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REVIEW

Immunosuppression and tumor development in organ transplant recipients: the emerging dualistic role of rapamycin

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Summary

Cancer morbidity and mortality are increasingly apparent risks in transplant recipients, thus reducing life quality and overall survival. These risks have largely been attributed to long-term immunosuppressive drug therapy, which remains necessary to prevent organ allograft rejection. Interestingly, however, recent studies challenge the premise that all immunosuppressive drugs necessarily promote cancer. A particular class of immunosuppressants, referred to as mammalian target of rapamycin (mTOR) inhibitors, has been shown to have potent anti-cancer effects that are presently being tested in clinical studies. The focus of this review is to present current evidence that allows us to understand better the dual immunosuppressive and anti-cancer functions of this class of drugs used to prevent allograft rejection. We will concentrate on the different functions of mTOR that allow it to simultaneously control the immune system and tumor development. We will also discuss results from current clinical studies that either support or refute this potential dualistic role.

Introduction

Organ transplantation has become a well-established procedure for curing patients of life-threatening diseases such as cardiomyopathy, chronic renal failure, or even cancer. However, to maintain the function of a transplanted allograft without immunologic rejection, the vast majority of transplant recipients are kept on various levels and combinations of immunosuppressive drugs for their entire life. Calcineurin inhibitors (CNIs), such as cyclosporine A (CsA), have been widely used on account of their excellent immunosuppressive properties mediated principally by inhibition of T-cell stimulation [1]. Unfortunately, one of the associated side-effects of immunosuppressive drugs, like CsA, is the development of post-transplant malignancy [2]. Regarding CNIs, they appear to pose an increased risk for cancer with cumulative exposure [3]. Whether this is on account of a long-term compromise of tumor-associated immunity [4], or to direct effects that promote tumor-cell aggressiveness [5], reduced DNA repair [6], or increased tumor angiogenesis [7], remains unclear. A strong case can be made for the hypothesis that immunosuppressive drugs in general nonspecifically promote cancer development. This argument is based on data indicating that nearly all immunosuppressive drugs are associated with high rates of cancer whether they are used to prevent transplant rejection or for the treatment of other inflammatory diseases. For instance, older generation drugs such as azathioprine and corticosteroids have also been linked to the development of cancer [8]. Indeed, it has been known for several decades that malignancies are a serious problem occurring in immunosuppressed transplant patients, inspiring the creation of the Israel Penn International Transplant Tumor Registry. Data from the Israel Penn registry indicate that cancer development occurs at a younger age in transplant recipients and tends to be more aggressive [9]. The incidence of cancer increases with time after transplantation [10], where skin cancer incidence after 20–30 years approaches 50% in regions of low sun exposure, and 80% in areas of high sun

exposure [11]. In fact, it has been estimated that ca 90% of all post-transplant malignancies are either squamous cell or basal cell carcinomas [12]. In the balance, lip carcinomas, post-transplant lymphoproliferative diseases, Kaposi sarcoma, anal and vulva carcinomas, and hepatocellular carcinomas (HCCs) are also elevated, not to mention a wide-variety of other cancer types [13]. Most importantly as a consequence, skin cancer, and the variety of other types of cancer, result in a death rate in transplant patients with functioning grafts that compares closely to cardiovascular disease. Therefore, to improve long-term transplant recipient survival, and to reduce associated morbidity, we need to optimize for immunosuppressive drugs that at least minimize the risk for tumor development. One potential breakthrough in this respect is the use of immunosuppressive mammalian target of rapamycin (mTOR) inhibitors (mTORis), which have immunosuppressive and tumor suppressor functions [8]. In the following review, we focus on the immunosuppressive and anti-cancer properties of mTORis, and their future perspectives in organ transplantation for decreasing the ever-increasing problem of cancer.

