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ORIGINAL ARTICLE

Impact of cyclosporine versus tacrolimus on the incidence of *de novo* malignancy following liver transplantation: a single center experience with 609 patients

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calcineurin inhibitor, cancer development, immunosuppression, liver transplantation, long-term survival, multivariate analysis, risk factors.

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Summary

De novo malignancies are a major cause of late death after liver transplantation. Aim of the present study was to determine whether use of cyclosporine versus tacrolimus affects long-term tumor incidence considering potential confounders. *De novo* malignancies in 609 liver transplant recipients at Munich Transplant Centre between 1985 and 2007 were registered. In 1996, the standard immunosuppressive regimen was changed from cyclosporine to tacrolimus. Different effects of those drugs on long-term tumor incidence were analyzed in multivariate analysis. During 3765 patient years of follow-up (median 4.78 years), 87 *de novo* malignancies occurred in 71 patients (mean age 47.5 ± 13.3 years, mean time after liver transplantation 5.7 ± 3.7 years). The cumulative incidence of *de novo* malignancies was 34.7% for all tumor entities after 15 years as compared to 8.9% for a nontransplanted population. The most frequent tumors observed were nonmelanoma skin cancers (44.83%). Moreover, post-transplant lymphoid disease, oropharyngeal cancer ($n = 6$, 6.9%), upper gastrointestinal tract cancer ($n = 4$, 4.6%), lung cancer ($n = 4$, 4.6%), gynecological malignancies ($n = 4$, 4.6%), and kidney cancer ($n = 3$, 3.45%) were detected. Multivariate analysis revealed recipient age [hazards ratio (HR) 1.06], male gender (HR 1.73), and tacrolimus-based immunosuppression (HR 2.06) as significant risk factors. Based on those results, a tacrolimus-based immunosuppression should be discussed especially in older male patients. Whether reducing tacrolimus target levels may reduce the risk for de novo malignancies has yet to be determined in prospective trials.

Introduction

Improving long-term results in transplant medicine has been among the major medical accomplishments of the past decades. Experience in surgery, perioperative management, and the introduction of better immunosuppressive agents and regimens in liver transplantation has improved results and led to decreased complication rates and enhanced long-term organ survival. In addition, the optimization of postoperative intensive care including the use of vasoactive drugs and mechanical ventilation continuously improved long-term graft function [1–3]. Moreover, recipient-related risk factors, for instance the underlying cause of liver failure and recipient's age and general state of health have been considered critically. In all, these positive developments have resulted in current overall 10-year survival rates of approximately 60% following liver transplantation [4–6].

In light of this long-term survival, malignant tumors have become a significant cause of late mortality in transplant patients [7–9]. Organ transplant recipients have an increased risk of cancer compared to an age- and sex-matched population, with a tumor incidence between 2% and 16% [10–12]. Although nonmelanotic skin cancers

with good prognoses remain the most common, other *de novo* malignancies significantly contribute to late mortality in transplant patients [12,13]. A three- to fourfold increased risk for developing cancer in general and an up to 500-fold higher incidence of certain cancers have been described in transplant recipients [14]. Tumor incidence in transplant patients depends on length of follow-up and on the time period in which the transplantation was performed [13,15]. In addition, recipient age and smoking history have been reported as potential risk factors for the development of cancer [9,16].

With respect to immunosuppressive medication, studies in kidney transplant recipients show a lower incidence of tumor development in patients receiving cyclosporine compared with tacrolimus [8]. In liver transplant patients, the influence of different types of calcineurin inhibitors on *de novo* malignancies remains controversial [17,18].

Clinical trials have been recently initiated to evaluate calcineurin inhibitor-free immunosuppressive regimens using m-TOR inhibitors [19,20], which may be associated with a decreased risk of cancer development. Nevertheless, calcineurin inhibitors remain the gold standard for immunosuppression in liver transplant patients. In 1996, our institution's routine immunosuppressive regimen was altered, to replace the calcineurin inhibitor cyclosporine with tacrolimus. The aim of the present study was to determine whether this change in immunosuppression affects the incidence of *de novo* malignancies after liver transplantation. Furthermore, other potential risk factors for tumor development were evaluated.

