for different factors, page 4.

A) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by period of transplantation.

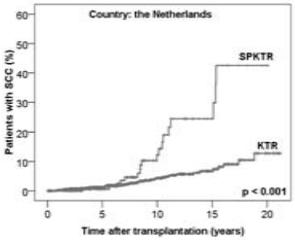


For the period of transplantation between 2002-2005 no Kaplan-Meier curve could be calculated.

B) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by women and men.



C) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by country of origin.



For the Mediterranean and Asia, Suriname or Africa group no Kaplan-Meier curve could be calculated.

Fig S2 a-c: Risk of SCC in SPKTR compared to KTR stratified for different factors, page 5.

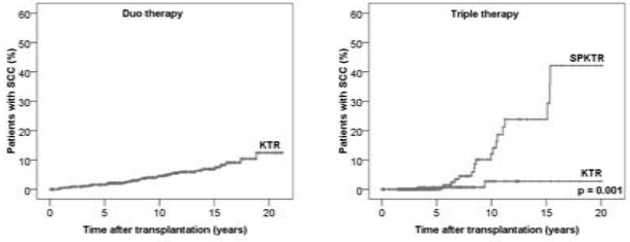
D) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by age period.

E) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by number of mismatches.

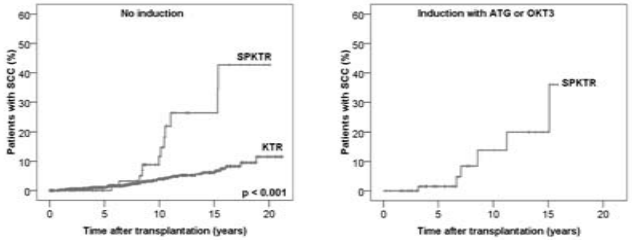
F) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by immunosuppressive therapy.

Fig S2 d-f: Risk of SCC in SPKTR compared to KTR stratified for different factors, page 6.

G) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by duo or triple maintenance immunosuppressive therapy.



H) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by induction therapy.



For the group induction without ATG or OKT3, i.e. induction with methylprednisolone, daclizumab, basiliximab, no Kaplan-Meier curve could be calculated.

I) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by number of rejections.

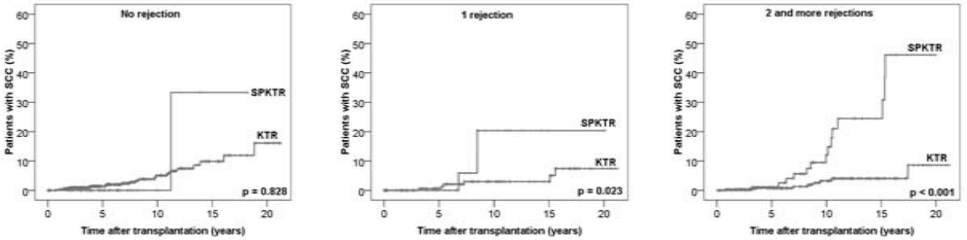


Fig S2 g-i: Risk of SCC in SPKTR compared to KTR stratified for different factors, page 7.

J) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by induction or rejection therapy with or without OKT3 or ATG.

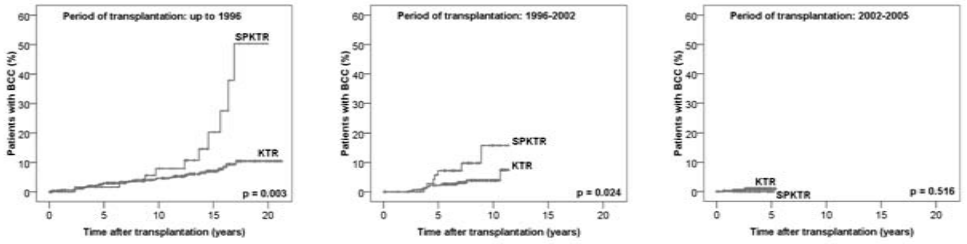
K) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by level of immunosuppression*.

Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, SCC = squamous cell carcinoma, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

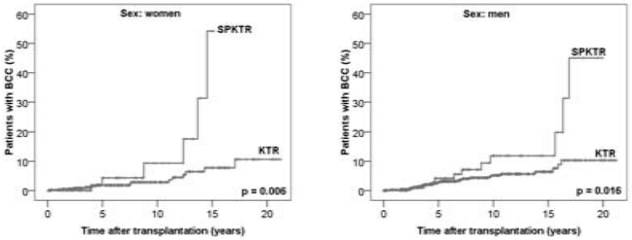
*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

Fig S2 J-k: Risk of SCC in SPKTR compared to KTR stratified for different factors, page 8.