Historical perspectives on rapamycin

Rapamycin, the first known mTORi, was found during a discovery program for anti-microbial agents from natural resources in 1975 [14]. A strain of Streptomyces hygroscopicus was isolated from a soil sample collected at Easter Island (Rapa Nui), from which the active substance rapamycin was named. More than 10 years later the role of rapamycin as a potential immunosuppressive agent was described [15]. Since these early days of development, more than 2000 publications can be found in the literature about the role of this drug as an immunosuppressant in transplant recipients. In addition, pharmaceutical companies have developed several chemical analogs. Today this class of mTORis consists primarily of rapamycin (Rapamune®/sirolimus, Wyeth, Madison, NJ, USA) and its derivates (CCI779/temsirolimus, Wyeth; RAD001/everolimus, Novartis, Basel, Switzerland), as well as the analogue ap23573 (ARIAD Pharmaceuticals, Cambridge, MA, USA). Other analogues such as 32 deoxy-rapamycin (SAR943) or zotarolimus (ABT-578, Abbott Laboratories, Abbott Park, IL, USA) have been developed to prevent allergic inflammation [16], or for cardiovascular stent implantation [17], but have not been used for the treatment of transplant recipients.

Molecular biology of mTOR

A key to understanding the multiple roles of rapamycin and its derivates as a class of drugs is in their capacity to inhibit an integral part of the cell-signaling machinery, namely mTOR. mTOR is a 289 kD serine/threonine kinase that maps to the human chromosome 1p36.2. The molecule is a downstream effector of the phosphatidylinositol 3-kinase (PI3K)/AKT (protein kinases B) signaling pathway, which mediates essential cell survival and proliferation signals. Phosphorylation of PI3K is induced by many tyrosine kinase receptors [e.g., epidermal growth factor receptor (EGFR), and vascular endothelial growth factor receptor (VEGFR)], as well as integrins and G-protein coupled receptors. Downstream effectors of mTOR are the protein 70S6 kinase (p70S6K), as well as the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). In response to proliferative stimuli initiated by a variety of growth factors/hormones, both p70S6K and 4E-BP1 are phosphorylated, leading to active translation of mRNA (Fig. 1). IL-2 is one cytokine that triggers cells (T cells) via the mTOR pathway, along with a host of other growth factors, some of which are critical for neoplasm formation. Importantly, rapamycin acts to inhibit mTOR by binding to FKBP12, forming a drug/immunophillin complex that modulates the activity of intracellular targets in various cell types.

Effects of rapamycin on the cell cycle

Mammalian target of rapamycin is a pivotal regulator of cell growth and proliferation for different cell types, including particularly lymphocytes, endothelial cells, and tumor cells. One end-effect of mTOR inhibition is a 15–20% inhibition of overall protein translation, leading to G1 cell cycle arrest [18]. There are also specific regulatory effects on the synthesis of essential cell cycle proteins, such as cyclin D1 and c-myc [19]. Cyclin D1 in association with cyclin-dependent kinase 4 (CDK4) is essential for retinoblastoma protein phosphorylation (pRb). In addition, rapamycin stabilizes p27, which inhibits the activity of the cyclin/CDK complex [20]. Rapamycin also blocks the elimination of CDK inhibitor p27 and facilitates the formation of cyclin/CDK-p27 complexes [21,22]. Moreover, it has been shown that rapamycin inhibits signal transducer and transcription activator 3 (STAT3) via mTOR [23], which in-turn mediates the stabilization of cyclin D1 and up-regulates c-myc (Fig. 1). Cell cycle arrest after administration of the rapamycin analogue CCI-779 has been observed in myeloma cells, showing up-regulation of p27 CDK inhibitor, which contributes to G1 arrest. In keeping with this observation, c-myc and cyclin D1 show a marked decrease in expression with low doses of CCI-779 [24]. It is also interesting to note that STAT3 is constitutively active in many cancers, and therefore may serve as one of several indirect molecular targets of rapamycin in malignancy. Supporting the hypothesis