Material and methods

Study design

In this retrospective study, 609 patients were analyzed with respect to the development of *de novo* malignancy after successful liver transplantation. The data were obtained from the prospectively conducted liver transplant database and included all patients who underwent liver transplantation between 1985 and 2007 at the Department of Surgery, Campus Grosshadern, Ludwig-Maximilians University, Munich. The observation period ended in December 31, 2009 or at the time of patient death. All new malignancies diagnosed after liver transplantation were captured for the study. Already existing tumors, their metastases or recurrences, i.e. hepatocellular carcinoma (HCC) were registered, but not counted as *de novo* neoplasia. Multifocal manifestations (especially in nonmelanotic skin cancers) or metastasis of a post-transplant *de novo* malignancy were counted as one tumor. Two different *de novo* malignancies in one patient were counted as two tumor entities.

Structured detection of *de novo* malignancies

The following steps were taken to detect *de novo* malignancies during the observation period:

1. All liver transplant recipients underwent routine follow-up care including at least one visit at our hospital's outpatient clinic per year. During these visits, all forms of newly diagnosed cancer were registered prospectively in the database.
2. Between 2006 and 2008, individualized screening for the detection of *de novo* malignancies was performed in 228 patients of 371 living patients. This included abdominal ultrasound, dermatological examination, and the systemic tumor markers CEA, CA 19-9, CA 72-4, AFP, SCC, NSE, ProGRP, CA 15-3 und CA 125 in women and prostate-specific antigen in men. Telephone interviews including a detailed questionnaire on the development of malignancies were conducted with patients who did not present for screening, or with their family physician. A total of 91.1% of patients could be adequately interviewed. To what extend this screening program influences the detection rate of *de novo* malignancies has to be evaluated in further studies.
3. All transplanted patients were matched with the Bavarian Regional Tumor Registry (Munich Cancer Registry, Tumor Centre Augsburg and Regensburg). According to Bavarian Cancer Registry Law, all pathologists, hospitals and physicians are obliged to report all cases of carcinoma to this tumor registry. Similar to all solid malignancies, nonmelanoma skin cancers once diagnosed by pathologist or clinicians have to be reported to the Bavarian tumor registry.

Immunosuppressive regimens

Standard immunosuppression following liver transplantation was divided into two periods: immunosuppression from 1985 to 1996 was based on the calcineurine inhibitor cyclosporine (target trough serum level: 100–150 ng/ml). In 1996, cyclosporine was replaced by tacrolimus, with a target trough serum level of 8–10 ng/ml; these target levels were maintained throughout the whole investigation time.

Data analysis

Patients with a newly diagnosed malignancy after transplantation were analyzed for pre-existing malignancies, etiology of underlying liver disease, and documented alcohol abuse.

Statistics

The results are expressed as mean ± SD when not indicated otherwise. Chi-square test was used for raw

estimations of related variables on tumor incidence after transplantation and patient survival. The cumulative tumor risk among liver transplant patients was identified for solid and hematological tumors, as well as for nonmelanotic skin cancer. The Kaplan–Meier method was performed to estimate the probability for cumulative tumor incidence, and comparison between subgroups was calculated using the Breslow test (Generalized Wilcoxon). To determine the influence of different variables on the relative risk for tumor development, a Cox proportional hazard model was conducted for confounders with $P < 0.2$ in univariate analysis. $P < 0.05$ was considered statistically significant. All calculations were performed with SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

To assess the relative tumor risk, our population was compared with a standard, age- and sex-matched population from the greater Munich area (data obtained from the Munich Cancer Registry: http://trm.web.med.uni-muenchen.de). Age match was achieved by clustering the population into 5-year age periods. Within those clusters, the standardized morbidity ratio was established. The matching procedure was performed gender specifically.

Results

Patient characteristics

A total of 609 patients with a mean age of 47.5 ± 13.3 years at primary liver transplantation were analyzed in this study. At the time of analysis, the male population was 1.7-fold larger than the number of female patients included. Average patient age was slightly higher in the male population, but this was without statistical significance. These 609 patients underwent 727 liver transplantations between 1985 and 2007, including 87 re-transplantations. In 14 patients, three liver transplantations were performed and one patient received four transplantations. The median follow-up was 4.78 (0–22.5) years with 3765 patient-years in total. Indications for liver transplantation were mainly end stage liver diseases caused by viral hepatitis (23.5%) and nutritive toxic liver damage (19.4%) or primary tumors of the liver (20%) i.e. HCC or cholangiocarcinoma. Demographic and clinical characteristics are summarized in Table 1, divided for patients with and without tumor (Table 1A) or for the era of orthotopic liver transplantation (OLT; Table 1B).

