Immunosuppressive therapy and post-transplant malignancy

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Introduction

Organ transplantation is a well-established method for the therapy of end-stage organ failure. The emergence of novel immunosuppressive regimens has reduced the risk of rejection and extended the life expectancy of organ recipients. The long-term outcome of these patients is now challenged by life-threatening complications such as cardiovascular disease, infections and post-transplant malignancies. Malignancy is a well-recognized complication of transplantation and can manifest as de novo cancer, as a recurrence of a pre-existing malignancy or from transmission of malignancy from the donor. Recent studies show that tumour incidence increases with time after organ transplantation and is related to the intensity of immunosuppression [1,2]. Overall, a 3- to 4-fold increased incidence of cancer has been observed in transplant patients compared with age-matched controls in the general population. During immunosuppressive therapy, there is a higher frequency of some relatively rare tumours that tend to be biologically more aggressive than those that occur in the general population [3].

The relative risk for developing skin cancer in allografted patients is increased up to 70% in regions with high sun exposure [4]. Post-transplantation lymphoproliferative disorder (PTLD) occurs in up to 11% of renal transplant recipients and holds the major cause for cancer-related mortality. There is a 400- to 500-fold increase in Kaposi sarcoma compared with controls of the same ethnic origin and a 100-fold increase in vulval and anal carcinomas in transplant recipients [5]. The aetiology of post-transplant malignancy is believed to be multifactorial in nature and probably involves impaired immunosurveillance of the host, direct carcinogenic effects of some immunosuppressive agents and depressed antiviral immune activity referring to a number of common viral-related post-transplant malignancies.

To minimize the risk of malignancy after transplantation, it is important to evaluate the effects of an immunosuppressive agent on cancer incidence and tumour growth. However, the common use of combination therapies limits the interpretation of single-agent effects on the tumorigenesis process. There is now growing evidence that newer immunosuppressive drugs exert anti-neoplastic effects and could therefore simultaneously address the risk of allograft rejection and cancer in transplant recipients. The focus of this review is to illustrate known pro- and anti-neoplastic mechanisms attributed to some currently used immunosuppressive drugs.

mTOR inhibitors

The mammalian target of rapamycin (mTOR) is a critical modulator of protein translation. Emerging data indicate that mTOR plays a pivotal role in regulating a plethora of signalling pathways activated in response to growth factors and metabolic changes [6]. Inhibitors of mTOR (mTORis) exert their immunosuppressive activity by impeding the response to interleukin-2 (IL-2) and thereby blocking the activation of T- and B-cells. After binding of the mTOR inhibitor to the 12-kDa immunophilin FK506-binding protein (FKBP12), this complex interacts with and inhibits the serine/threonine kinase mTOR [6]. Currently used mTOR inhibitors in transplantation medicine include rapamycin (Rapamune®, Wyeth, Madison, NJ, USA), rapamycin-derivatives RAD001 (Everolimus®, Novartis, Basel, Switzerland) and CCI779 (Temsirolimus®, Wyeth), and the rapamycin analogue ap23573 (Deforolimus®, ARIAD Pharmaceuticals, Cambridge, MA, USA).

Multiple effects of mTORis on cellular signalling have been described. Rapamycin induces inhibition of the phosphatidylinositol-3-kinase (PI3K) signalling pathway that plays a key role in regulation of proliferation, survival, mobility and angiogenesis [7]. Rapamycin further inhibits signal transducer and transcription activator 3 (STAT3) signalling [8]. STAT3 mediates the expression of a variety of genes in response to cell stimuli and is involved in many cellular processes such as cell growth and apoptosis.
transplant showed a significantly reduced risk of developing any post- or even in combination with calcineurin inhibitors (CNI) the mTOR inhibitor drugs sirolimus and everolimus alone types of agents. Maintenance of immunosuppression with inhibitors alone (CsA or tacrolimus) or a combination of both setting of organ transplantation, mTOR is anticipated [20]. In contrast to their status in clinical oncology, in the setting of organ transplantation, mTORs are anticipated to exert a dual role by providing adequate immunosuppression to prevent organ rejection and simultaneously impair tumour development. A multivariate analysis of renal transplant patients examined the incidence of post-transplant malignancies in patients receiving mTOR inhibitors alone (everolimus or sirolimus), calcineurin inhibitors alone (CsA or tacrolimus) or a combination of both types of agents. Maintenance of immunosuppression with the mTOR inhibitor drugs sirolimus and everolimus alone or even in combination with calcineurin inhibitors (CNI) showed a significantly reduced risk of developing any post-transplant de novo malignancy and non-skin solid malignancy compared to a therapy with CNI alone [21]. Analysis of five multicentre studies of patients after renal transplantation revealed that patients receiving a rapamycin-based therapy without CsA or a rapamycin maintenance therapy after early CsA withdrawal had a significantly lower rate of malignancy in the first 2 years after transplantation [22]. Recent clinical trials in renal transplant recipients have further confirmed the significantly reduced risk of developing any malignancy under treatment with mTOR inhibitors [23,24]. However, in the Symphony study that investigated the efficacy and relative toxicity of four different immunosuppressive regimens in 1645 renal transplant recipients, the highest cancer incidence was noted in the rapamycin group after 12 months of follow-up [25].