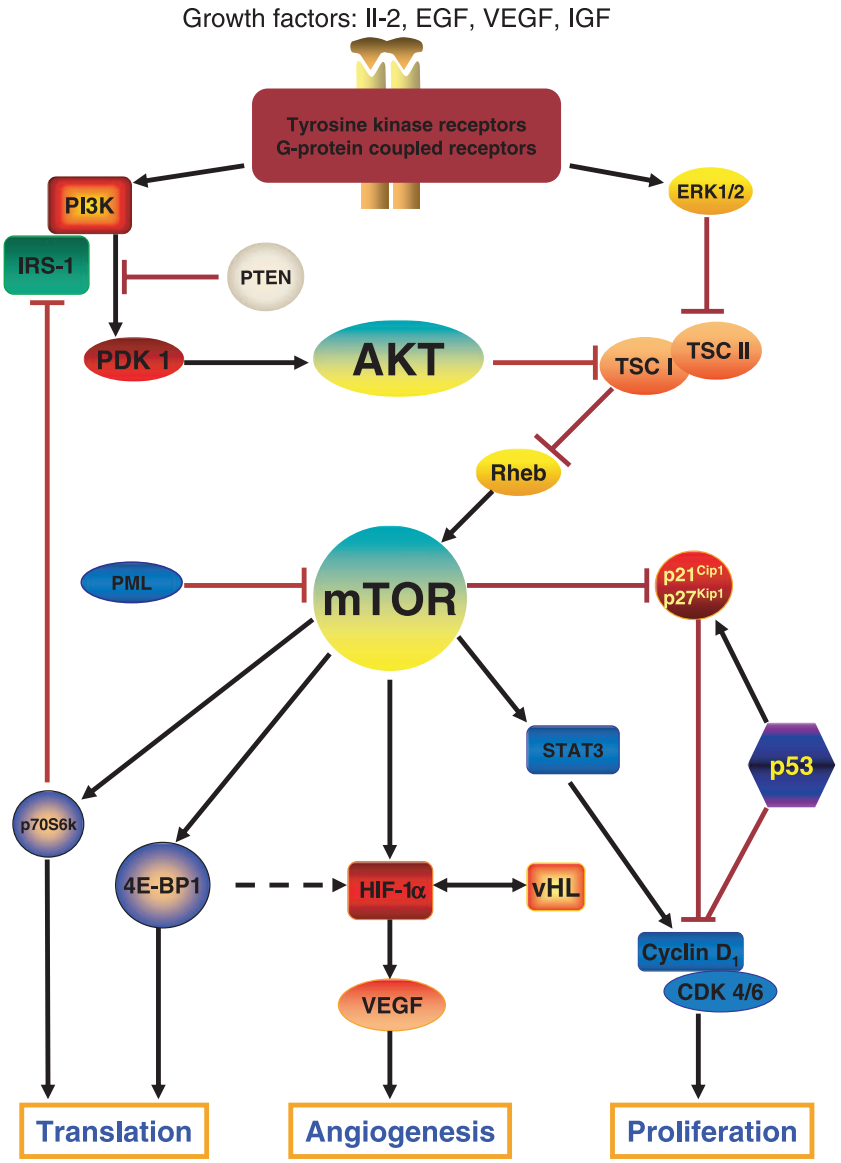

Figure 1 The integral role of mTOR in malignancy development and growth.

that rapamycin acts against cancer cells, it has been shown that IFN-γ is able to dephosphorylate STAT3 when mTOR is blocked with rapamycin, thus inducing cell apoptosis [25].

In more specific relation to transplantation, post-transplant lymphoproliferative disorders (PTLDs) are a serious problem in renal transplant recipients, with a particularly poor prognosis in older patients and recipients with systemic disease [26]. Excessive growth of Epstein–Barr virus (EBV) transformed B-lymphocytes is often the cause of this life-threatening disorder [27]. An increase in PTLDs has most often been associated with the use of CNIs [28], and is especially associated with 'over-immunosuppression' that is thought to block the ability of the immune system to fight EBV infection. This hypothesis is evidenced by the fact that PTLDs are known to respond in some cases to a reduction in immunosuppression. More recently, however, mTOR inhibition has been suggested to have potential effects against EBV-infected cells. There is now evidence that rapamycin inhibits the proliferation of EBV-induced B-cell lymphomas by down regulation of CDK4, and by increasing p27 expression [29]. It has also been demonstrated that RAD001 has anti-proliferative effects on EBV-transformed B-cells *in vitro* and *in vivo*, inducing apoptosis and growth arrest in the G0/G1 phase [30]. El-Salem *et al.* [31] suggest that mTOR is constitutively activated in the entire spectrum of PTLD subtypes, regardless of their EBV expression, indicating that this molecule could be an excellent therapeutic target. Indeed, some patients with PTLD have already

been successfully treated with rapamycin and rituximab [32], although controlled randomized studies are needed to make any final conclusions.

