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Immunosuppressive Therapy and Malignancy in Organ Transplant Recipients

A Systematic Review

Alex Gutierrez-Dalmau and *Josep M. Campistol*

Department of Nephrology and Renal Transplantation, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, Barcelona, Spain

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Abstract

Post-transplant malignancy is recognised as being a major limitation to the success of solid organ transplantation and it is currently considered one of the unavoidable costs of long-term immunosuppressive therapy. However, the continual introduction of new immunosuppressive drugs and the growing knowledge about their different oncogenic profiles, requires a continuous evaluation of the available evidence on this topic.

The incidence and risk of malignancy is elevated in solid organ transplant recipients compared with the general population. As proof of the relationship between immunosuppressive therapy and post-transplant malignancy, epidemiological data reveal that the length of exposure to immunosuppressive therapy and the intensity of therapy are clearly related to the post-transplant risk of malignancy, and that once cancer has developed, more intense immunosuppression can translate into more aggressive tumour progression in terms of accelerated growth and metastasis and lower patient survival. The association between malignancy and immunosuppressive therapy is mediated through several pathogenic factors. Indirectly, immunosuppressive drugs greatly increase the post-transplant risk of malignancy by impairing cancer surveillance and facilitating the action of oncogenic viruses. However, the direct pro- and anti-oncogenic actions of immunosuppressants also play an important role. The cancer-promoting effect of calcineurin inhibitors, independently of depressed immunosurveillance, has been demonstrated in recent years, and currently only mammalian target of rapamycin (mTOR) inhibitors have shown simultaneous immunosuppressive and antitumour properties. Reports of the initial results of the reduced incidence of cancer in organ transplant recipients receiving mTOR inhibitor therapy strongly indicate separate pathways for pharmacological immunosuppression and oncogenesis. The role of mTOR inhibitors has been firmly established for the treatment of post-transplant Kaposi's sarcoma and its role in the management of patients with other post-transplant malignancies should be clarified as soon as possible.

Prevention of morbidity and mortality resulting from post-transplant malignancy should become a main endpoint in solid organ transplant programmes, and the choice and management of immunosuppressive therapy in each phase of transplantation plays a central role in this objective. Although comprehensive and rigorous information about the management of immunosuppressive therapy in transplant recipients at risk of or affected by cancer is still lacking, new experimental and clinical data about mTOR inhibitors offers novel approaches to this problem

1. Malignancy in Organ Transplant Recipients: The Dark Side of Success

Clinical organ transplantation is currently considered the best, and maybe the only, therapeutic option in most types of end-stage organ failure. An important part of this success is attributable to the growing number of immunosuppressive drugs that have been developed and introduced into the clinical scenario. Largely because of their use, loss of organs

as a result of acute, irreversible rejection is now uncommon and 1-year graft survival rates of 80–90% are the norm for all types of organ transplantation.[1] The dark side of this scenario is the cost of long-term treatment with immunosuppressive drugs in terms of impaired immune function and the direct secondary effects of exposure to these drugs. One aspect of this cost is often fatal malignant disease.

At the end of 2005, approximately 160 000 people in the US were living with functioning solid organ allografts, up from 62 000 in 1993.[2] Similar progress has been reported in Europe, Australia and many Asiatic countries.[3] As long-term survival with functioning allografts increases, more patients will be at risk of developing malignancies. Post-transplant cancer is thus a growing concern in the scientific community and continuous evaluation of the available evidence on this topic is essential.

This article aims to provide a systematic review all the available information on the issue of immunosuppressive therapy and malignancy in organ transplant recipients. We searched MEDLINE (1966 to January 2007) by combining different lists of words grouped by different topics: (i) 'cancer', 'neoplasm' or 'malignancy'; (ii) 'transplant' or 'transplantation'; (iii) 'renal', 'kidney', 'heart', 'cardiac', 'liver', 'gut', 'pancreas' or 'lung'; and (iv) 'lymphoglobulin', 'thymoglobulin', 'OKT3', 'daclizumab', 'basiliximab', 'corticosteroids', 'azathioprine', 'mycophenolate', 'calcineurin inhibitors', 'cyclosporine', 'FK506', 'tacrolimus', 'rapamycin', 'sirolimus', 'RAD001' or 'everolimus'. The search results were restricted to the presence of these words in the title or abstract of the articles. The search was not restricted to studies published in English. The articles retrieved were selected for review if they contained information relevant to the issues discussed in this review:

- epidemiology of malignancy in organ transplant recipients;
- epidemiology of malignancy in non-transplanted, pharmacologically immunosuppressed patients;
- malignancy-related morbidity and mortality in organ transplant recipients;
- risk factors for malignancy in organ transplant recipients;
- pathogenic factors for malignancy in organ transplant recipients;
- experimental and clinical data on the pro- and anti-oncogenic effects of different immunosuppressive drugs.

2. An Old but Controversial Story: The Epidemiological Relationship Between Immunosuppressive Therapy and Malignancy

2.1 Malignancy in Organ Transplant Recipients

The concept of malignancy as a complication of organ transplantation emerged early in the history of this therapeutic modality,[4-8] and one of the major contributors to the discovery of this association was Israel Penn (1930–99), through what is currently known as the 'Israel Penn International Transplant Tumour Registry' (IPITTR) [http://www.ipittr.uc.edu/Home.cfm]. This registry is the largest database devoted to post-transplant malignancy in the world.[9] Although the IPITTR contains huge amounts of valuable information, it does not include information on person-years of follow-up, and therefore cannot provide incidence rates of malignancy or estimates of the relative risklative risk of developing tumours.

Large registries are the best source of information about post-transplant malignancy incidence rates; however, with few exceptions,[10-13] studies performed using registries are subject to several limitations (table I): (i) contribution to the registry is often voluntary;[14,15] (ii) the database does not include information on the entire population of organ

Table I. Characteristics of the main transplant registry-based studies published in recent years

Study (year)	Data source	Geographic origin	General population database	Date of transplant	Organ	No. of pts	No. of cancers	Patient-years	Other characteristics of the study
Moloney et al.[10] (2006)	National renal transplant database of Ireland	Ireland	Irish national cancer registry	1994–2001	Kidney	1 558	702	NR	
Robson et al.[20] (2005)	OPTN/UNOS	US	NR	1996–8	Kidney	8 246	322	NR	Observational cohort study
	CTS	Europe and Canada	NR	1996–8	Kidney	5 256	250	NR	First-time renal transplant pts
Chapman and Webster[21] (2004)	ANZDATA	Australia and New Zealand	NCSCH	1980–2003	Kidney	13 077	1545[a]	110 395	Only records the first diagnosis of SCC and BCC
Kasiske et al.[19] (2004)	USRDS	US	USCS	1995–2001	Kidney	35 765	NR	NR	First kidney transplants; Medicare enrollees; 3y of follow-up
Bustami et al.[22] (2004)	SRTR	US	Comparison with general population not performed	1996–2002	Kidney	41 686	797	NR	Pts receiving first kidney transplants with >6mo of follow-up; cases of solid tumours diagnosed in the first 6mo post-transplant were excluded
Adami et al.[17] (2003)	Swedish inpatient register	Sweden	Swedish cancer registry	1970–97	Kidney, liver, lung, heart, pancreas	5 931	692	40 360	Pts with history of cancer and cancer cases in the first 30d post-transplant were excluded. Only records first cancer cases
Kyllonen et al.[12] (2000)	Finland transplant registry	Finland	Finish cancer registry	1967–97	Kidney	2 844	230	20 817	
Birkeland et al.[11] (2000)	Danish registry on regular dialysis and transplantation	Denmark	Danish cancer registry	NR–1995	Kidney	1 821	209	13 734	
Hoshida et al.[23] (1997)	Multicentre study	Japan	Osaka cancer registry	1970–95	Kidney	2 744	46	12 982	
Birkeland et al.[13] (1995)	Nordic registry	European Nordic countries	National cancer registries	1964–82	Kidney	5 692	471	32 392	
Brunner et al.[24] (1995)	EDTA-ERA registry	UK, Germany, Italy, Sweden	Cancer registries of England and Wales, Sweden, Germany, Democratic Republic, and northern Italy	NR	Kidney	NR	NR	NR	Average annual cancer incidence 1985–9

a Excluding non-melanoma skin cancer.

ANZDATA = Australian and New Zealand Data Registry; **BCC** = basal cell carcinoma; **CTS** = Collaborative Transplant Study; **EDTA-ERA** = European Dialysis and Transplant Association-European Renal Association; **NCSCH** = National Cancer Statistics Clearing House; **NR** = not reported; **OPTN** = Organ Procurement and Transplantation Network; **pts** = patients; **SCC** = squamous cell carcinoma; **SRTR** = Scientific Registry of Transplant Recipients; **UNOS** = United Network for Organ Sharing; **USCS** = US Cancer Statistics; **USRDS** = US Renal Data System.

transplant recipients in a geographical area;[15,16] (iii) some types of tumour, such as non-melanoma skin cancers (NMSC),[16] are excluded; and (iv) only first-cancer cases are included,[14,17,18] or the periods of follow-up are short.[19,20] On the other hand, single-centre studies are generally retrospective and therefore the true frequency of post-transplant malignancy may be underestimated. Furthermore, in some types of rare tumour, a single new case can result in enormous variations in tumour rates. Finally, clinical trials rarely include a sufficient number of patients to detect significant differences in patient survival or malignancy, and generally do not provide extended follow-up.

Another way to explore post-transplant malignancy is to estimate the cancer risk in organ transplant recipients compared with that in the general population. In this case, the most appropriate parameter is the standardised incidence ratio (SIR), defined as the ratio of the observed number of tumours to the expected number of tumours in transplant recipients compared with age- and sex-matched controls in the same geographical area. Unfortunately, few countries with national cancer registries are able to compare cancer rates accurately between a subgroup, such as organ transplant recipients, and the national rate (see table I).