Azathioprine and mycophenolic acid

Azathioprine (AZA) is a purine analogue that is incorporated into cellular DNA where it inhibits the purine nucleotide synthesis and interferes with RNA synthesis and metabolism. Its strong correlation with tumour incidence in transplant recipients is reflected by the fact that it is listed as a human carcinogen in the 11th Report on Carcinogens of the US Department of Health and Human Services [26]. Two large prospective epidemiological studies reported a high incidence of non-Hodgkin’s lymphoma, squamous cell cancers of the skin, hepatobiliary carcinomas and mesenchymal tumours in renal transplant patients, who were treated with AZA and prednisone [26]. The high incidence of skin cancers in the course of treatment with AZA is proposed to be due to its synergism with UV radiation in carcinogenesis. It has been shown that AZA causes the accumulation of 6-thioguanine in patients’ DNA, and 6-thioguanine and UVA are synergistically mutagenic [27]. Furthermore, the intercalation of thioguanine into the DNA inhibits repair and induces codon misreads that could encounter for AZA-mediated cytotoxicity and carcinogenesis [28]. However, in the era of combination therapies the effects of lower doses of AZA on tumour development in a transplanted patient are difficult to determine. Compared with the more powerful immunosuppressant CsA, AZA has been associated with a lower cumulative incidence of tumours after transplantation [29]. Hence, a clear correlation of less intensive AZA use with cancer development in transplant recipients has not been established.

Mycophenolic acid (MPA) reversibly inhibits inosine 5’-monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in the de novo synthesis of guanine nucleotides. MPA displays high lymphocyte specificity and cytotoxicity due to the higher dependence of activated lymphocytes on both salvage and de novo synthesis of guanine nucleotides relative to other cell types. The morpholinoethyl ester of MPA, mycophenolate mofetil (MMF), has potent immunosuppressive properties and is used worldwide in transplantation medicine to prevent organ rejection. In addition to the well-known immunosuppressive effects, an increasing number of studies document the antiproliferative properties of MPA on a variety of cell lines [30]. IMDPDH activity is increased significantly in cancer cells, and it was thus considered a sensitive target for chemotherapy [31]. In fact, MPA has been shown to inhibit the growth of tumour cells in vitro and in mouse xenografts [32]. This observation led to testing of MPA
in small cohorts of patients with a variety of cancers in the 1970s. Considerable dose-limiting gastrointestinal toxicity limited the success of these trials due to the use of the diethanolamine salt of MPA rather than the currently prescribed MMF prodrug [33,34].

One molecular mechanism contributing to MPA’s antitumour activity might be its ability to induce apoptosis as demonstrated in human neuroblastoma-, B-lymphoma- and multiple myeloma cells [35–37]. Furthermore, the antitumour activity of MPA is linked to its antiangiogenic properties. MPA was found to potently inhibit endothelial cell proliferation in vitro and block tumour-induced angiogenesis in vivo [38]. We have recently shown a preferential sensitivity of endothelial cells to MPA treatment compared to different tumour cell lines. A glioblastoma cell line (U87) displayed resistance to MPA treatment in vitro but MPA significantly inhibited U87 tumour growth in vivo suggesting an important role for the tumour microenvironment in MPA response [39]. In another study that used RNA interference, the knockdown of one of the two known isoforms of inosine monophosphate dehydrogenase (IMPDH-1) was sufficient to cause endothelial cell cycle arrest. In addition to that data, anti-endothelial activity of MPA was detected in two recent pharmacological screens among FDA-approved drugs that aimed to identify compounds with antiangiogenic properties [40,41].