In further regard to lymphoid neoplasms, mantle cell lymphomas (MCLs) have been shown to be particularly sensitive to the use of mTORis. It is notable that MCLs show a t(11;24)(q13;q32) translocation which is associated with constitutive overexpression of cyclin D1. As mTOR promotes cell cycle progression via cyclin D1 [33], it is logical that mTORis have shown activity against MCL. Clinical trials with relapsed MCLs have been based on the use of CCI-779 [34] and RAD001 [35]. Synergistic inhibition of tumor cell growth in MCLs was achieved when RAD001 was combined with conventional chemotherapeutic drugs [36].

Also linked to the effects of mTOR on the cell cycle, it has recently been shown that RAD001 causes a significant G1 cell cycle accumulation and marked reduction of S-phase activity in HCC cell lines (Hep3B and SNU398) [37]. The apoptotic effects of mTOR inhibition could be enhanced by combining treatment with doxorubicin. This is consistent with a report from Beuvink *et al.* [38] who showed that RAD001 has a chemo - sensitizing effect when combined with oxaliplatin. Moreover, p53 status may determine the fate of cancer cells with regard to rapamycin-induced apoptosis. Cells with wild-type p53 that arrest in G1 remain viable, whereas p53 mutant sarcoma cells undergo apoptosis [39]. Most recently, it has been shown that p53 status in leiomyosarcomas determines the sensitivity of these cancers to mTORi treatment [40], and this observation is consistent with results we published previous to this report, where rapamycin/sirolimus prevented the spontaneous occurrence of sarcomas in a p53 knock-out mouse model [41]. In line with these findings, Hernando *et al.* [40] have reported up-regulation of mdm2, a repressor of p53, in smooth muscle tumors of PTEN - knockout mice, which was a direct effect of AKT phosphorylation leading to a stabilization of mdm2. Together, these data indicate that the effects of mTORis on the cell cycle are a critical component of their anti-neoplastic actions.

The effect of rapamycin on PTEN/AKT signaling

In addition to the downstream activities of mTOR, important upstream regulators of its activity may be altered in different cell types. Numerous growth factors and cytokines, such as VEGF, EGF, and IGF, trigger via PI3K through their receptor tyrosine kinase and G-protein coupled receptors [42]. PI3K activation leads to an accumulation of phosphatidylinositol-tri-phosphate (PIP3) and subsequent activation of AKT via pyruvate dehydrogenase kinase 1 (PDK1). PTEN, a tumor suppressor gene, counteracts AKT activation through PI3K elimination. This part of the pathway is directly upstream of mTOR and is constitutively active in various cancers, as well as hereditary disorders such as Cowden syndrome [40,43]. Dysregulation often stems from aberrations in the PTEN gene, where deletions or methylations are associated with development of malignancies [44]. Relative to this discussion, increased upstream activity is likely to lead to increased mTOR activity, which will promote cell proliferation and help to drive essential processes for tumors, including angiogenesis. Therefore, mTOR inhibition could provide a target to turn-off commonly observed dysregulation via the PI3K–AKT axis. For example, it has been shown that increased AKT activity significantly sensitizes multiple myeloma cells to mTOR inhibition by CCI-779 and rapamycin [45]. Not only is multiple myeloma cell proliferation inhibited by these substances, VEGF-mediated angiogenesis is also reduced in a dose-dependent manner, which, consistent with our reasoning, is more pronounced with PTEN mutations. *In vivo* experiments substantiate these findings by showing that CCI-779 significantly decreases proliferation and angiogenesis in a multiple myeloma tumor model [24]. A crucial role for the AKT-mTOR pathway has also been shown for leiomyosarcomas. Conditional smooth muscle PTEN knock-out mice reveal smooth muscle tumors of the gastrointestinal tract with constitutive up-regulation of AKT and subsequent mTOR activity [40]. These investigators linked the activation of TSC II (also upstream of mTOR, but downstream of AKT) phosphorylation to sarcoma formation, which could be abrogated with rapamycin treatment. AKT inhibits TSCI/II, which inhibits Rheb (Ras homologue enriched in brain) activation, leading to an increase in mTOR activity (Fig. 1). VEGF is up-regulated and angiogenesis promoted with a loss of function mutation of TSCI or TSCII [46]. Most recently Sodhi *et al.* [47] showed that signaling through G-protein coupled receptor and TSCII is a critical step of the mTOR activation process in transformed endothelial cells of Kaposi sarcoma. In connection to this signaling axis scheme, a number of investigators have now shown that renal transplant recipients receiving CsA, and subsequently developing Kaposi sarcomas, show remarkable tumor regression responses when later switching them to sirolimus [48–50]. In the Stallone *et al.* [48] study, the authors suggest that VEGF may have been elevated with AKT in these lesions, accompanied by p70S6K phosphorylation, thus providing at least one mechanistic explanation for the tumor responsiveness to mTOR inhibition. Even more remarkable and fundamental is the fact that tumor regression was possible in the presence of a full immunosuppressive regimen; controlled studies, however, need to be performed to confirm this observation.