As a result of these limitations, the large number of studies published on malignancy in organ transplant recipients show wide variability in rates (figure 1 and figure 2). To facilitate interpretation of these studies, the data discussed in this section of the article are limited to articles that analyse malignancy rates mainly in renal transplant recipients: the differences between the distinct types of organ transplant are discussed in section 3.

Some of the differences observed in malignancy incidence rates may also be because of different follow-up times, since the duration of immunosuppression is considered to be one of the most important factors in the increase in the incidence of malig-

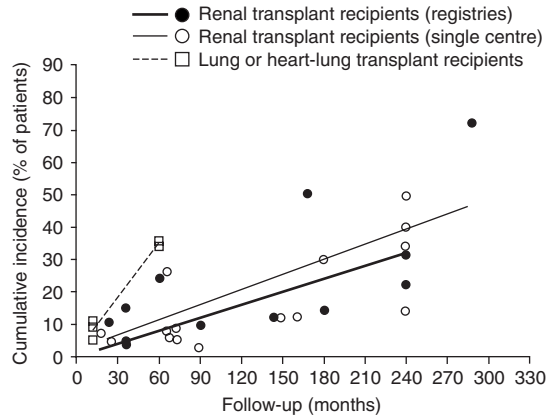

Fig. 1. Incidence of cancer in organ transplant recipients reported by different registries and single-centre studies. Data from renal transplant recipients (registries[10,11,17-20,25-29] and single centres[18,23,30-43]) and lung or heart-lung transplant recipients.[43,44]

nancies in organ transplant recipients.[47-50] As early as 3 years post-transplant, the cumulative risk of cancer in renal transplant recipients has been shown to be 3.9–14.9%, increasing to 3.9–39% at 10 years, 13.9–50% at 20 years and reaching 80% at 30 years after transplantation (figure 1).[10,11,17-20,23,25-42] A recent study by Kasiske et al.[19] aimed to avoid underestimating the incidence by using a different strategy. To detect the occurrence of cancers after kidney transplantation, these authors linked data from the US Renal Data System (USRDS) to Medicare billing claims. The accuracy of the data from the Medicare billing claim relies on physicians, hospitals and clinics to bill for services related to the diagnosis and treatment of cancer. The 3-year cumulative incidence was 14.9%, the highest published to date. This rate is in agreement with a rate of approximately 13% in a study with the same duration of follow-up performed using the Australian and New Zealand Data Registry (ANZDATA),[51] in which quality control for accuracy and completeness has recently been published.[14] In contrast, this incidence was much higher than rates obtained in previous analyses of the Collaborative Transplant Registry (CTS) or the Organ Procurement and Transplantation Network/United Network for Organ Sharing (OPTN/

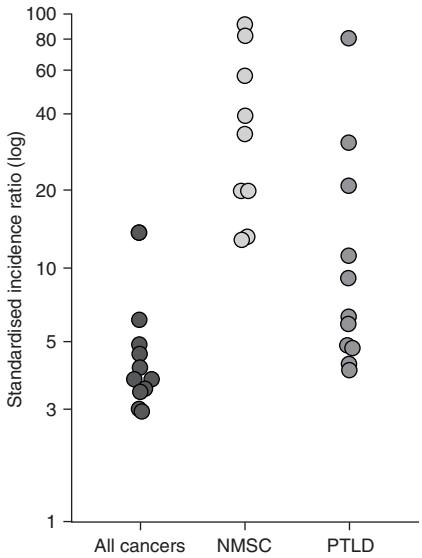

Fig. 2. Comparison of standardised incidence ratios for all post-transplant *de novo* cancers, non-melanoma skin cancer (NMSC) and post-transplant lymphoproliferative disease (PTLD).[10-14,17-19,21,23,35,45,46]

UNOS) – 4.7% and 3.9%, respectively – with the same duration of follow-up.[20]

Calculations of the malignancy SIR in renal transplant recipients compared with those in the general population show similar variability. The risk of *de novo* malignancy after transplantation varies between 2.7-fold and 13.7-fold with respect to the general population (figure 2).[10-13,17,18,23,45,46] Another valid method for assessing the risk of cancer after organ transplantation is to compare malignancy rates in renal transplant patients with those in patients receiving dialysis or those on transplant waiting lists. In both cases, the transplanted population shows an increased risk in the overall incidence of cancer compared with the untransplanted population.[11,14,19,30,52-54] In the ANZDATA registry, the SIR for all tumours except NMSC was 2.14 in patients receiving dialysis versus 3.46 in renal transplant recipients.[14] In the USRDS, renal transplant recipients have been reported to have a 2.6-fold higher risk of NMSC and a 1.2-fold higher risk of any non-skin cancer than patients on the US transplant waiting list.[19]

The elevated risk and incidence of malignancy in organ transplant recipients have a tremendous clinical impact. Malignancy causes a substantial proportion of the late mortality after transplantation (10–47%), mainly depending on the duration of post-transplant follow-up.[14,25,31,32,55,56] In most studies, the primary cause of overall mortality is undoubtedly cardiovascular disease, although malignancy is gaining ground as long-term survival is achieved in a greater number of organ transplant recipients. Indeed, malignancy is the foremost cause of death in the ANZDATA registry,[14] and is usually among the first three causes of death – along with cardiovascular and infectious diseases – in other registries.[25,31,32,55,56] In summary, malignancy is currently one of the major factors that limits life expectancy in organ transplant recipients.

2.2 Malignancy in Pharmacologically Immunosuppressed, Non-Transplanted Patients

The notion that immunosuppressive therapy can increase the risk of malignancy in organ transplant recipients would be reinforced if this risk was shown to be increased in medical conditions other than transplantation in which the patient receives immunosuppressive drugs. A study that included data from 59 000 patients included in the population-based North Jutland Prescription Database and the Danish Cancer Registry found that overall risks of developing squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) of the skin, and non-Hodgkin's lymphoma (NHL), were increased among individuals who received prescriptions for corticosteroids but excluding those patients who were also receiving other immunosuppressive drugs. In these patients, the SIRs were 2.45 for SCC, 1.52 for BCC and 2.86 for NHL.[26] Similar findings have been reported in other population-based stud-

ies[57] and in a case control study.[58] Other small retrospective studies have described an association between Kaposi's sarcoma and chronic corticosteroid therapy in non-transplanted patients.[59] However, a recently published case-control study that was designed to assess the risk of lymphoma associated with corticosteroid treatment for polymyalgia rheumatica and temporal arteritis, found no appreciable increase in the risk of lymphoma, even at considerable cumulative doses.[60]

Long-term ciclosporin exposure has been associated with an increased risk of NMSC, and especially with SCC,[61,62] as well as with an increased risk of both NMSC and NHL.[63] The data on azathioprine are conflicting: several studies did not find a significant increase in the risk of malignancy in non-transplanted patients treated with azathioprine; however, other studies report that azathioprine therapy is associated with a tendency towards higher cancer rates and cancer-related mortality.[64-66] Patients treated for multiple sclerosis were found to have a progressive rise in their risk of malignancy that was related to the duration of exposure to azathioprine.[66] The relative risk of cancer was 1.3 in patients treated for <5 years, 2.0 in those treated for 5–10 years, and 4.4 in those who received >10 years of treatment, although none of these increases in risk were statistically significant. A recent meta-analysis has demonstrated a pooled relative risk for lymphoma of 4.18 associated with azathioprine or mercaptopurine use in patients with inflammatory bowel disease,[67] although the increased risk of lymphoma in this study could be the result of the immunosuppressive therapy administered or the severity of the underlying disease, or both. Taken together, these results suggest that immunosuppressive therapy carries a low but significant risk of cancer in non-transplanted patients. These data are even more striking if we consider that, unlike the majority of non-transplanted patients, organ transplant recipients receive combinations of several immunosuppressive drugs.

3. Links Between Cancer Pathogenesis and Immunosuppressive Therapy

3.1 Intensity of Immunosuppressive Therapy and Cancer Risk.

Assessing cancer pathogenesis in organ transplant recipients is difficult because of the mixture of pathogenic factors in these patients. The presence of environmental and genetic risk factors common to the general population and the complex interaction established between the effect of depressed immunosurveillance, the action of pro-oncogenic viruses, and possibly direct carcinogenic effects of immunosuppressive drugs, converge in organ transplant recipients. The final effect of these factors is manifested as an increased risk of malignancy in organ transplant recipients. Further evidence of the relationship between immunosuppressive therapy and the risk of malignancy is that the latter increases with the intensity of the former. One of the most solid arguments for this association was reported by Dantal et al.[32] in a prospective, open, randomised trial of two ciclosporin regimens (low-dose [trough blood concentrations of 75–125 ng/mL] and normal-dose [trough blood concentrations of 150–250 ng/mL]) in renal transplant recipients. At 66 months of follow-up, malignancy was more frequent in the normal-dose group. In addition, several retrospective studies have also shown increased malignancy rates associated with more intense exposure to immunosuppressive drugs.[68-70] In other cases, use of a more intensive immunosuppressive regimen, rather than of a higher dose of only one drug, has been associated with higher malignancy rates. Thus, the addition of ciclosporin to classical dual protocols of azathioprine plus corticosteroids was followed by an increase in the cumulative incidence of malignancy, and especially in the incidence of NMSC.[33,71,72]