The interaction of MMF with adhesion molecules seems to play an important role in preventing integrin-dependent tumour dissemination and metastasis. MMF was shown to induce alterations of the beta1 integrin profile and thereby block tumour cell adhesion to vascular endothelium [42].

Another mechanism discussed for the antitumour activity of MPA is the induction of differentiation in tumour cells. Addition of MPA to cultures of HL-60, neuroblastoma and melanoma cells resulted in a decreased cellular level of guanine nucleotides and the induction of cell differentiation [43–45].

In a phase I clinical trial, MPA was tested as an anti-cancer agent in patients with advanced multiple myeloma. Partial response to the drug adds evidence that inhibition of the IMPDH pathway may be a promising novel targeted therapy to overcome drug resistance in multiple myeloma [46]. Furthermore, synergy between imatinib and MPA in the treatment of chronic myelogenous leukaemia (CML) has been reported [47].

Evidence of potential anti-neoplastic effects of MPA in transplant recipients are provided by a number of clinical studies. In a large cohort study that examined the risk of malignancies with MMF in renal transplant recipients, a trend towards a lower risk of malignancy and a significant increase in time to malignancy in the MMF group were found. [48]. Data from the transplant registry of the International Society for Heart and Lung Transplantation (ISHLT) showed in a multivariate analysis that the use of MMF in standard immunosuppressive regimens is associated with a significantly lower risk of developing malignancy after orthotopic heart transplantation [49]. An independent report compared the cancer incidence in AZA- versus MMF-treated patients using data from the SRTR database [the SRTR database contains information collected by the Organ Procurement and Transplantation Network (OPTN)]. In 17 145 adult renal transplant recipients with pre-existing diabetes mellitus, the incidence of any post-transplant malignancy was higher in AZA-treated patients compared with MMF-treated patients (3.7% versus 2.2%, respectively). This report also indicated a significant difference in lymphoproliferative malignancies (0.6% versus 0.3%) and de novo solid tumours (2.5% versus 1.6%) [50]. However, the Tricontinental Multicenter study and the US Randomized study that compared the treatment of MMF with AZA in cadaveric renal recipients showed no difference in the incidence of any malignancy between the two drugs [51,52]. Since MMF is more effective than AZA in preventing allograft rejection [53] and might even lower the risk of malignancy, it offers a favourable profile in transplantation medicine.

**FTY720**

The immunosuppressive agent FTY720 is a synthetic analogue of sphingosine and was isolated from culture filtrates of the ascomycete *Isaria sinclairii*. FTY720 inhibits the egress of lymphocytes from lymph nodes to efferent lymphatics and blood via modulation of sphingosine-1-phosphate (S1P) receptors on lymphocytes [54]. The efficacy and safety of FTY720 in de novo kidney transplant patients was proven in combination with CsA and corticoids in a phase II study [55]. However, the multifaceted mode of action of FTY720 opens up other possibilities for its use, e.g., as anti-cancer agent.

FTY720 was shown to exert potent anti-tumour and anti-metastatic activities in different tumour types in vitro and in vivo. Pro-apoptotic activity of FTY720 was linked to its ability to inhibit Akt- or downregulate Bcl2 survival signalling [56].

In multiple myeloma cell lines, Yasui and colleagues showed that FTY720 induces mitochondria-mediated apoptosis followed by the release of mitochondria proteins, cleavage of PARP and activation of caspase-8, -3 and -9. FTY720 also inhibits other survival signals triggered by cytokines such as IL-6 via inhibition of STAT3, Akt and ERK phosphorylation in a dose-dependent manner [57].

The potent antiangiogenic activity of FTY720 is linked to its functional antagonism of vascular S1P receptors and transactivation of the CXCR4 chemokine receptor [58,59]. Recent studies showed that FTY720 decreases vascular permeability induced by the key angiogenic protein VEGF [60].

Azuma and colleagues showed that FTY720 caused cytoskeletal changes in cancer cells that resulted in mis-shaped cells with reduced filopodias. In addition, it reduced the expression of integrins on the surface of cancer cells and impeded their ability to adhere and migrate to extracellular matrix proteins [61]. Evidence that FTY720 could inhibit cancer metastasis process comes from a study of Zhou et al. in 2006. They showed that FTY720 markedly inhibited prostate cancer cell invasion by down-regulating GTP-bound RhoA, a protein that is associated with metastasis in many types of malignancies [62].