The survival rates for the entire group of 609 patients are illustrated in Fig. 1a. As patients developing *de novo* malignancies are a selected collective who survived at least the first 6 months, a direct comparison with all patients is not reasonable. About 22% of our patients after OLT died within 6 months postoperatively. Therefore, these patients have been excluded for survival analysis. Censored survival curves for patients with and without *de novo* malignancies are displayed in Fig. 1b.

Incidence of *de novo* malignancies

During the observation period, 87 cases of *de novo* malignancy after liver transplantation were diagnosed in 71 patients. Cancer was diagnosed an average of 5.7 ± 3.7 years after the first transplantation, and at a mean age of 59.2 ± 9.6 years. The incidence of cancer was significantly higher in male compared to female transplant recipients (13.4% vs. 8.8%, $P = 0.010$). Recipients' age at transplantation or the time to primary diagnosis of cancer did not differ with respect to gender.

In comparison with a sex- and age-adjusted population (data from the Munich Cancer Registry), a significantly higher cumulative tumor incidence was observed in liver transplanted individuals [years after transplantation]: 10.0% vs. 2.1% [5], 26.4% vs. 5% [10] and 34.7% vs. 8.9% [15] ($P < 0.05$) (Fig. 2). The risk for tumor development continuously increased over the observation period.

Nonmelanotic skin cancer, i.e. squamous cell carcinoma ($n = 15$; 17.2%) or basal cell carcinoma ($n = 24$; 27.6%) were the most frequent *de novo* malignancies, followed by post-transplant lymphoid disease (PTLD), melanotic melanoma, tumors of the otolaryngological tract, and tumors of the prostate (each $n = 6$; 6.9%). Moreover, cancer of the upper gastrointestinal tract (GI) ($n = 4$; 4.6%), lung cancer ($n = 4$; 4.6%), gynecological malignancies ($n = 4$; 4.6%), and kidney cancer ($n = 3$; 3.45%) were diagnosed, as well as myeloproliferative disorder ($n = 2$; 2.3%), and single cases of sarcoma, colorectal, breast, central nervous system cancer, and cancer of unknown primary (each $n = 1$; 1.15%) (Fig. 3a).

The onset of *de novo* malignancy after liver transplantation differed with respect to tumor type (years after transplantation ± SD): malignant melanoma (2.5 ± 1.0), PTLD (4.2 ± 2.5), and tumors of the upper GI tract (3.9 ± 4.1) developed early after liver transplantation followed by nonmelanotic skin cancers [first diagnosis of squamous (6.2 ± 3.4) or basal cell carcinoma (5.1 ± 3.3)], head and neck (6.8 ± 1.9), and prostate cancers (6.8 ± 2.6). Gynecological tumors, (7.2 ± 4.3), lung (7.7 ± 3.5) and especially kidney cancer (11.2 ± 3.0), and myeloma (14.6 ± 2.7) occurred with a later onset after liver transplantation (Fig. 3b).

Potential risk factors for *de novo* malignancies

Univariate analysis – risk factors for de novo malignancies

The incidence of malignant diseases was significantly higher in male compared to female transplant recipients: 12.3% vs. 5.4% 5 years and 32.3% vs. 17.4% 10 years after

Table 1. Patient characteristics and univariate analysis.

		A		B	
	All patients	Patients with tumor	Patients without tumor	OLT 1985–1995	OLT 1996–2007
Number of patients	609	71	538	359	250
Gender (female/male)	227/382	20/51	207/331	118/241	109/141
Age at transplantation	47.5 ± 13.3	53.2 ± 9.9	46.6 ± 13.6	47.45 ± 12.2	47.56 ± 14.0
Primary diagnosis for LTx					
Virus hepatitis (C/B/other)	143 (92/39/12)	23 (18/4/1)	120 (74/35/11)	96 (69/23/4)	47 (23/16/8)
Tumors in the liver (HCC/CCC/metastasis)	122 (98/15/9)	12 (10/2/0)	110 (88/13/9)	59 (50/1/8)	63 (49/13/1)
Alcohol/nutritic toxic	118	15	103	82	36
Acute/cryptogen liver failure	68	8	60	33	35
PBC	38	7	31	16	22
Acquired defects	37	3	34	21	16
PSC	31	1	30	24	7
Genetic defect	30	1	29	16	14
Autoimmune hepatitis	22	1	21	12	10
Leading immunosuppression: cyclosporine A/tacrolimus	359/250	39/32	320/218	359	250
Observation time (median)	4.78	9.91	3.57	9.05	3.67
Age at diagnosis of tumor		59.19 ± 9.6	59.2 ± 11.2	59.2 ± 8.0	
Potential risk factors					
Alcohol abuse	161	22	139	103	58
Hepatitis B	68	6	62	33	35
Hepatitis C	122	21	101	87	35
Pre-existing tumor of the liver (HCC/CCC)	139 (122/17)	16 (14/2)	123 (108/15)	77 (74/3)	62 (48/14)
Pre-existing extrahepatic cancer	23	3	20	8	15