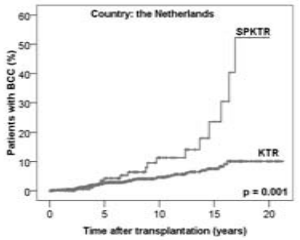
A) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by period of transplantation.



B) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by women and men.



C) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by country of origin.



For the Mediterranean and Asia, Suriname or Africa group no Kaplan-Meier curve could be calculated.

Fig S2 a-c: Risk of BCC in SPKTR compared to KTR stratified for different factors, page 9.

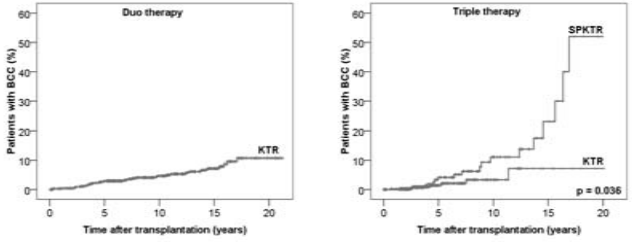
D) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by age period.

E) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by number of mismatches.

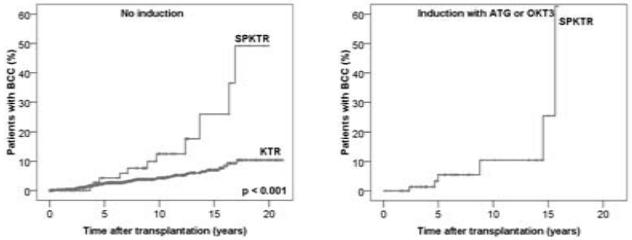
F) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by immunosuppressive therapy.

Fig S2 d-f: Risk of BCC in SPKTR compared to KTR stratified for different factors, page 10.

G) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by duo or triple maintenance immunosuppressive therapy.



H) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by induction therapy.



For the group induction without ATG or OKT3, i.e. induction with methylprednisolone, daclizumab, basiliximab, no Kaplan-Meier curve could be calculated.

I) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by number of rejections.

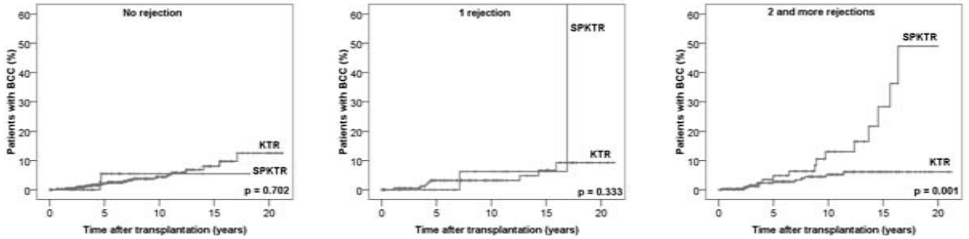
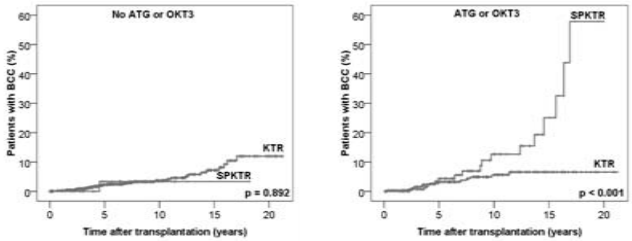
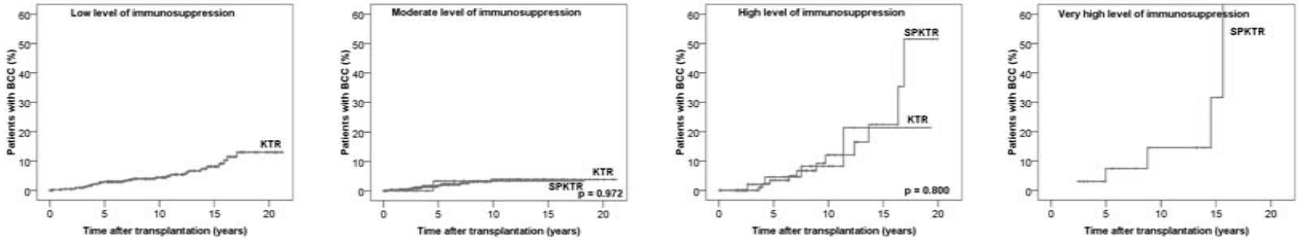


Fig S2 g-i: Risk of BCC in SPKTR compared to KTR stratified for different factors, page 11.

J) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by induction or rejection therapy with or without OKT3 or ATG.



K) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by level of immunosuppression*.



Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, BCC = basal cell carcinoma, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

Fig S2 j-k: Risk of BCC in SPKTR compared to KTR stratified for different factors, page 12.

Table S1a Risk factors of skin cancer in kidney transplant recipients adjusted for age and sex using Cox proportional hazard analysis.

Risk factors	Non melanocytic skin cancer	Squamous-cell carcinoma as first event	Basal-cell carcinoma as first event
Sex			
Women	1	1	1
Men	1.2 (0.69;1.9)	1.5 (0.67;35)	0.94 (0.48;1.8)
Age			
Up to 50	1	1	1
50 – 60	1.3 (0.69;2.4)	2.2 (0.90;5.6)	0.80 (0.32;2.0)
60 and older	2.9 (1.5;5.5)	4.6 (1.7;12.5)	2.2 (0.03;5.0)
Country of origin			
Netherlands	1	1	1
Mediterranean	0.36 (0.05;2.6)	No events	0.59 (0.08;4.3)
Suriname, Africa, Asia	0.35 (0.05;2.6)	No events	0.55 (0.07;4.0)
HLA mismatching			
0	1	1	1
1-3	0.92 (0.48;1.7)	1.5 (0.51;4.3)	0.63 (0.28;1.4)
4-6	1.5 (0.63;3.6)	0.56 (0.06;5.0)	1.9 (0.70;5.1)
ATG or OKT3 as induction or rejection treatment			
No	1	1	1
Yes	0.92 (0.53;1.6)	0.84 (0.35;2.0)	0.97 (0.46;2.0)
Type of maintenance immunosuppression*			
Aza in any combination	1	1	1
MMF in any combination	0.35 (0.16;0.77)	0.15 (0.04;0.59)	0.57 (0.19;1.7)
CsA or Tac	0.53 (0.28;0.99)	0.35 (0.15;0.84)	0.71 (0.28;1.8)
Level of immunosuppression			
Low	1	1	1
Moderate	0.47 (0.26;0.86)	0.42 (0.17;1.0)	0.50 (0.23;1.1)
High or very high	1.8 (0.72;4.7)	0.95 (0.13;7.3)	2.5 (0.83;7.3)

*Aza: azathioprine; MMF: mycophenolate mofetil; CsA: cyclosporine; Tac: tacrolimus.

Table S1b Risk factors of skin cancer in simultaneous pancreas kidney transplant recipients adjusted for age and sex using Cox proportional hazard analysis.

Adjustments	Non melanocytic skin cancer	Squamous-cell carcinoma as first event	Basal-cell carcinoma as first event
Sex			
Women	1	1	1
Men	0.75 (0.33;1.7)	0.64 (0.21;2.0)	0.84 (0.24;2.9)
Age at transplantation			
Up to 50	1	1	1
50 – 59	1.9 (0.56;6.5)	2.5 (0.53;11.6)	1.2 (0.15;9.8)
60 and older	No patients	No patients	No patients
Country of origin			
Netherlands	1	1	1
Mediterranean	No events	No events	No events
Suriname, Africa, Asia	No events	No events	No events
HLA mismatching			
0-3	1	1	1
4-6	0.90 (0.38;2.2)	0.65 (0.21;2.0)	1.8 (0.38;8.3)
ATG or OKT3 as induction or rejection treatment			
No	1	1	1
Yes	1.6 (0.46;5.3)	1.3 (0.27;5.8)	2.6 (0.33;20.6)
Type of maintenance immunosuppression			
Aza in any combination	1	1	1
MMF in any combination	1.1 (0.42;3.1)	No events	4.2 (0.80;22.1)
CsA or Tac	No SPKTR in this group	No SPKTR in this group	No SPKTR in this group
Level of immunosuppression			
Low or moderate	1	1	1
High	0.69 (0.16;2.9)	0.98 (0.15;6.3)	0.35 (0.03;3.6)
Very high	1.1 (0.42;2.9)	1.3 (0.34;5.1)	0.89 (0.22;3.7)

*Aza: azathioprine; MMF: mycophenolate mofetil; CsA: cyclosporine; Tac: tacrolimus.