Recent published data support the investigation of FTY720 as a novel therapeutic approach for patients with
blasto crisis in chronic myelogenous leukaemia (CML) or adult acute lymphoblastic leukaemia that express the BCR-ABL oncogene. The prognosis in these patients is usually poor. FTY720 was shown to induce cell death and reduce clonogenicity in myeloid and lymphoid cell lines and patient-derived progenitor cells that were either sensitive or resistant to imatinib and/or dasatinib. Of note, no adverse effects of FTY720 on normal bone marrow progenitor cells were detected in this study [63].

Although Novartis discontinued the development of FTY720 for allograft recipients due to a lack of benefits compared with MMF in a large phase III clinical trial, the multifaceted activities of this compound described here warrant further clinical testing.

**Calcineurin inhibitors**

CsA and tacrolimus are among the most widely used immunosuppressive agents belonging to the family of CNI. Their major effect is to block the IL-2 expression and to increase the production of transforming growth factor beta (TGFβ), thereby impairing immune response [64,65]. CNIs are linked to a higher incidence and aggressive progression of neoplasm. These effects were mainly attributed to the impairment of the organ recipient’s immune surveillance [66–68]. However, Hojo and colleagues were able to prove that CsA-treated cells underwent significant morphological alterations, including induction of pseudopodial protrusions, increased cell motility and invasive growth, thereby inducing cancer progression by a cell-autonomous mechanism probably mediated by TGFβ1 [69]. TGFβ is a pleiotropic cytokine that has a dual role in carcinogenesis. Initially, it acts as a tumour suppressor and causes growth arrest of epithelial cells and cells in the early stages of cancer [70]. In established tumours, TGFβ exerts an effect that is favourable for the survival, progression and metastasis of the tumour [71]. These data suggest that TGFβ-mediated effects of CsA might rather promote the growth of dormant tumours than inducing de novo cancer. Possible mechanisms for enhanced de novo cancer development induced by CsA include its ability to inhibit the DNA repair apparatus that facilitates the accumulation of DNA mutations [72,73]. Combined with depletion of activated T-cells, this could result in decreased clearance of altered cells.

The results of clinical studies evaluating the risk of cancer development in patients treated with CNIs are conflicting. In a prospective, randomized study, patients receiving full-dose CsA had an increased incidence of post-transplant malignancy, as compared with those receiving low doses (32% versus 20% of patients, respectively). This trend was apparent for all types of cancers, including skin cancer, PTLD and other cancers [1]. In a series of 106 patients with hepatocellular carcinoma who underwent liver transplantation, a higher cumulative dosage of CsA showed a negative effect on the recurrence-free survival rate [74].

The comparison of cancer incidence in patients treated with CsA versus tacrolimus yielded conflicting results. Although tacrolimus is associated with a slightly higher immunosuppressive potency [75], there are studies that report a reduction in the incidence of de novo solid tumours if these patients were treated with tacrolimus versus CsA [76,77].

On the other hand, tacrolimus treatment was associated with a higher rate of PTLD that might be correlated with its greater immunosuppressive effect. The analysis of 41 686 renal allograft recipients included in the Scientific Registry of Transplant Recipients (SRTR database) demonstrated that, in patients who did not receive induction therapy, the cumulative incidence of PTLD was lower in CsA-treated patients than in tacrolimus-treated patients. This difference disappeared if the patient had received induction therapy, which itself poses the recipients to a 1.8-fold higher risk for PTLD development. A possible explanation could be the strong immunosuppressive effect of induction therapy that might override the differences among the maintenance drugs [76].

**Biological agents and small molecules**

Lymphocyte-depleting antibodies are strongly associated with a higher risk of post-transplant malignancy, in particular with virally induced cancer. Furthermore, the use of induction regimens containing lymphocyte-depleting antibodies is one of the best-known risk factors for the development of PTLD. A striking rise in the frequency of PTLD was first described in 1990 in heart transplant recipients, who received the monoclonal antibody against CD3 (OKT3, muromonab) in addition to standard immunosuppressive therapy [78]. Subsequent studies investigating the effect of anti-thymocyte globulins and OKT3 on PTLD development supported this data [79]. In contrast, treatment with anti-IL-2 receptor antibodies such as basiliximab or daclizumab was not correlated with an increased risk for post-transplant malignancy [2,80,81]. These antibodies target specifically activated T-cells, macrophages and monocytes whereas the total number of lymphocytes remains unaffected. Using the Collaborative Transplant Study (CTS) database, a recent study has evaluated the influence of different induction therapies on graft survival and the incidence of non-Hodgkin lymphoma in 112 122 renal allograft recipients. The highest rates of lymphoma were found in patients treated with equine-/rabbit-anti-thymocyte globulin or OKT3, whereas IL-2 receptor antibodies offered the best risk-to-benefit ratio with regard to graft survival and lymphoma incidence [82].