It is well known that more intensive immunosuppression is used to prevent and treat allograft rejection in following transplantation of organs other than the kidney,[73-75] and this phenomenon is correlates with a progressive increase in malignancy rates in kidney, liver, pancreas, heart, lung and combined heart-lung transplantation. The cumulative incidences of *de novo* malignancy development in lung or heart-lung transplant recipients reported by the registry of the International Society of Heart and Lung Transplantation (ISHLT) are the highest published in any organ transplant recipient registry.[44] For lung and heart-lung transplant recipients, the cumulative incidence rates for malignancy were 35.1% and 34.2%, respectively, at 60 months after transplantation (figure 1). A nationwide study in Sweden reported SIRs for various cancers in kidney and non-kidney transplant recipients compared with age- and sex-matched populations. For cancer overall, the SIR was 3.9 (95% CI 3.6, 4.2) in kidney transplant recipients versus 4.9 (95% CI 3.7, 6.4) in non-kidney transplant recipients.[17] In this and other studies, the increase in risk was greater for post-transplant NHL than other cancers,[17,75] but was not limited to this malignancy.[76-80]

Finally, more intense immunosuppression can translate not only into an increased risk of malignancy, but also into more aggressive tumour progression, in terms of accelerated growth and metastasis,[81] and lower patient survival.[34,82-84] In contrast, a reduction of immunosuppression might have a positive impact on the clinical course of the tumour and on the prognosis for survival, at least in certain types of cancer.[82]

3.2 Cancer Immunosurveillance: Tumours of Non-Viral Origin

Impaired cancer surveillance as a result of immunosuppressive therapy is one of the better established concepts in the classical understanding of the pathogenesis of malignancies in organ transplant recipients (recently reviewed by Dunn et al.[85]). However, only recent work in the 1990s has lent support to this concept. The definitive evidence came from the study of gene-targeted mice that completely lacked natural killer T (NKT) cells, T cells and B cells.[86] After receiving injection of a carcinogen, these mice developed sarcomas more rapidly and with greater frequency than did genetically matched wild-type control mice, and also developed far more spontaneous epithelial tumours than did wild-type mice.[87] Thus, lymphocytes in mice not only protect the host against the formation of chemically induced cancers but also prevent the development of spontaneous epithelial tumours.

Immunosurveillance is also involved in defending against the early steps of the metastatic processes, which include vascular emboli, lymphatic invasion, and perineural invasion (collectively referred to as 'VELIPI').[88] Recent data suggest that tumours without evidence of VELIPI, compared with tumours showing these signs, contain significantly more memory T cells.[88] Prolonged survival and the absence of pathological signs of early metastatic invasion were associated with increased levels of mRNA for the products and markers of T helper-1 (T_h1) effector T cells (CD8, the transcription factor T-box expressed in T cell [T-Bet], interferon regulatory factor 1, interferon-γ, granulysin and granzyme B).[88]

From an epidemiological point of view, the difficulty in assessing cancer immunosurveillance in organ transplant recipients arises from their greater susceptibility to endemic viruses and other pathogens, as well as viral reactivation. Several analyses in transplant recipients from Australia,[14] New Zealand,[14] the US,[19] Ireland,[10] Nordic countries[11,13,17,46] and Japan[23] have included a sufficient number of patients and had a long enough duration of follow-up to show an increased risk for a wide range of malignancies with no known viral aetiology (table I). When compared with the general

population using relative risk ratios, organ transplant recipients have been reported to have greater risks for a broad subset of tumours with no apparent viral origin (figure 3).[10-12,17,18,21,23,35]

Stricter assessment of immunosuppressive therapy-related cancer risk could be extrapolated by comparisons between renal transplant recipients and populations receiving dialysis or, even better, on the kidney transplant waiting list. However, like immunosuppressive therapy, end-stage renal disease and uraemia are also associated with immune system abnormalities, which could increase susceptibility to malignancies and confound the analysis.[89,90] In addition, candidates for kidney transplantation are usually extensively studied to detect – and, if necessary, to treat – malignant disease before their inclusion on the waiting list, which could result in bias, leading to lower cancer rates in renal transplant recipients than in patients receiving dialysis. However, assessment of 13 077 renal transplant recipients from 1980 to 2003 versus 33 820 patients undergoing dialysis in the same period in Australia and New Zealand showed numerically higher SIRs for a wide range of non-viral tumours, including melanoma, cancers of the digestive and respiratory tracts, leukaemia, and tumours of the bone and soft tissues, in transplant recipients.[21] A similar comparison in Denmark, performed in a smaller number of pa-

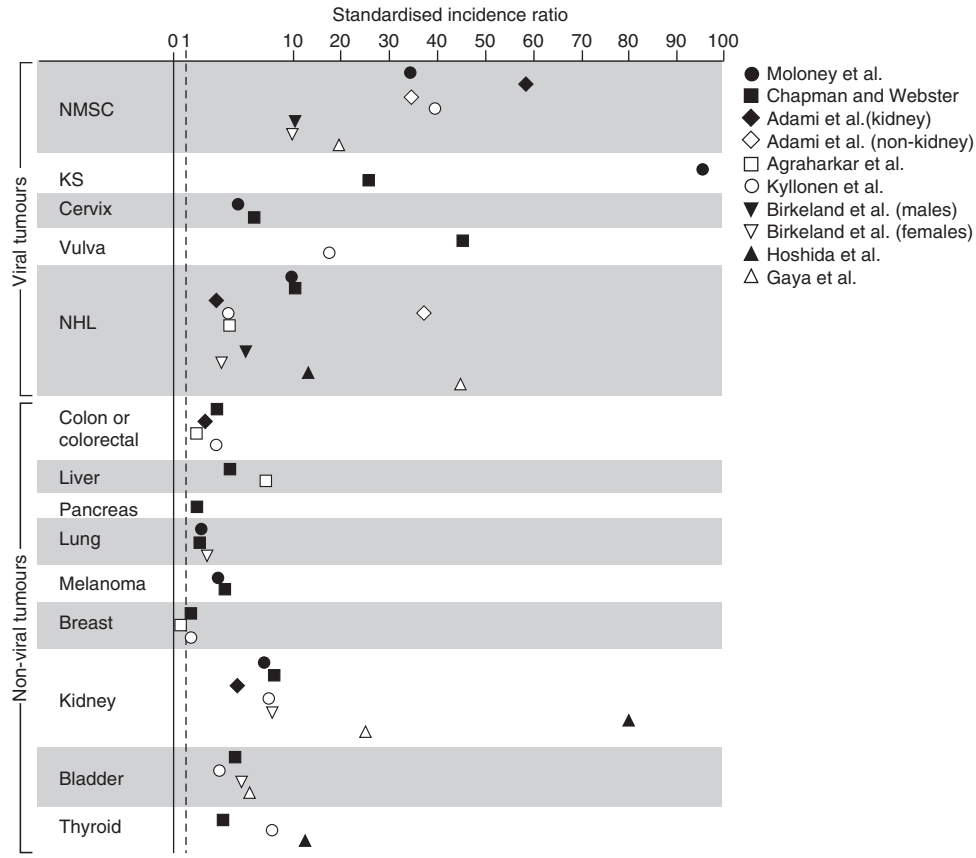

Fig. 3. Standardised incidence ratios for tumours of viral and non-viral origin. Data from Moloney et al.,[10] Chapman and Webster,[21] Adami et al.,[17] Agraharkar et al.,[35] Kyllonen et al.,[12] Birkeland et al.,[11] Hoshida et al.[23] and Gaya et al.[18] **KS** = Kaposi's sarcoma; **NHL** = non-Hodgkin's lymphoma; **NMSC** = non-melanoma skin cancer.

tients, showed a higher risk for lung cancer in transplant recipients.[11] Even kidney cancer, which is strongly related to uraemia,[91,92] is more common in kidney transplant recipients than in patients on the kidney transplant waiting list.[11,19]

3.3 Infection: Virus-Induced Malignancies

A limited number of viruses have been related to different malignancies in organ transplant recipients and the general population (table II). According to the mechanism through which these viruses induce tumours, they can be grouped into two categories: (i) direct oncogenic viruses; and (ii) viruses that are only indirectly carcinogenic.[93] Acting through direct gene regulation or through interaction with oncogenes and tumour-suppressor gene products, direct oncogenic viruses are able to modify the proliferation-antiproliferation pathways of the host cell as a strategy for maintaining their own replication.[94] These viruses are able to deactivate tumour-suppressor gene proteins such as retinoblastoma and p53.[95] In contrast, indirect oncogenic viruses are not able to cause malignant transformation directly, but their presence increases the probability of specific types of malignancy several-fold. Other oncogenic factors probably also contribute to multistep carcinogenesis.

In this context, immunosuppressive therapy is an important cofactor in viral oncogenesis. Depressed immunosurveillance has its impact through a double mechanism, firstly by increasing the risk of infections and their persistence, and secondly by increasing the probability that the transformed cell will escape cancer surveillance, allowing cancer cells to proliferate and clonally expand. As a result, virally induced malignancies are the most frequent post-transplant cancers, and compared with their relatively low incidence in the general population, the relative risk of these malignancies shows a substantial increase (figure 3).[10-12,17,18,21,23,35]

3.3.1 Post-Transplant Lymphoproliferative Diseases

Post-transplant lymphoproliferative diseases (PTLDs) are a heterogeneous group of disorders that include a morphologically and clinically heterogeneous spectrum of diseases ranging over the four categories that were established by the WHO 2001 classification.[97] Three factors, intrinsically related and of special interest, characterise PTLDs: firstly, the central pathogenic role played by Epstein-Barr virus (EBV) in a large number of PTLD patients;[98-101] secondly, the close association between immunosuppression and the development of this entity; and finally, some authors have underlined the fact that transplantation involves the placement of a foreign antigen mass in the host and therefore a

Table II. Oncogenic infections and related malignancies[96]

Microbe	Transforms target cell	Tumours in animal models	Malignancy
Human papillomaviruses	+	+	Cervical carcinoma, NMSC, ano-genital cancer
Human polyomaviruses (BKV, JCV, SV40)	+	+	Mesotheliomas, brain tumours
Epstein-Barr virus	+	+	B-cell lymphoproliferative diseases, nasopharyngeal carcinoma
Herpesvirus (HHV8)	+	+	Kaposi's sarcoma, primary effusion lymphomas
Hepatitis B virus	−	−	Hepatocellular carcinoma
Hepatitis C virus	−	+	Hepatocellular carcinoma
Human T-cell leukaemia Virus-1	+	?	T-cell leukaemias
Helicobacter pylori	−	−	Gastric carcinoma

HHV8 = human herpesvirus 8; **NMSC** = non-melanoma skin cancer; **+** indicates that the virus (microbe) has such an effect; **−** indicates the virus (microbe) does not have the effect; **?** indicates it is unknown whether the virus has such an effect.

marked degree of chronic antigen stimulation.[102,103] There is evidence that chronic antigenic stimulation with eventual clonal selection can lead to lymphoma,[104] so this phenomenon may also be relevant to consider as a factor in the development of PTLDs.