Expression of these same signaling molecules has also been analysed in patients with HCC undergoing liver transplantation. AKT/mTOR signaling was found to be active in approximately 40% of these patients, but did not show any relation to the patient outcome [37]. *In vitro* experiments with HCC cell lines (Hep3B and SNU398) showed a marked reduction in p70S6K protein phosphorylation, but up-regulation of AKT after treatment with RAD001. We have recently reported a similar phenomenon with rapamycin use and gastric cancer cells [51]. One explanation for AKT up-regulation under mTOR inhibition relates to p70S6K inhibition of IRS-1 (insulin receptor substrate 1). Release of p70S6K inhibition on IRS-1 leads indirectly to an increase in AKT. This negative regulatory loop could explain the relative benign nature of tumors associated with TSC mutations, and also may provide a means for tumor resistance to mTOR inhibition [52] (Fig. 1). An experimental example of this potentially detrimental feedback loop comes from TSCII-deficient cells where rapamycin treatment re-establishes otherwise disabled AKT signaling, resulting in their protection from death on account of DNA damaging effects [53]. Nonetheless, clinical data in cancer patients showing that rapamycin may actually increase tumor resistance by increasing AKT activity have not been corroborated. We should also not conclude this subject without mentioning that the multi-protein mTOR complex (mTORC1) that is downstream of AKT, and is inhibited by rapamycin, has an 'associate' growth-factor sensitive complex (mTORC2), which appears to have positive upstream activity on AKT and is not sensitive to rapamycin. As a consequence of this discovery, although rapamycin has shown some ability to disrupt the mTORC2 complex in specific cell types, the entire mTOR pathway is not as completely blocked with rapamycin as originally suspected. Therefore, the mTOR-integrated pathway is very complex and we are most likely only seeing the 'tip of the iceberg'. At present, we can only conclude that while a detailed basis for the mTOR network is being worked out, there remains reason to believe from a molecular perspective that mTOR inhibitors have a realistic potential in treating certain types of cancer. Only further dissection of these pathways, and clinical studies, will provide the final answers.

The effect of mTORis on hypoxia signaling

An additional pathway influenced by mTOR involves hypoxia-inducible factor-1α (HIF-1α) and VEGF. Besides the direct effect of mTORis on tumor cell growth, the disruption of HIF-1α signaling in endothelial cells leads to a decrease in VEGF production and thus impaired angiogenesis [54]. VEGF transcription and translation levels are regulated via 4EB-P1 and HIF-1α [55], which are downstream of mTOR. HIF-1α, in particular, is stabilized under hypoxic conditions leading to nuclear transfer, and subsequent transcriptional activation of VEGF [56]. The regulation of VEGF through transcriptional activation by HIF-1α has been attributed a central role in the development of tumor blood vessels [57], and interestingly, has been linked to familial polyposis syndromes [58]. These molecules are regulated via mTOR-dependent and independent pathways [46]. In this respect our group has recently shown that disruption of HIF-1α signaling via rapamycin significantly impairs tumor cell proliferation and angiogenesis in an experimental model of gastric cancer [51]. It has also been shown that the responsiveness of renal cell carcinomas in clinical studies is linked to expression of HIF-1α [59]. This hypothesis is related to the von Hippel-Lindau (vHL) complex, which under normoxic conditions rapidly degrades HIF-1α. With loss of vHL function, as is commonly observed in clear cell renal cancer, there is accumulation of HIF-1α [60], leading to an increase in VEGF [61]. This effect is augmented by mTOR activation through promotion of protein stabilization and translation, thus, further increasing HIF-1α activity [62]. Therefore, mTOR is a molecular target for renal cell cancer patients and has important implications for clinical trials with mTORis, as vHL mutations and HIF-1α can serve as a biomarker for patient selection.