The emergence of novel targeted biologicals and small molecular agents has fuelled hope to develop therapies that deliver immunosuppression without long-term toxicity. Currently, a number of interesting biologicals are investigated in preclinical and clinical studies including (i) belatacept, a fusion protein of the Fc-fragment of human IgG1 immunoglobulin with the extracellular domain of CTLA-4 that blocks the interaction between CD80/86 and CD28 costimulatory pathways; (ii) anti-interleukin-15; (iii) anti-CD40 and (iv) efalizumab, a humanized anti-LFA1 monoclonal antibody. Further, targeted inhibition of a series of pathways is tested using novel small molecular inhibitors of pyrimidine synthesis (FK778), Janus kinase 3 (JAK3, CP-690550) and protein kinase C (PKC, AEB-071). It remains to be elucidated whether these promising novel agents will
reduce the incidence of post-transplant malignancies as well as infectious and cardiovascular-related complications [83].

Conclusion

The advent of novel immunosuppressive regimens has steadily reduced the incidence of acute transplant rejection. With the increased life expectancy of allograft recipients, post-transplant malignancy has emerged as an important cause for mortality in these patients. The tremendous growth of knowledge in cancer biology and the molecular mechanisms underlying anti-neoplastic effects of immunosuppressive agents offers new possibilities to address this issue. Therefore, prospective clinical studies are necessary to evaluate the impact of selected immunosuppressive agents on cancer development and progression.

Conflict of interest statement. None declared.

References


Ethnic advantages in kidney transplant outcomes: the Hispanic Paradox at work?

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Introduction

Kidney graft loss in the first 10 years following transplantation is a significant problem despite tremendous scientific advances in treating acute and chronic rejection. While short-term acute rejection has come under control with 1-year survival rates surpassing 91%, long-term graft survival remains inadequate [1]. The 3-year, 5-year and 10-year deceased donor unadjusted graft survival rates are 78.6%, 67.1% and 40.8%, respectively, among all kidney recipients in the United States (USA) [2].

The loss of transplanted grafts is a major public health problem. Kidney transplantation is the treatment of choice for most patients with end-stage renal disease (ESRD) since it provides a longer length of life, better quality of life [3,4] and is more cost-effective than dialysis [5,6]. When kidney grafts fail, patients return to dialysis, creating an even greater demand for scarce kidney (re-)transplants and further burdening society with greater costs [7–9]. Mortality rates on dialysis following a failed kidney transplant are significantly higher than those prior to transplant [10]. The shortage of donated kidneys provides a moral and societal imperative to optimize their use. These concerns underscore the need to maximize long-term graft survival [11]. Understanding factors that contribute to long-term graft survival is critical to public health and its expectation that scarce resources be utilized efficiently. This paper examines the possible underlying demographic and socioeconomic factors associated with better graft survival among Hispanic kidney transplant recipients.

The presence of sociodemographic and socioeconomic disparities in graft survival rates compounds the problem of inadequate long-term graft survival. Health disparities can be defined as 'potentially avoidable differences in health (or in health risks that policy can influence) between groups of people who are more or less advantaged socially; these differences systematically place socially disadvantaged groups at further disadvantage on health' [12]. Most research on disparities in transplant outcomes has focused on African Americans who experience higher rates of chronic allograft nephropathy (CAN) compared to whites and other minorities. The mean time to CAN is shorter in African American recipients than in white recipients (18 versus 37 months), although the incidence of CAN is comparable [13]. African Americans also experience lower graft survival [14,15]. Beyond 3 years, African American recipients experience a 5–15% lower graft survival rate than that in whites [16,17]. The conditional half-lives for deceased donor kidneys in adult African American and white recipients are 8 years and 14 years, respectively [18]. Compared to whites, minorities receive poorer quality