PTLDs are the most common malignancy in the first year post-transplant, when concentrations and doses of immunosuppressive drugs are highest.[17,75] Moreover, nonrenal transplant recipients show a higher increase in risk compared with kidney transplant recipients, again suggesting a strong association between the intensity of the immunosuppressive regimen and PTLD risk.[17,75] In addition, the type and cumulative dose of the immunosuppressive regimen influence the incidence and latency of PTLDs. Since 1990, when an increased incidence of PTLDs in heart transplant recipients after the addition of muromonab CD3 (OKT3) to an immunosuppressive regimen was reported,[102] the use of induction regimens containing lymphocyte-depleting antibodies has been one of the best known risk factors for PTLD. This risk has been demonstrated for muromonab CD3 and antithymocyte globulin in several studies.[22,73,75,105,106] Finally, the observed total or partial clinical remission of PTLD with reductions of immunosuppressive treatment, especially in the more benign types, shows the dependence between PTLDs and immunosuppression.[107-110]

3.3.2 Non-Melanoma Skin Cancer

NMSC is the most frequent post-transplant malignancy in countries with predominantly White populations.[10,11,17,19,27,30-32,35-37,111,112] The risk of NMSC is 12- to 90-fold greater in organ transplant recipients than in the general population (figure 2). The onset of NMSC occurs at a younger age in organ transplant recipients and the disease is more aggressive, manifested by an increased risk of multiple lesions, local recurrence, regional and distant metastasis, and mortality.[28,29,48,83,111,113-116] All these phenomena result from the convergence of several pathogenic factors for this particular type of malignancy, such as UV radiation,[117] human papillomavirus (HPV) infection and immunosuppressive therapy.

Indeed, the intensity and duration of immunosuppression appear to correlate with the risk of NMSC. In a retrospective study of >295 patients, the addition of ciclosporin to dual therapy with azathioprine and prednisone increased the incidence rate of NMSC from 29 cases per 1000 person-years to 48 cases per 1000 person-years and decreased post-transplant time to NMSC development.[71] In a prospective randomised trial, the use of a long-term maintenance regimen with normal-dose versus low-dose ciclosporin led to a higher frequency of NMSC, pre-epitheliomatous lesions and warts, and the number of patients with multiple skin lesions was also significantly higher in this group.[32] Finally, more frequent NMSC and earlier post-transplant onset of this malignancy were observed in nonkidney organ transplant recipients, who usually receive more intense immunosuppression.[77-80]

Immunosuppressive therapy might exert a pro-oncogenic action at different levels in NMSC. In experimental studies, several immunosuppressive drugs, including azathioprine and calcineurin inhibitors, have been demonstrated to be able to directly increase skin mutagenic activity and/or the tumourogenesis of UV radiation or chemical carcinogens.[118-124] The role of immunosuppressive therapy in impairing surveillance, and of subsequently increasing the probability and spread of infection by HPV, has already been mentioned in this article (section 3.3). Indirect evidence suggests that immunosuppression could favour the role of HPV in NMSC development in organ transplant recipients. Studies using distinct methodologies have detected epidermodysplasia verruciformis (EV)-associated HPV DNA in a significant proportion of skin tumours,[125-127] and gene expression of EV-HPV has also been demonstrated in SCCs.[128] In addition,

the percentage of premalignant lesions and NMSC from organ transplant recipients that are positive for HPV DNA is about 80%,[126,129-131] sometimes even reaching 100%.[132] In contrast, in the general population, detection rates for HPV DNA in NMSC are about 30%.[126,133,134] Finally, depressed immunosurveillance also favours the survival and proliferation of transformed cells.

4. Pro- and Anti-Oncogenic Effects of Immunosuppressive Drugs

Few of the different issues tackled in this review are as complex and sensitive as the direct cancer-promoting effects of certain immunosuppressive drugs, independent of depressed immunosurveillance. Epidemiological data from organ transplant recipients may reveal only some of the information necessary to draw rigorous conclusions. Randomised controlled trials (RCTs) are generally too small and have insufficient follow-up to detect differences, and most registries lack sufficient information about drug prescriptions and dosages. To perform comparisons between different drugs it is important to not forget that the newer drugs were introduced simultaneously with the emergence of better pathology and focus on diagnoses, such as PTLD, which were not used in earlier eras.[103] Moreover, immunosuppressive drugs are generally used in different combinations and at various dosages throughout follow-up. In contrast, there is growing experimental evidence of the different oncogenic effects of immunosuppressive drugs, which could be of great value in assessing this question and of relevance in the clinical setting.

4.1 Biological Agents

Lymphocyte-depleting antibodies have been shown, as a group, to clearly increase the risk of malignancy, mainly of virally induced cancers. The striking rise in the frequency of PTLD and NMSC associated with the use of lymphocyte-depleting antibodies as induction or anti-rejection therapy has previously been described in this review (sections 3.3.1 and 3.3.2). Unfortunately, there are very few data from direct comparisons among these drugs, and most studies do not analyse polyclonal agents individually.[75,105,135] A single-centre study showed differences in lymphoma incidence and delay to cancer diagnosis between two different antithymocyte globulins. Thymoglobulin carried a higher relative risk (2.16; 95% CI 1.04, 4.48) of malignancy, mainly lymphoma, than did antithymocyte globulin (antithymocyte globulin Fresenius).[136] This initial finding has recently been corroborated by a study performed using the CTS database that analysed the incidence of NHL according to the type of induction therapy used in 112 122 kidney transplant recipients.[137] Muromonab CD3, antithymocyte globulin (Atgam®)[1] and thymoglobulin were each associated with a significantly higher risk than was not receiving induction therapy, whereas antithymocyte globulin Fresenius was not associated with an increased risk. In contrast, using data from the Scientific Registry of Transplant Recipients (SRTR) database, no differences in the relative risk of developing PTLD were found between any specific lymphocyte-depleting antibodies.[22] Nevertheless, this latter study included only patients from the US, where antithymocyte globulin Fresenius is not commercially available.

The underlying mechanism of these differences is not known. Their variable oncogenic activity can probably be explained by differences in the range of activity of the different polyclonal and monoclonal antibodies against lymphocyte surface antigens. Antithymocyte globulin Fresenius displays a significantly narrower spectrum of activity against lymphocyte antigens than do Atgam® and thymoglobulin,[138] whereas muromonab CD3 has a powerful T-

1 The use of trade names is for product identification purposes only and does not imply endorsement.

cell depleting activity and also blocks the function of NKT cells.[139] Importantly, these drugs not only deplete T-cell populations, but also induce hyporesponsiveness in the T cells that escape depletion *in vivo*, an action that is possibly related to down-regulation of T-cell functional surface receptors, co-receptors, and other molecules that control T-cell activation.[140]

The only antibodies that show immunosuppressive efficacy in reducing acute rejection rates[141,142] without consistent evidence that they increase malignancy risk[75,105,135,137,140,142] are the non-lymphocyte-depleting anti-CD25 monoclonal antibodies. The difference, compared with the previously mentioned antibodies, is that the activity of anti-CD25 is restricted to activated T cells and macrophages/monocytes. Treatment with basiliximab significantly reduced the number of circulating T lymphocytes expressing the interleukin (IL)-2 receptor, but the numbers of total lymphocytes, lymphocyte subtypes or T lymphocytes expressing activation antigens other than the IL-2 receptor were unaffected.[142,143] Opelz et al.[137] recently suggested that the increased risk of lymphoma could be linked to activity against CD3, present in Atgam®, thymoglobulin and muromonab CD3, which is almost absent in antithymocyte globulin Fresenius, and nonexistent in anti-CD25 antibodies.

4.2 Corticosteroids

Corticosteroids have been extensively used in the prevention of rejection following organ transplantation and are an essential part of most immunosuppressive regimens. Unfortunately, for this reason and because they are almost always used in combination with other immunosuppressants, there are few epidemiological data that would allow their potential pro-oncogenic role in organ transplant recipients to be evaluated. However, the use of corticosteroids in non-transplanted patients who are not receiving other immunosuppressive drugs has been shown to be related to an increased risk of malignancy, mainly concerning NMSC.[26,57-59]

Corticosteroids have been proposed to play a dual role in oncogenesis. These drugs are used in oncological practice on the basis of their pro-apoptotic effects in lymphoid cells and their effectiveness in ameliorating several tumour-related complications. However, recent data have demonstrated anti-apoptotic and proliferation-promoting effects of corticosteroids in carcinoma cells from a wide variety of tumours (recently reviewed by Rutz and Herr[144]). In addition, corticosteroids enhance tumour cell resistance to apoptosis in solid tumours,[143,145] inactivate B and T lymphocytes (including activated T killer cells),[146] reduce the expression of major histocompatibility class I antigen *in vivo*,[147] and decrease immunosurveillance even at very low doses.[148] In conclusion, through a direct pro-oncogenic action in cells or by facilitating tumour cell escape from immunosurveillance, corticosteroids could significantly contribute to the increased malignancy risk observed in organ transplant recipients.