The promyebcytic leukemia (PML) tumor-suppressor gene has also been linked to the mTOR/HIF-1α pathway. Dysregulation of this tumor suppressor gene has been observed in several human cancers through effects on growth arrest, senescence, and apoptosis. PML apparently affects p53, pRb (Retinoblastoma) and SMAD (mothers against decapentaplegic homologue) [63]. Bernardi *et al.* [64] clearly demonstrated that PML is a negative regulator of HIF-1α and thus, loss of PML function induces neoangiogenesis. Furthermore, PML and mTOR physically interact and co-localize in the nucleus under hypoxic conditions. $PML^{-/-}$ cells show an increase in phosphorylation of mTOR, S6K, and AKT, causing a pro-cancer effect, which can be abrogated by rapamycin [64]. Further studies will be needed to determine whether this suppressor gene is also involved in transplantation-associated tumor development.

Mirshahi *et al.* [65] have proposed another interesting mechanism involving angiogenesis. They described a dramatic increase of VEGFR-1 and VEGFR-3 on human bone marrow endothelial cells after exposure to supernatants obtained from multiple myeloma and chronic lymphocytic leukemia cell cultures, causing an increase in endothelial cell proliferation and migration. This effect was blocked by the administration of inhibitors to ERK1/2, or mTOR, and was attributed to the inhibition of VEGF signaling. These findings underscore the notion

that lymphoid neoplasms are able to maintain an angiogenic phenotype in a paracrine fashion, even in the bone marrow, and may prove to be responsive to mTOR inhibition.

mTORis and viral-associated cancer in transplant recipients

Although the scope and emphasis of this review does not permit an adequate examination of mTORi effects on viruses associated with cancer, a few words must be directed towards this topic. In fact, the most common cancers in transplant recipients are associated with viral infections, namely nonmelanoma skin cancer (human papilloma virus), PTLD (EBV), Kaposis sarcoma (herpes virus 8), and HCC (hepatitis B/C virus). Interestingly, there is an extreme paucity of information as to how the immunosuppressive effects of mTORis, or other immunosuppressants, affect the immune responses to these viruses. Also, it is unknown how mTORis may affect viral proliferation, which could be a critical point as there are hints in the literature that some viruses may operate at least partially through the mTOR axis [47,66]. A strong call for research on this important topic is in order and will be essential for designing strategies to reduce post-transplant cancer.

Are mTORis effective against cancer in transplant recipients?

A problem in treating transplant patients is to provide effective immunosuppression, while not promoting cancer development. The evidence just presented suggests that mTORis might be capable of playing the dual role of immunosuppressant and anti-tumor agent, thus addressing this critical problem in organ transplantation. Indeed, there is now evidence that mTORis can protect allografts against rejection, while simultaneously displaying anti-tumor effects [7], and the literature is increasing with regard to the anti-tumor effects of mTORis in various experimental tumor models [67].

In humans, for the most part, there is insufficient data to make clear conclusions on the effectiveness of mTORis against cancer. Publications thus far on transplant recipients are in the form of case reports, or studies not statistically powered for this purpose. Nonetheless, there are hints that mTORis could be useful in a dualistic role. For example, as previously mentioned, multiple groups have reported on CNI-immunosuppressed renal transplant recipients with Kaposi sarcoma, demonstrating tumor regression after switching immunosuppression from CNIs to sirolimus [48–50]. This is a fundamental observation because tumor regression occurred in the face of full immunosuppression with sirolimus, thus not increasing the risk for organ allograft rejection. Kaposi sarcoma is a logical target for mTORi therapy as it is a highly vascular tumor, and we have shown mTORis to be strongly anti-angiogenic [54]. There are also reports of partial or complete remission after treatment with sirolimus in adult [68] and child [69] PTLD. Others have reported a partial or complete remission for metastasizing hepatocelluar carcinoma after transplantation with sirolimus treatment alone [70], or in combination with MMF in individual patients [71]. A pilot study from Edmonton suggests that sirolimus may be effective in reducing HCC recurrence after liver transplantation [72]. The hypothesis that sirolimus can improve HCC-recurrence-free survival following liver transplantation is presently being tested in a prospective-randomized international study (SiLVER Study; http://www.silver-study.org) sponsored by our center at the University of Regensburg. In addition, possible beneficial effects of sirolimus against skin cancer are being tested in at least three controlled clinical trials in Europe. More randomized clinical trials of this sort will be needed in transplant patients to rigorously test the dualistic role theory for mTORis. The results of these trials are much anticipated, and are essential, but will require some years to complete.