OLT, orthotopic liver transplantation; HCC, hepatocellular carcinoma; CCC, cholangiocellular carcinoma; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

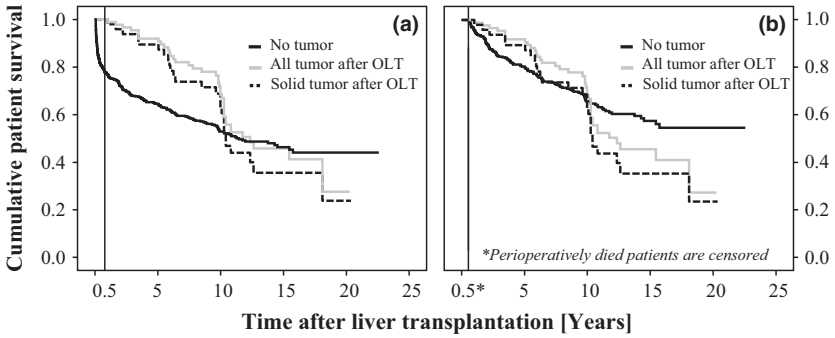

Figure 1 Cumulative patient survival after liver transplantation for patients without *de novo* tumor development (black line), all patients with *de novo* tumors (gray line) and the subgroup of solid tumors (dotted line). (a) All patients; (b) excluding patients, who died within 6 months postoperatively. OLT, orthotopic liver transplantation.

transplantation, $P = 0.01$ (Fig. 4a). The time interval between liver transplantation and primary tumor diagnosis did not show significant gender differences.

Pre-existing malignancy, i.e. HCC, was associated with an increased rate of *de novo* tumors (8.9% vs. 13.3% 5 years and 23.5% vs. 41.3% 10 years after liver transplantation, $P = 0.02$) (Fig. 4b). Within the group of patients transplanted because of benign liver diseases (i.e. nutritive toxic, viral hepatitis, etc.), there was no significant difference in the incidence of *de novo* malignancy according to the type of liver disease before transplantation.

The incidence of *de novo* malignancies was significantly higher in the 359 patients transplanted in the era from 1996 to 2007 and who were treated with a tacrolimus-based immunosuppression compared to 250 patients, transplanted between 1985 and 1996 and treated with cyclosporine (12.4% vs. 6.2% 5 years and 36.3% vs. 18% 10 years after liver transplantation, $P < 0.01$) (Fig. 4c). As the

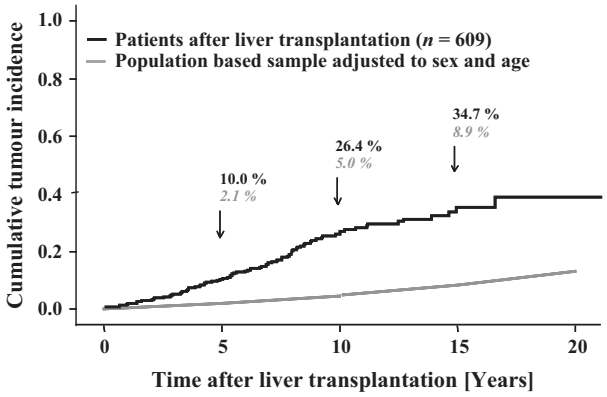

Figure 2 Cumulative tumor incidence in patients after liver transplantation compared to a population-based sample adjusted to sex and age.

number of patients is unequally distributed between the study periods, more patients are at risk for developing *de novo* malignancies transplanted after 1996. Nonetheless, the longer observation period for patients transplanted before 1996 will in part balance this disparity.