4.3 Antimetabolites

4.3.1 Azathioprine

Azathioprine has been used for the prevention of rejection in clinical transplantation for more than 30 years. Azathioprine is a purine analogue that is incorporated into cellular DNA, where it inhibits purine nucleotide synthesis and interferes with RNA synthesis and metabolism.[149] Azathioprine could directly promote cancer through several mechanisms. One of the first mechanisms proposed consists of a synergism with UV radiation in carcinogenesis. In UV-induced carcinogenesis in mice, azathioprine shortened the period for tumour development and increased the number of skin cancers, even in comparison with ciclosporin.[124] Recently, O'Donovan et al.[118] demonstrated that azathioprine enhances the effect of UVA light in generating

mutagenic oxidative DNA damage. Oxidative stress and mutagenic DNA lesions formed by reactive oxygen species are linked to human malignancy. Clinical treatments that induce chronic oxidative stress may therefore carry a risk of therapy-related cancer. These authors suggest that immunosuppression by azathioprine may be one such treatment. Azathioprine causes the accumulation of 6-thioguanine in patients' DNA, and 6-thioguanine and UVA are synergistically mutagenic.[38]

Azathioprine could also increase the susceptibility of DNA to mutagenesis through other mechanisms that are unrelated to UV exposure. An early report suggested that the carcinogenicity of azathioprine involves the postreplicative DNA mismatch repair system.[150] Azathioprine is partly converted to thioguanine, which could be intercalated into DNA, thus inhibiting repair splicing and eliciting codon misreads.[150] Azathioprine therapy can also select cell clones with DNA mismatch repair deficiencies.[76] Inactivation or a diminished activity of the DNA mismatch repair system greatly increases spontaneous mutation rates. Indeed, defective DNA mismatch repair is characteristic of transplant-related acute myeloid leukaemia/myelodysplastic syndrome (AML/MDS).[76] This phenomenon could be related to the increased AML/MDS rates observed in organ transplant recipients: after reviewing AML/MDS cases reported to the CTS database since 1985, Offman et al. found a significant correlation between azathioprine doses and the incidence of AML/MDS.[76]

In contrast, epidemiological data do not allow the immunosuppressive effects of azathioprine to be analysed separately from its probable direct oncogenic action. Undoubtedly, the early years of organ transplantation history, before the introduction of ciclosporin, provide sufficient evidence of an azathioprine-related increase in several post-transplant malignancies.[8] In the previously discussed study by Dantal et al.,[32] two groups of patients were randomised to normal or low ciclosporin doses, combined with azathioprine and prednisone. Higher ciclosporin doses were associated with a greater incidence of cancer; furthermore, although the two groups received equal azathioprine doses, cancer was more frequent in patients who received the highest mean doses of azathioprine. However, as would be expected, compared with the more powerful immunosuppressant ciclosporin, azathioprine has been associated with similar,[72] or generally lower, cancer incidence rates.[33,69,151]

4.3.2 Mycophenolate Mofetil

Mycophenolate mofetil (MMF) is an ester prodrug of the immunosuppressant mycophenolic acid. Mycophenolic acid is a selective and reversible uncompetitive inhibitor of inosine monophosphate dehydrogenase, an enzyme that is crucial for the proliferation of T and B lymphocytes.[149] The antiproliferative effects of MMF are translated to *in vitro* antitumoural activity in several cancer cell lines.[152,153] Data on the pro- or anti-oncogenic activity of MMF are conflicting: on the one hand, it has been associated with potential enhancement of tumour cell invasiveness[154] and a mutagenic effect *in vitro*,[155] while on the other hand, it has been related to possible prevention of adhesion receptor-dependent tumour dissemination.[156,157] In addition, MMF has been suggested to enhance the anti-herpes activity of aciclovir and ganciclovir, which could be of value in preventing the development of EBV-induced PTLD.[158]

Several clinical studies have tried to clarify whether MMF is pro- or anti-oncogenic. An RCT of azathioprine versus MMF combined with ciclosporin and prednisone conducted in 580 heart transplant recipients found similar overall cancer incidence rates in both groups, 15.6% versus 12.5%, respectively, after 36 months of follow-up.[159] In agreement with these data, a systematic review of RCTs of MMF versus azathioprine in renal transplant recipients also found no significant differences

in the incidence of skin cancer between patients receiving these two immunosuppressants.[160]

The relationship between MMF treatment and the risk of developing PTLD has been extensively studied, with conflicting results. In two large case-control studies in the US population, the incidence of PTLD did not differ according to MMF use. First, Funch et al.[100] found no significant differences in the incidence of PTLD in 15 000 US renal transplant recipients receiving triple therapy immunosuppressive regimens, whether they contained MMF or not. Similarly, in another case-control study that analysed 8246 patients included in the UNOS/OPTN databases, lymphoma and overall malignancy rates did not differ according to MMF use.[20] In contrast, data analysis of the population included in the CTS database that was presented in this latter study showed that MMF therapy was associated with a decreased risk of PTLD and of any cancer compared with patients not taking MMF. This result is in agreement with a review of the incidence of PTLD in the first 38 500 kidney transplants reported to the SRTR: compared with azathioprine, MMF at discharge was associated with a lower risk of PTLD.[135] However, the results of this study should be interpreted with caution. A higher proportion of patients receiving MMF than those receiving azathioprine had been treated with aciclovir or ganciclovir, which would have decreased the frequency of PTLD patients. Moreover, patients taking MMF or azathioprine had a lower risk of PTLD in 25 127 Medicare-enrolled renal transplant recipients between 1996 and 2000.[105]

In the paediatric population, the incidence of post-renal transplant PTLD was unmodified by MMF use.[161]

In conclusion, MMF does not seem to be associated with an increased risk of malignancy and could be associated with a slight reduction in the incidence of PTLD. Since MMF is much more effective than azathioprine in preventing allograft rejection,[162] this information is highly important. MMF improves immunosuppressive efficacy without increasing the risk of malignancy.

4.4 Calcineurin Inhibitors

The two clinically available calcineurin inhibitors are ciclosporin and tacrolimus. The immunosuppressive effects of these drugs depend on calcineurin inhibition,[149] but an additional action of calcineurin inhibitors, which partly mediates their immunosuppressive effect, is enhanced production of transforming growth factor β1 (TGFβ-1).[163-166] This pleiotropic cytokine has been implicated in the development of tumour invasiveness and metastatic spread.[167,168] High-grade and biologically aggressive tumours have been shown to contain significantly higher levels of TGFβ-1 than more highly differentiated tumours, and the dynamic relationship between levels of TGFβ-1 protein and TGFβ-1 receptors is considered to be significant in tumour progression.[167-171] Calcineurin inhibitor-dependent increased production of TGFβ-1 and TGFβ-1 involvement in carcinogenesis were the basis of a study by Hojo et al.[172] These authors demonstrated that ciclosporin induces phenotypic changes in cells, including inducing invasiveness in non-transformed cells, through a cell autonomous mechanism. This effect is independent of the action of ciclosporin on the host's immune cells and is probably mediated by TGFβ-1.

In vitro evidence was complemented by *in vivo* experimental data in severe combined immunodeficient (SCID)-beige mice, which are deficient in T, B and NKT cells, minimising the interference of the immune system. Ciclosporin treatment increased the number of metastases in SCID-beige mice that had been treated with several cancer cell lines and, again, the mediator involved was TGFβ-1.[172] This pioneering work was subsequently confirmed by studies from the same[173,174] and other[175] groups, and was followed by similar find-

ings in studies using tacrolimus.[165] Maluccio et al.[165] showed that tacrolimus has a dose-dependent effect on TGFβ-1 expression and tumoural spread in immunodeficient mice. However, in contrast with ciclosporin, the cancer-promoting effect of tacrolimus was demonstrated only with doses that were higher than the dosage required to promote allograft acceptance in experimental transplantation models.[176] This finding could be translated into a clinically different cancer risk profile between the two calcineurin inhibitors.

TGFβ-1 involvement in the cancer-promoting effects of calcineurin inhibitors involves factors other than cellular changes. Accumulating evidence suggests that tumour progression is governed not only by genetic changes intrinsic to cancer cells, but also by epigenetic and environmental factors. Calcineurin inhibitors increase TGFβ-1 secretion by malignant cells and surrounding cells.[166,174] TGFβ-1 acts on the host to suppress antitumour immune responses, enhance extracellular matrix production and augment angiogenesis.[168,170] These changes resemble some of the environmental tissue changes induced by calcineurin inhibitor-induced fibrogenesis, which also involves TGFβ-1.[164,177,178]

Before these studies, calcineurin inhibitors had been associated with clear promoting effects on the development of spontaneous or chemically induced tumours *in vivo*, although the mechanisms were not completely known.[179-181] Guba et al.[182] ascribed some of the cancer-promoting effect of ciclosporin in mice to enhancement of tumour angiogenesis. The increased expression of vascular endothelial growth factor (VEGF) observed in ciclosporin-treated animals could be responsible for enhanced angiogenesis.[182,183] Koehl et al.[174] also demonstrated that TGFβ-1 is at least partially responsible for the increased angiogenesis associated with ciclosporin treatment.

Calcineurin inhibitors could be involved in other general mechanisms of carcinogenesis. A recent study showed that proline oxidase (p53-induced gene-6) mediates apoptosis in cancer cells through a calcineurin-dependent pathway.[184] Both proline oxidase and p53-induced activation of nuclear factor of activated in T cells (NFAT) were sensitive to ciclosporin and tacrolimus, which abolished proline oxidase-mediated apoptosis and reduced p53-induced apoptosis,[184] thus favouring tumour growth and spread. These results are in agreement with the study by Sugie et al.,[123] which demonstrated that ciclosporin suppressed p53-dependent apoptosis and DNA repair following UVB irradiation, and are complementary to those observed in the study by Yarosh et al.[119] These authors demonstrated that calcineurin inhibitors decrease DNA repair and apoptosis in human keratinocytes following UV irradiation. All the evidence together partly explain the increased photocarcinogenesis associated with calcineurin inhibitors *in vivo*[122,124,185] and account for the increased skin cancer rates observed in organ transplant recipients.