Outside of organ transplantation there are increasing reports on the use of mTORis as anti-cancer agents. The most successful applications have been for the treatment of MCL and renal cell carcinoma. One phase II trial has reported successful treatment of relapsed MCL with CCI-779. The response rate was 38% with three patients showing a complete, and 12 patients a partial, remission [34]. It should be realized that the doses used in these oncology studies far exceed that given to transplant recipients, and are generally administered in a bolus-dosing regimen. Therefore, caution must be taken when comparing expectations in transplant recipients, who receive much lower doses of mTORis on a daily basis. It is also worth adding, however, that we have found low daily dosing of rapamycin, similar to a regimen used in transplant recipients, to be more effective in experimental models than bolus dosing [73]. Nonetheless, others, using high-dose mTORi therapy have found similar results with CCI-779 in phase II trials with different advanced solid tumors refractory to standard therapy [74–76]. Examples of relapsed hematologic cancers have also been successfully treated RAD001 in a phase II study [35]. As mentioned earlier while discussing molecular reasoning for mTOR inhibition, one of the most effective clinical applications for this class of drugs appears to be in the treatment of renal cell carcinoma [75,77]. Temsirolimus has shown effectiveness in clinical trials that has resulted in fast-track FDA approval for compassionate use in renal

cell cancer (http://www.cancer.gov/cancertopics/druginfo/fda-temsirolimus).

Other oncology trials have been reported. Several phase I and II studies have been performed with the National Cancer Institute (Bethesda, MD, USA) in patients with recurrent malignant glioma. In these trials sirolimus or temsirolimus were used. Dose escalation was performed with a maximum tolerated dose of 330 mg/week. CCI-779 treatment resulted in side effects such as hypercholesteremia and hypertriglyceridemia [78], but Galanis *et al.* [79] reported that CCI-779 was well - tolerated in patients with recurrent gliomas and 36% of the patients showed clinical improvement by radiologic examination. Interestingly, tumor specimens with high levels of p70S6K appear to predict a patient population more likely to derive benefit from the treatment, suggesting this as a biomarker for optimal therapeutic guidance. As another example for oncologic trials, a study using CCI-779 on metastatic melanoma has been reported recently with a dose of 250 mg/week [80]. Unfortunately, only one

Table 1. Reported clinical studies using mTOR inhibitors.

Type	Tumors	Drug	Additional information
Case report	Kaposi sarcoma [49]	Sirolimus	Conversion to rapamycin leads to tumor regression
Phase I	Hepatocellular carcinoma metastatic renal cell carcinoma, metastatic sarcoma (meeting report 2004)	AP23573	Dose escalation, preliminary meeting report
Phase I	Renal cell carcinoma, breast adenocarcinoma [76]	CCI-779	Safety, tolerability and pharmacokinetics
Phase I	Advanced solid tumors (including GI tumors) [84]	CCI-779	Combination with leucovorin and 5-fluorouracil; discontinued because of toxicity
Phase I	Fibrosarcoma, nonsmall cell lung carcinoma (meeting report 2003)	RAD001	Dose escalation, toxicity pharmacokinetics, pharmacodynamics
Phase I/II	Advanced GI tumors (meeting report 2004)	RAD001	Combination with imatinib in imatinib refractory tumors
Phase II	Refractory renal cell carcinoma [75]	CCI-779	Efficacy, safety and pharmacokinetics of multiple doses
Results from 5 multi-center studies (phase II and III)	Renal transplant patients [85]	Sirolimus	Rate of malignancy after transplantation, 2-year results; sirolimus + CsA
Retrospective single-center analyses	PTLD, hepatoblastoma (pediatric liver transplant patients) [69]	Sirolimus	Only sirolimus-treated patients analysed
Phase I	Recurrent gliomas [78]	CCI-779	Maximum dose 250 mg/week, combined with anti-epileptic drugs
Phase I/II	Refractory hematologic malignancies [35]	RAD001	Typical side effects, 6/9 patients clinically improved after treatment
–	Kaposi sarcoma [48, 50]	Sirolimus	Renal-transplant recipients switched from CsA to sirolimus; complete regression of Kaposi sarcoma
Phase II	Metastatic melanoma [80]	CCI-779	No effect on OS and EFS in advanced melanomas
Phase II	Advanced or metastatic breast cancer [74]	CCI-779	10% of patients with 250 mg had side effects, but 0% of patients treated with 75 mg/week; both doses were effective
Phase II	Recurrent glioblastoma multiforme [79]	CCI-779	Slightly longer survival for responder; pS6kinase suggested as a biomarker for response to treatment
Phase II	Relapsed mantle cell lymphoma [34]	CCI-779	Good response of mantle cell lymphoma; anti-proliferative activity; thrombocytopenia as side effect
Phase I	Recurrent malignant gliomas [86]	Sirolimus	Safe co-administration with Gefitinib; 6% of patients responded, 38% with stable disease
–	Hepatocellular carcinomas of patients undergoing liver transplantation [72]	Sirolimus	Uncontrolled trial, inhibition of tumor recurrence suggested