Recipient age was a significant risk factor for patients older than 34 years compared to younger recipients ($P < 0.05$). For patients older than 51 years, a further increase in the incidence of *de novo* malignancies was evident although this difference was not significant ($P = 0.051$) (Fig. 4d).

Multivariate analysis

Potential confounders with a $P < 0.20$ in the univariate analysis were included in the multivariate model (gender, recipient age, immunosuppressive regimen, pre-existing malignancy, alcohol abuse, and viral hepatitis C). Male gender ($P = 0.046$), recipient age at liver transplantation, ($P < 0.001$) and tacrolimus versus cyclosporine-based immunosuppression ($P = 0.003$) were identified as independent risk factors for the development of malignant disease in the covariate-adjusted model. In contrast, pre-existing tumors of the liver, alcohol abuse as well as a hepatitis C infection could not be shown to be independent prognostic factors after liver transplantation in multivariate analysis (Table 2).

Discussion

De novo malignancies represent a serious complication in the late postoperative period after liver transplantation. The elevated risk and incidence of *de novo* malignancy after solid organ transplantation have an immense clinical impact and account for 10–47% of late mortality depending on the duration of the post-transplant follow-up [9,16,21]. In liver recipients an incidence of cancer of 2–16% has been published, much higher than that in healthy age-matched patients [10,13,22]. Recently, Watt *et al.* published a malignancy rate of 22% within 12 years after liver transplantation [16].

The present study demonstrated a cumulative incidence of *de novo* neoplasms of 10% within 5 years, 26.4% at 10 years and 34.7% at 15 years after liver transplantation. The higher incidence of *de novo* malignancies compared to previous studies may reflect the systematic and reliable cancer documentation by law in Bavaria. Therefore, not only

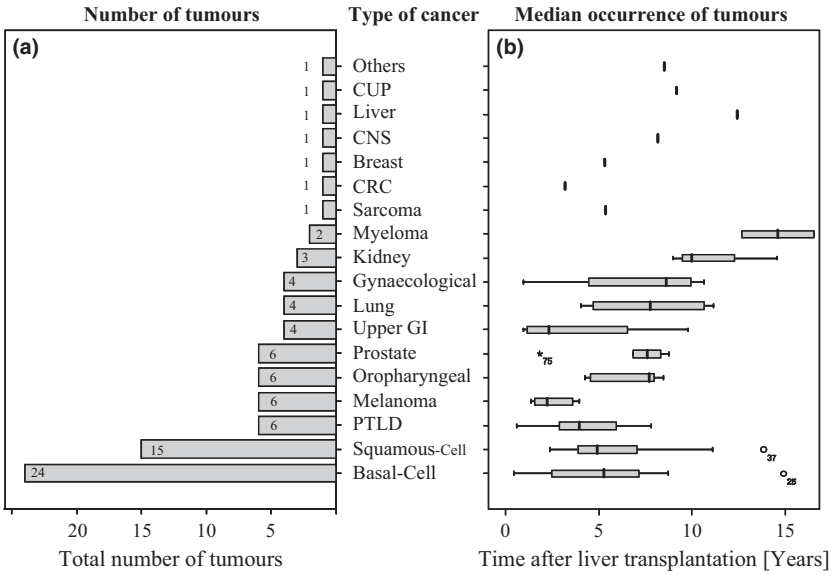

Figure 3 Total numbers (a) and median time period until occurrence in years (b) after liver transplantation sorted by different types of cancer. CUP, cancer of unknown primary; CNS, central nervous system; CRC: colorectal cancer; GI, gastrointestinal tract; PTLD, post-transplant lymphoproliferative disorder; skin tumors, melanoma, squamous cell carcinoma, and basal-cell carcinoma.