For the last few decades calcineurin inhibitors have been the cornerstone of immunosuppressive therapy because of their immunosuppressive potency. Consequently, comparisons of ciclosporin-based therapies with the previously used azathioprine-based regimens have generally shown higher malignancy rates and a lower mean time to tumour development with ciclosporin,[12,33,36,37,69,151] although some studies found no significant differences.[72] Therefore, epidemiological data provide insufficient evidence of a calcineurin inhibitor-specific pro-oncogenic effect that is independent of the immunosuppressive effect of these drugs.

Although it has been hotly debated in the past, tacrolimus has a slightly greater immunosuppressive effect than ciclosporin;[186,187] however, differences in malignancy rates, which would be expected, are less clear. A recent meta-analysis of all RCTs comparing tacrolimus with ciclosporin for the initial immunosuppressive treatment of kidney transplant

recipients included 123 reports from 30 trials (4102 patients). This analysis found no differences in the occurrence of malignancy between the two drugs.[187] Registry-based studies can analyse a larger number of patients and longer follow-up periods, but are subject to a different bias in the selection of therapy. An analysis of 25 127 Medicare renal transplant recipients between 1996 and 2000,[105] and of 41 686 patients included in the SRTR database who received their first kidney transplant between 1996 to 2002,[22] demonstrated that, in patients who did not receive induction therapy, the cumulative incidence of PTLD was lower in ciclosporin-treated patients than in tacrolimus-treated patients. This difference disappeared if the patient had received induction therapy.[22,105] Similar results were found in over 200 000 organ transplant recipients included the CTS database, in whom the risk of PTLD development associated with tacrolimus was approximately twice that associated with ciclosporin, irrespective of induction therapy.[75] Cherikh et al.[135] found a 24% increase in the incidence of PTLD in recipients of a first kidney transplant in the OPTN/UNOS database who received tacrolimus compared with those who received ciclosporin, but this increase was not statistically significant.

The results seem to differ in non-PTLD cancer. Several studies found no differences in the occurrence of solid tumours between ciclosporin- and tacrolimus-based regimens.[188,189] Furthermore, in patients who did not receive induction therapy, Bustami et al.[22] demonstrated a 30% reduction in the incidence of *de novo* solid tumours if these patients were treated with tacrolimus versus ciclosporin. Similarly, data from 35 765 Medicare first kidney transplant recipients between 1995 and 2001 demonstrated that tacrolimus-based regimens were associated with a clear and significant reduction in the risks of malignancy overall and skin cancer.[19]

A clear explanation for these differences is currently lacking, but a possible explanation is that the greater immunosuppressive effect of tacrolimus is manifested by a higher rate of PTLD, a virus-induced malignancy, which is highly dependent on the overall level of immunosuppression induced. However, the lower pro-oncogenic effect of tacrolimus[165] may be able to counterbalance its greater immunosuppressive effect, thus equalising or reducing the rates of solid tumours associated with tacrolimus compared with ciclosporin.

4.5 Mammalian Target of Rapamycin (mTOR) Inhibitors: Are Different Mechanisms for Immunosuppression and Cancer Promotion Possible?

4.5.1 mTOR in Oncogenesis

The development of an oncogenic state is a complex process involving the accumulation of multiple independent mutations that lead to deregulation of the cell signalling pathways that are central to the control of cell growth and cell fate.[190] The last few decades of research have placed the mammalian target of rapamycin (mTOR) as a central element at the crossroads of the multiple signalling pathways that control cell growth. The serine/threonine kinase mTOR is a highly conserved integrator of both mitogenic and nutrient inputs in yeast and mammalian cells, and has been shown to control cell growth in response to various environmental cues (figure 4).[191]

The mitogenic signal is initiated by growth factor-mediated activation of receptor tyrosine kinases (RTKs) located on the cell surface.[191,192] RTKs activate two key signal-transduction components, Ras and phosphatidylinositol 3 kinase (PI3K). Downstream of both factors, the signal is modulated by several negative regulators, such as phosphatase and tensin homologue (PTEN) or neurofibromin 1 (NF1). Recent discoveries indicate that the Ras and PI3K pathways converge to activate mTOR to stimulate cell growth. Notably, several tumour suppressors whose function was previously unknown, such

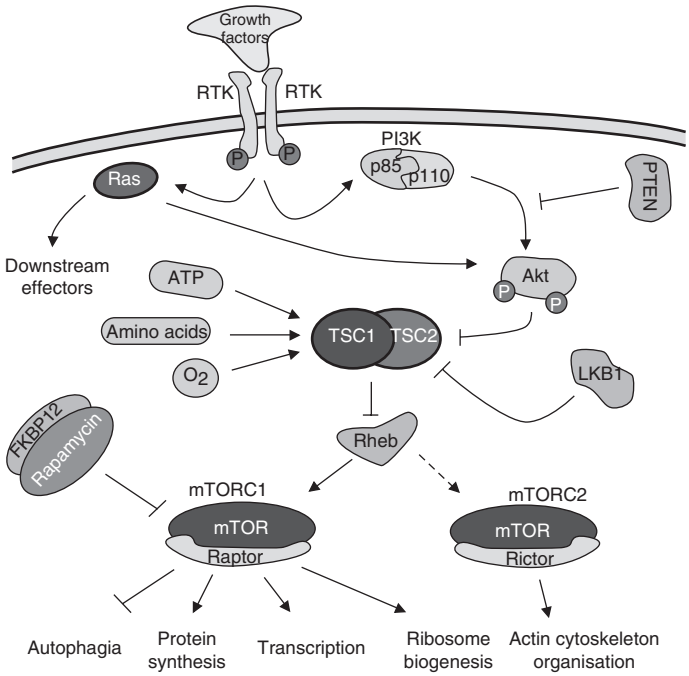

Fig. 4. The mTOR (mammalian target of rapamycin) pathway. The mitogenic signal is initiated by growth factor-mediated activation of receptor tyrosine kinases (RTKs). RTKs activate Ras and phosphatidylinositol 3 kinase (PI3K). Both pathways converge to activate mTOR, which stimulates cell growth under favourable conditions (i.e. when there are available nutrients and energy). The signal is modulated by several negative regulators, such as phosphatase and tensin homologue (PTEN), tuberous sclerosis complex 1 (TSC1), TSC2 and serine/threonine protein kinase 11 (LKB1). Only mTOR complex 1 (mTORC1) is sensitive to rapamycin inhibition. **ATP** = adenosine triphosphate; **FKBP12** = FK 506 binding protein 12; **Rheb** = Ras homologue enriched in brain.

as tuberous sclerosis complex 1 (TSC1, also known as hamartin), TSC2 (tuberin) and serine/threonine protein kinase 11 (LKB1), have recently been shown to attenuate mTOR signalling in nutrient-poor conditions (reviewed by Shaw and Cantley[191]). All of these elements build a signalling network, in which mTOR is strategically positioned at the intersection and is strictly controlled by several inputs and regulators, and functions as a gatekeeper, regulating cell growth, metabolism and proliferation.

Consequently, this network has evolved to ensure that cell proliferation occurs only under environmentally favourable conditions. When a mutation occurs in one or more elements in this biochemical network, cell growth may be driven in a manner unrestricted by environmental cues. Indeed, components of the Ras, PI3K and mTOR signalling pathways are mutated in most human cancers.[193,194] The preponderance of mutations in these interconnected pathways in human cancer suggests that the loss of growth-control checkpoints and promotion of cell survival in nutrient-limited conditions may be an obligate event in tumourigenesis.

Knowledge of the process of mTOR activation is continuously increasing. Biochemical investigation has identified two distinct mTOR complexes (mTORCs), mTORC1 and mTORC2, each composed of mTOR, a common regulatory subunit called LST8, and at least one further subunit that specifies the downstream substrates. These substrate-defining subunits are raptor for the mTORC1 complex and rictor for mTORC2. Put simply, the

former regulates when a cell grows and the latter regulates where a cell grows.[192]

Sirolimus, otherwise known as rapamycin, and other structurally similar compounds (everolimus, temsirolimus), called mTOR inhibitors, are available in the clinical setting. Their class name, mTOR inhibitors, describes their action in a non-rigorous manner, since only mTORC1 is sensitive to inhibition by rapamycin.[195,196] mTORC2 is insensitive to rapamycin and, as previously mentioned, regulates spatial aspects of cell growth, mainly through controlling cell cycle-dependent polarisation of the actin cytoskeleton.[195] mTORC1 signalling plays a role in various growth-related processes (reviewed by Wullschleger et al.[192]):

- Translation: the best-studied targets of mTOR are 40S ribosomal S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1), which are both involved in the mTORC1 regulation of translation.
- Ribosome biogenesis: mTORC1 promotes biosynthesis of ribosomes by controlling the activity of several RNA polymerases (polymerases I, II and III) in a coordinated manner.
- Macroautophagy: mTORC1 negatively controls this catabolic process, promoting the uptake of nutrients such as glucose, amino acids, lipoprotein and iron.
- Transcription: mTORC1 signalling controls transcription of many genes involved in metabolic and biosynthetic pathways.
- Metabolism: mTORC1 appears to play an important role in the control of cellular metabolism, including amino acid biosynthesis and glucose homeostasis, and recent findings suggest that it also participates in adipogenesis.