EFS, event-free survival; OS, overall survival; CsA, cyclosporine A; GI, gastrointestinal.

patient from this cohort showed a partial response to the therapy, although it is notable that most patients had advanced disease with liver metastases. The authors concluded that CCI-779 did not achieve sufficient anti-tumor activity in advanced melanoma to warrant further evaluation as a single agent, at least at the doses and scheduling tested. In contrast, others have shown *in vitro* and *in vivo* that the BRAF (v-raf murine sarcoma viral oncogene homologue B1) inhibitor BAY43-9006 and rapamycin (or CCI-779), in combination with cisplatin, synergistically inhibit melanoma proliferation [81,82]. Moreover, Bedogni *et al.* [83] demonstrated that constitutively active AKT is present in transformed melanoma cells, suggesting the AKT pathway could at least be partially blocked with mTORis. Clinical trials have also been performed for the treatment of breast cancer. Unfortunately, although responses have been detected with CCI-779 treatment in a phase II trial [74], a phase III trial using the drug in combination with letrozole in the first-line treatment of postmenopausal, hormone-receptor positive, metastatic disease has since been stopped because early trial data suggested no additional benefit was likely with the addition of the mTOR inhibitor. As with all potential anticancer agents, variable success is to be expected with each protocol and each type of cancer, Nonetheless, early clinical data suggest mTOR inhibitors may become an important oncologic tool for the treatment of specific types of cancer, but more studies are clearly needed. A summary of important studies reported since 2004 is given in Table 1. We further emphasize that numerous studies and clinical trials are presently ongoing.

Conclusions

Advances in our knowledge of molecular signaling linked to mTOR support the hypothesis that inhibitors of mTOR can play a unique dualistic role in organ transplant recipients, having both an immunosuppressive and anti-neoplastic function. What is critical is that our understanding of the mTOR pathway allows for evidence-based application of mTORis in situations where specific molecular dysregulation is recognized in particular types of cancer. Therefore, the decision to use mTOR inhibitors in transplant patients with (or at high risk for) cancer is not without experimental evidence and molecular reasoning. Furthermore, in the setting of organ transplantation, mTORis are the only currently approved anti-rejection substances that have demonstrated a capacity to both inhibit the immune system, and interfere with cancer growth. What is also encouraging is that the daily doses used in transplant recipients have been shown to be experimentally effective against cancer. However, we still have much to learn and in the end must demonstrate that mTORis can be successfully applied in prospective randomized clinical transplantation trials. Although these trials will require significant time and resources to complete, we must accept this challenge if we are to get reliable answers. As the mTOR pathway is now being recognized for its complexities, determining a bottom-line regarding a dualistic role for mTOR inhibitors can only be found in the complex setting of clinical transplantation. What we can conclude from this review of the literature is that there is substantial evidence to conduct such clinical trials.

Authorship

AG, HJS and EKG contributed to the writing of the review manuscript.

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Conflict of Interest

None of the authors have any conflict of interest in connection with the submitted manuscript, in particular no commercial association that pose a conflict of interest.

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