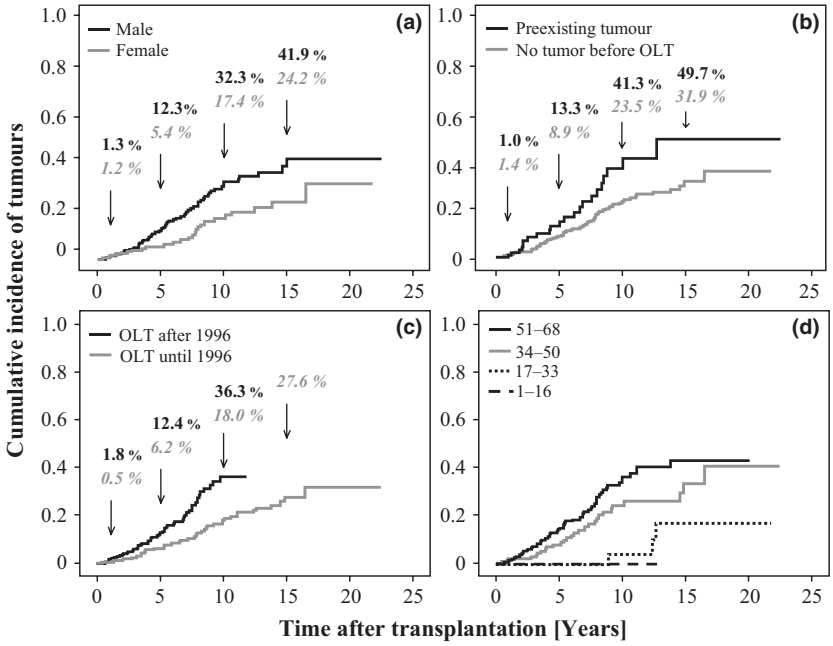

Figure 4 Cumulative tumor incidence after liver transplantation (years). Cancer incidence in males versus females (a) in patients with history of malignant disease versus patients without history of malignant disease (b) in patients transplanted prior to and after 1996 (c) with respect to recipients' age (years) 1 ≤ 16, 17 ≤ 33, 34 ≤ 50, 51 ≤ 68 (d).

Table 2. Multivariate analysis.

	P-value	Hazards ratio	Confidence interval
Age at liver transplantation (per year)	0.001	1.06	1.04–1.09
Gender (male versus female)	0.046	0.59	0.35–0.99
Time period of immunosuppressive regimen	0.003	0.48	0.26–0.78
Tumor of the liver prior to transplantation	0.293	1.31	0.79–2.17
Alcohol abuse	0.615	0.87	0.53–1.46
Hepatitis C	0.906	1.03	0.61–1.74

patients followed up at our center were included. The comprehensiveness of the tumor documentation in this large study sets it apart from many of the albeit larger transplant registry studies in which the potentially limited detail and completeness of the data may result in an underestimation of tumor incidence [15]. The current study demonstrated that tumor incidence increases continuously over time following liver transplantation. This emphasizes the importance of extended follow-up periods of more than 10 years to adequately document the risk of *de novo* malignancy after solid organ transplantation. As other previous studies reported lower tumor incidence rates, one would speculate that malignancies have been lost to follow-up limiting data value. Moreover, the number of patients included as well as the long median observation period represent unique features of this manuscript.

Cases of *de novo* malignancy have been shown to cluster around certain tumor entities. Previous studies have described up to 10-fold increases in the incidence rate for skin cancer and threefold higher rates of lymphoma in transplanted patients compared to a nontransplanted population [8,10,14,23]. The present data also show increased risk for cancers of the head and neck, urogenital tract, and kidney in transplanted patients. Both previously published data and the current study show that the risk of other more common malignancies, such as prostate and breast cancer, does not seem to be increased in liver transplant recipients [9,24].

In agreement with previous studies [10,12,25], male gender and recipient age were associated with a significantly increased risk for *de novo* malignancies after liver transplantation. Recipient age over 34 years was identified as cutoff for increased *de novo* malignancy rates in the present study. A further increase was evident above the age of 51 years although this finding was not statistically significant. A similar finding was described by Collett *et al.*, who demonstrate an increased incidence of *de novo* malignancies in 37 617 recipients of solid organs in males and recipients older than 50 years [15].