Signalling components upstream and downstream of mTORC1 are often altered in human cancers. Indeed, preclinical studies suggest that the sensitivity of tumours to mTORC1 inhibitors may correlate with aberrant activation of the PI3K pathway and/or with altered expression of cell cycle regulatory or anti-apoptotic proteins.[191,192] Using this rationale, sirolimus and its derivatives temsirolimus (CCI-779), everolimus (RAD-001) and AP-23573 are currently being evaluated in clinical trials as cancer treatments. The results of these trials show that mTORC1 inhibitors may induce prolonged stable disease and even tumour regression in a subset of patients.[197]

4.5.2 mTOR Inhibitors and Cancer in Organ Transplantation

The two mTORC1 inhibitors available in for the prevention of rejection following organ transplantation are sirolimus and everolimus. Both drugs are able to exert their actions after associating with the isomerase FK506-binding protein-12 (FKBP12).[198] The immunosuppressive effects of the complex of sirolimus or everolimus and FKBP12 depend on inhibiting mTORC1 kinase activity. By blocking mTORC1, sirolimus and everolimus are able to interfere with IL-2 and other mitogen-mediated antigen receptor stimulation of T and B cells.[198] These effects have been proven to prevent acute rejection effectively, through different immunosuppressive regimens, following clinical organ transplantation.[199,200]

After mTOR inhibitors were introduced in organ transplantation, several studies aimed to demonstrate their dual role as immunosuppressors and antitumoural drugs. For this purpose, *in vivo* experiments were performed at doses that provide effective immunosuppression. In these conditions, Guba et al.[182] demonstrated *in vivo* that sirolimus inhibited tumour growth through an anti-angiogenic mechanism. The anti-angiogenic action of sirolimus was due to interference with VEGF signalling. These results were subsequently confirmed by Luan et al.[173] and Boffa et al.[201] using different *in vitro* and murine models of cancer. Notably, these studies confirmed not only the restriction of tumour growth, but also the inhibition of the metastatic pro-

cess.[173,182] Angiogenesis is the key initiating process in tumour growth and metastasis,[202] and VEGF has been identified as a central mediator in tumour angiogenesis.[203] Expression of VEGF by tumour cells leads to the formation of new blood vessels, and tumour cells may then spread from the tumour into the circulation, leading to metastasis.[202] Remarkably, the effects of ciclosporin in these models were the opposite of those of sirolimus, promoting tumoural growth, angiogenesis and metastasis at immunosuppressive doses. Moreover, in a combined murine model of cancer and heart transplantation, Koehl et al.[174] showed that sirolimus simultaneously protected allografts from rejection and inhibited tumour progression, while ciclosporin promoted cancer at doses even lower than those optimal for immunosuppression. Importantly, the deleterious effect of ciclosporin was abrogated by simultaneous administration of sirolimus in the different *in vitro* and *in vivo* experimental models previously described.[173,174,182,201]

In addition to the direct antitumoural effects of mTOR inhibitors, these drugs exhibit other properties that might be of value in reducing malignancy rates in organ transplant recipients. Sirolimus significantly suppressed the UVB-mediated increase in p70S6K activity, an essential step in the DNA damage-dependent signalling pathway responsible for the UVB-mediated increase in interstitial collagenase (matrix metalloproteinase; MMP-1) and stromelysin-1 (MMP-3) protein levels in human dermal fibroblasts.[204] Matrix-degrading metalloproteinases promote UVB-triggered detrimental long-term effects such as cancer formation and premature skin aging. Moreover, sirolimus inhibits UVB-induced p53 phosphorylation, preventing the induction of the immunosuppressive cytokines tumour necrosis factor (TNF)-α and IL-10 in the skin in response to DNA damage.[205] On the other hand, sirolimus and everolimus profoundly inhibit the proliferation, cell cycle progression, and survival of EBV B cell lymphomas *in vitro* and *in vivo*.[206,207] Taken together, these data indicate the potential utility of mTOR inhibitors in two of the most frequent post-transplant cancers, PTLD and skin cancer.

De novo cancer incidence rates in transplant recipients who have received sirolimus therapy seem to confirm experimental data. Two RCTs comparing ciclosporin and sirolimus used in combination with azathioprine or MMF and corticosteroids in renal allograft recipients (n = 161), showed a 5% *de novo* cancer incidence in the ciclosporin group versus 0% in the sirolimus group after a 2-year follow-up.[208] Two further RCTs (n = 1295) examined the continuous combination of ciclosporin and corticosteroids with the addition of sirolimus, azathioprine or placebo. Two years post-transplantation, the incidence of skin cancer was significantly lower in patients receiving sirolimus than in those receiving placebo. However, the cumulative incidence of all cancers did not differ between the groups.[208]

The malignancy-related 5-year follow-up results of another RCT have recently been reported by Campistol et al.[209] In this study, enrolled patients (n = 525) who were initially treated with a triple regimen of ciclosporin, sirolimus and corticosteroids, were randomly assigned at 3 months to remain on the initial regimen or to have ciclosporin withdrawn. This latter strategy reduced the relative risk of skin cancer (0.35; 95% CI 0.23, 0.53) and delayed the median time to the development of a first skin carcinoma compared with remaining on ciclosporin. The incidence of non-skin malignancies at 5 years after renal transplantation was also reduced in patients who received therapy without calcineurin inhibitors after ciclosporin withdrawal compared with that in those who received sirolimus therapy combined with ciclosporin.[209] A further RCT that assigned kidney transplant recipients with a stable graft to a sirolimus-based, calcineurin inhibitor-free conversion regimen (n = 555) or to continuation

with calcineurin inhibitors (n = 275) also assessed this question. At 18 months after randomisation, overall malignancy rates were significantly lower among patients receiving the sirolimus conversion therapy compared with patients who continued to receive calcineurin inhibitors, as were rates for NMSC (BCC and SCC) and other malignancies, except for PTLD.[210]

A retrospective, registry-based study (UNOS database) of 33 249 first kidney transplantations from 1996 to 2001 demonstrated that maintenance immunosuppression with mTOR inhibitors (sirolimus and everolimus) was associated with a significantly reduced risk of developing any post-transplant *de novo* malignancy or non-skin solid tumour.[211] The incidence rates of any *de novo* post-transplant malignancy were 0.60% in patients who received mTOR inhibitors without calcineurin inhibitors, 0.60% in patients who received mTOR inhibitors and calcineurin inhibitors, and 1.81% in patients who received calcineurin inhibitors without mTOR inhibitors; the rates of *de novo* solid tumours were 0%, 0.47% and 1.00%, respectively. These results, together with those of the abovementioned studies, suggest that there is a graded effect: malignancy rates are lower with mTOR inhibitor treatment and calcineurin inhibitor withdrawal than with maintenance of both drugs, and are much lower than with calcineurin inhibitor-based regimens that do not include mTOR inhibitors.

Kaposi's sarcoma is a rare, virus-induced malignancy that shows a disproportionately higher risk in organ transplant recipients compared with the general population.[212] The development of Kaposi's sarcoma after transplantation seems to be related to the action of human herpesvirus 8. This virus encodes a chemokine-like, G protein-coupled receptor (a homologue of the human IL-8 receptor CXCR2) that promotes the proliferation of endothelial cells through activation of the VEGF receptor, Flk-1/KDR.[213] Since VEGF is a key player in the pathogenesis of Kaposi's sarcoma, this angioproliferative disease probably provides mTOR inhibitors with a special opportunity to exhibit their antitumoural effects. The first report of post-transplant Kaposi's sarcoma regression after switching from ciclosporin to sirolimus in two kidney transplant recipients was published by our group in 2004.[214] This preliminary experience was subsequently confirmed by Stallone at al.,[215] who reported regression of cutaneous Kaposi's sarcoma in 15 kidney transplant recipients after a switch from an immunosuppressive regimen based on ciclosporin and myphenolate mofetil to a regimen based on sirolimus. Among the questions raised by these results is whether the Kaposi's sarcoma lesions regressed because sirolimus was given, because the calcineurin inhibitors or MMF was discontinued, or for both reasons. In the study by Stallone et al.,[215] ciclosporin and MMF were stopped and sirolimus was immediately started. Conversely, in six patients in our series, the immunosuppressive treatment was strongly minimised prior to initiating sirolimus therapy, but regression of Kaposi's sarcoma lesions was not obtained until sirolimus was introduced.[216,217] This finding suggests that the effect observed in Kaposi's sarcoma was due to the antitumour action of sirolimus rather than to cessation of ciclosporin or switching to less intensive immunosuppression. In the study by Stallone et al.,[215] expression of VEGF and a VEGF receptor was increased in samples of cutaneous Kaposi's sarcoma lesions, whereas activation of Akt and S6K1 was reduced. This observation suggests that sirolimus contributed to regression of the lesions by inhibiting the Akt–S6K1 signalling pathway.

5. Managing Immunosuppressive Therapy in the Transplanted Patient at Risk of or Affected by Cancer

The growing advances in knowledge of cancer pathogenesis and the different pro-oncogenic

profiles of distinct immunosuppressants have raised new questions:

- Are differences in the carcinogenic activity of immunosuppressive drugs clinically relevant?
- Should immunosuppressive therapy be adapted to patients' pre-transplant malignancy risk?
- What is the best approach to managing immunosuppressive therapy after a post-transplant *de novo* malignancy?

These are crucial, but difficult, questions for which completely satisfactory answers are currently lacking.

5.1 Preventing Post-Transplant *De Novo* Malignancy

To answer whether immunosuppressive therapy can be adapted to patients' pre-transplant malignancy risk, two key points must be known: the patient's risk and the corresponding therapy. The main risk factors for the development of post-transplant *de novo* malignancy have been clearly identified (table III). However, it is difficult to estimate the recipient's individual risk accurately, and it is even more difficult to establish a risk threshold for making decisions.