In the present data, the detection of a HCC in the recipients' explanted liver was an additional risk factor for *de novo* malignancies following transplantation in univariate analysis. Similarly, univariate analyses by Xiol *et al.*

suggested an increased risk of both nonskin and skin cancer in patients transplanted for HCC compared with patients transplanted for other reasons, however, the corresponding multivariate analysis confirmed only the increased risk of skin cancers [12]. A genetic predisposition for both hepatocellular and skin tumors may be present. Alternatively, both tumors may share common risk factors, for example, a history of smoking or alcohol abuse. In our patients, alcohol-induced liver cirrhosis was not associated with an increased incidence of *de novo* malignancies after liver transplantation. In contrast, a huge retrospective cohort study identified alcohol and nicotine as potential risk factors for increased lung and oropharyngeal tumor development following transplantation [11,13]. Although more patients were included in the manuscript of Benlloch *et al.*, the total tumor incidence rate was lower compared to the present study (5.3% vs. 14.3%) which may account for the observed differences. Moreover, increasing the number of patients with alcohol-induced liver cirrhosis may identify this genesis of cirrhosis as a risk factor of *de novo* malignancies also in our patient collective.

Our findings and previous data suggest that a recipient age above 50 years, male gender, and pre-existing malignancies should be considered when designing standards for tumor surveillance in liver transplant patients. As skin tumors and cancers of the head and neck are associated with modifiable behaviors such as unprotected sun exposure and smoking, patient education should be advocated. Sufficient surveillance programs should start early after liver transplantation and continue life long.

The results of the present study indicate that immunosuppression with the calcineurin inhibitor tacrolimus is associated with an increased rate of *de novo* malignancies following liver transplantation compared to cyclosporine, with a hazards risk ratio of 2.06 in multivariate analysis. The data were based on the initial immunosuppression at the time of discharge, and may fail to capture the exact history of a patient's immunosuppressive treatment over time. Furthermore, the different time intervals of liver transplantation (prior to and after 1996) may represent a systematic bias in this study. Nevertheless, previous studies in kidney transplantation were in accordance with the present findings [8]. In contrast to the present trial, a meta-analysis of 4102 kidney transplant recipients comparing initial immunosuppression with tacrolimus to cyclosporine failed to demonstrate a difference in the incidence of malignancies [26]. In contrast, a registry-based study including 35 765 patients demonstrated a decreased incidence of *de novo* malignancies with tacrolimus compared to cyclosporine (hazards risk ratio 0.94 vs. 1.01, respectively) [27]; however, this study included a post-transplant observation period of only 3 years, which may account for the different results. Tjon *et al.* also reported increased *de novo* malignancies with cyclosporine-based immunosuppression after liver transplantation compared to tacrolimus [17]. The significantly higher target trough levels for cyclosporine as well as the inclusion of an induction therapy have to be a considered as a limitation for direct comparisons with our data. Changes in the monitoring of cyclosporine blood levels may significantly affect *de novo* malignancy rates [17], and lower target trough levels of calcineurin inhibitors have been shown to reduce the incidence of *de novo* malignancies [28].

The mechanisms by which calcineurine inhibitors promote cancer development and growth remain poorly understood. The inhibition of the immune system and therefore reduction in its ability to react against cancer cells and their associated antigens [29] is one likely mechanism. Information on an indirect correlation between the rejection rate and tumor incidence may support this notion. There is also evidence that calcineurin inhibitors may directly promote the aggressive and invasive nature of cancer cells by depressing antiviral immune activity, supporting DNA-damage caused by immunosuppressive substances or up-regulating cytokines such as TGF-β, IL-10, or VEGF-2 [30]. Further experimental studies are needed to elucidate the mechanisms underlying tumor development in immunosuppressed patients. Alternate regimens, for instance the use of mTOR inhibitors such as sirolimus (SRL) as immunosuppressants, may help reduce tumor incidence in transplant recipients [31,32]. Whether m-TOR-inhibitors avoid the development of malignancies after liver transplantation is addressed by a multicentre-prospective trial in patients transplanted for HCC (SiLVER-Study) [19].

In summary, the present study identifies male gender, recipient age, and pre-existing tumors as risk factors for *de novo* malignancies in liver transplant patients. Moreover, transplantation with a tacrolimus-based immunosuppression was associated with a significantly increased *de novo* malignancy rate compared to cyclosporine. Whether reducing tacrolimus target levels or the substitution of mTOR inhibitors for immunosuppression in male recipients older than 50 years with HCC will decrease the rate of *de novo* malignancies in those patients at risk has yet to be determined in prospective trials.

Authorship

CDW: wrote the article, collected, and analyzed data. MKA: designed research/study, performed research/study. BS: collected data. SP: wrote the article. MR: analyzed data. MG: wrote the article. K-WJ: designed research/study. CB: analyzed data. CG: designed research/study, collected data.

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