Table III. Risk factors for the development of post-transplant *de novo* malignancy

Immunosuppressive therapy
antibody induction therapy
high exposure levels
number of drugs employed
pre-transplant use
Viral infection (table II)
Chronic renal failure
Conventional risk factors
smoking
sun exposure
analgesic abuse
previous malignancy
Demographic factors
age
male gender
White ethnicity
absence of diabetes mellitus

The clinical results of different immunosuppressive regimens available to date suggest that immunosuppressive therapies that contain mTOR inhibitors are associated with a lower *de novo* malignancy risk, and that this risk is even lower if the regimen does not contain calcineurin inhibitors.[208,209,211] The final decision in a patient should weigh the risk of cancer mainly with the immunological risk and, obviously, with the various spectrums of other pharmacological secondary effects. Certain patients are at exceptionally high risk for developing malignancy-related morbidity and/or mortality and could benefit from immunosuppressive therapy that has a low risk for malignancy:

- Patients with a history of several NMSC are at risk for multiple skin cancers in the future.[218,219] Since several RCTs show that the incidence of *de novo* NMSC is clearly reduced by the use of mTOR inhibitor regimens compared with other immunosuppressive therapies,[208,211] these regimens might significantly benefit these patients by reducing morbidity and mortality.
- Second transplant recipients with a history of PTLD and Kaposi's sarcoma are at high risk of cancer recurrence after the reintroduction of immunosuppressive therapy.[220-223] Experimental data has demonstrated the activity of mTOR inhibitors against EBV B cell lymphomas in several *in vitro* and murine models employing human-derived tumours.[206,207] However, the lack of clinical evidence of reduced PTLD incidence with mTOR inhibitor treatment, and previous reports – although in a small number of patients – showing the feasibility of re-transplantation without recurrence using other immunosuppressive therapies,[224,225] do not allow mTOR inhibitor regimens to be strongly recommended. In our opinion, mTOR inhibitors combined with MMF, which has shown a specific anti-herpes virus

activity,[158] would be the preferred immunosuppressive regimen in these patients. There is no clinical evidence of the value of mTOR inhibitors in preventing *de novo* Kaposi's sarcoma. However, the demonstrated efficacy of sirolimus conversion in patients with post-transplant Kaposi's sarcoma and its anti-angiogenic activity strongly supports its use in re-transplant patients with previous Kaposi's sarcoma.[214-216,226]

- Liver transplantation for hepatocellular carcinoma in patients with cirrhosis.[68,227] The general antitumoural activity of mTOR inhibitors has been specifically demonstrated *in vitro* for human hepatoma cells.[228] From a clinical point of view, a sirolimus-based immunosuppression protocol appeared to have beneficial effects on tumour recurrence and survival with an acceptable rate of rejection and toxicity in a series of 40 patients with hepatocellular carcinoma who underwent liver transplantation.[225] This benefit appeared be greater in patients with disease more advanced than the standard Milan criteria.[225] Anecdotal but spectacular responses to sirolimus conversion after hepatocellular carcinoma recurrence in several cases reported in the literature also encourage the use of mTOR inhibitors in this scenario.[225,229-231]
- Recipients with a history of pre-transplant tumour with high risk of post-transplant recurrence.[219] Several studies have reported reduced overall rates of any post-transplant *de novo* malignancy and non-skin solid malignancy in association with mTOR inhibitor therapy.[208-210] This evidence supports the preferential use of mTOR inhibitor-based regimens in the management of these patients.

5.2 Immunosuppressive Therapy Management in Recipients with *De Novo* Malignancies

Surprisingly, there is very little evidence on the management of immunosuppressive therapy in recipients with *de novo* malignancies. Currently, the reduction of immunosuppression in organ transplant recipients is a strong recommendation with *de novo* Kaposi's sarcoma or PTLD, and the benefit/risk balance of this measure is debated more for other solid tumours.[232] Although the efficacy of reduction or even cessation of immunosuppression in Kaposi's sarcoma or PTLD has been clearly established,[222,233] this approach could be associated with a significant risk of acute rejection or graft loss,[234] and Kaposi's sarcoma frequently recurs when immunosuppressive therapy is reintroduced or a second transplant is performed.[235] Alternative strategies based on conversion to mTOR inhibitor therapy and withdrawal of other immunosuppressive drugs, especially calcineurin inhibitors, could achieve a balance between adequate levels of immunosuppression to protect the allograft, and a potentially anti-oncogenic effect. In addition to experimental data, anecdotal clinical reports support the value of conversion to sirolimus as an adjuvant treatment for PTLD, in conventional combination with chemotherapy and/or to specific therapy targeting B cells, such as rituximab.[236,237] However, the efficacy of conversion to sirolimus and calcineurin inhibitor withdrawal in post-transplant cutaneous Kaposi's sarcoma has been strongly established.[214-216,226] The validity of this approach does not conflict with conventional treatment of Kaposi's sarcoma lesions, such as radiotherapy or surgical excision when indicated. Clinical data on visceral and severe forms of Kaposi's sarcoma are scarce. Lebbe et al.[226] recently reported 14 cases of Kaposi's sarcoma, including several cases of severe cutaneous Kaposi's sarcoma or visceral involvement. Three patients relapsed and a further three showed no response; the authors

suggested that the reasons for the ineffectiveness of sirolimus might have been a long delay before its introduction, and the extent and severity of the Kaposi's sarcoma lesions.[226]

NMSC is often easily resolved with surgical treatment. However, some patients have multiple skin cancers and others have cancers with a high risk of metastasis and even death.[28,29,48,83,111,113-116] In these patients, reduction of immunosuppression is a useful adjuvant strategy.[238] An international expert consensus for the reduction of immunosuppression for transplant-associated skin cancer has recently been published.[218] This exceptionally valuable publication has developed consensus on the level of tumour burden or metastatic risk of skin cancer warranting consideration of reduction of immunosuppression, and on the risks associated with reduction of immunosuppression in patients with multiple or high-risk skin cancers. However, this consensus does not address another reasonable strategy, consisting of conversion from a calcineurin inhibitor-based immunosuppressant regimen to one based on mTOR inhibitors.[239] In our opinion, despite the lack of direct evidence, there are enough data supporting a decreased risk of *de novo* NMSC with mTOR inhibitors compared with other immunosuppressive regimens to recommend this alternative.[208,211] mTOR inhibitors can be used in different doses or combinations, depending on the patient's immunological risk and risk of cancer, to ensure a minimal risk of rejection or graft lost and to lower morbidity and mortality associated with skin cancer. Several ongoing studies may support this suggestion.

Conventional oncological treatment is the cornerstone of post-transplant solid tumour management. However, evidence of faster and more aggressive progression of solid tumours in patients undergoing immunosuppressive therapy has already been discussed in this article. These data reaffirm the appropriateness of reducing immunosuppression in organ transplant recipients after the development of a solid tumour. The risks of rejection and graft loss, and the lack of evidence of a clear benefit to patient survival, frequently lead physicians to adopt a conservative attitude, even in kidney transplant recipients. In our opinion, a significant reduction of immunosuppression can be strongly recommended. This could improve response to appropriate oncological treatment for the specific tumour type. Importantly, experimental data from Koehl et al.[174] support the clinical experience of many physicians that kidney transplant recipients with solid cancers are able to undergo large reductions in immunosuppressive therapy for long periods without signs of rejection. In a combined murine model of cancer and heart transplantation, these authors demonstrated that the presence of tumours seemed to have a positive effect on allograft survival.

Experimental evidence of the efficacy of mTOR inhibitors in reducing tumour growth and metastasis should also be considered. mTOR inhibitors by themselves may have a positive impact on patient prognosis. In addition, they allow safer withdrawal of other immunosuppressive drugs with a demonstrated pro-oncogenic effect, especially calcineurin inhibitors. Finally, mTOR inhibitors seem to show a synergistic action when used in combination with anti-neoplastic agents.[239] Taken together, these findings support the use of mTOR inhibitors as an adjuvant therapy in the treatment of post-transplant solid tumours. However, only a comprehensive knowledge of the full circuitry of a signalling pathway allows the biological consequences of disturbing that pathway to be predicted. The existence of negative-feedback loops in the Ras-PI3K-mTOR signalling pathway means that prolonged mTOR inhibitor treatment would probably lead to enhanced PI3K-AKT activation in some tumours,[191] as has been observed in several tumour cell lines grown in culture, and in tissues from patients in clinical trials of rapamycin analogues.[240] In a subset of tumours,

this hyperactivation of PI3K-AKT signalling could make the tumour more aggressive. These findings suggest that the recommendation of the appropriate indications for mTOR inhibitors probably wait until results are available of clinical trials on the efficacy of this pharmacological group in different tumour types that are currently in progress. Finally, the use of mTOR inhibitors as antitumour agents in organ transplant recipients, specially in renal transplant recipients, would be limited by the presence of a significant degree of proteinuria or severe deterioration of renal function.[241]

6. Current Perspectives and Future Directions

In recent years, post-transplant malignancy has been recognised as a major limitation to the success of organ transplantation, and there has been an enormous growth in knowledge about cancer biology, the molecular effects of immunosuppressants and the intersections between both areas. Hope lies in the various efforts that are being directed to monitoring the minimal doses of immunosuppressants required to achieve allograft survival and to avoiding the secondary effects related to over-immunosuppression. Similarly, the search for some degree of tolerance could significantly reduce malignancy-related morbidity and mortality in organ transplant recipients. Reports of the initial results of studies of the effects of mTOR inhibitor therapy on the incidence of cancer in organ transplant recipients strongly indicate separate pathways for pharmacological immunosuppression and oncogenesis. The first evidence of a reduced incidence of *de novo* malignancy with mTOR-inhibitors should be followed by long-term results and studies in non-kidney transplant recipients. Finally, the information gap about the role of mTOR inhibitors in the management of patients with post-transplant malignancies must be closed as soon as possible.

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Correspondence: Dr *Alex Gutierrez-Dalmau*, Department of Nephrology and Renal Transplantation, Hospital Clinic, Villarroel 170, Barcelona, 08036, Spain.
E-mail: adalmau@clinic.ub.es