Walking on the Molecular Pathway: m-TOR Inhibition in the Liver Transplant Setting

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Abstract: Hepatocellular carcinoma (HCC) is a major problem worldwide, representing the fifth most common tumor among the general population [1]. Several molecular pathways seem to be involved in HCC growth and progression, realizing an intricate mechanism of proliferative and angiogenetic stimulation and inhibiting apoptosis. The recent identification of these molecular pathways has improved our knowledge of HCC tumorigenesis and has setted up a more specific approach to HCC treatment. However, since non advanced HCC is one of the most common indications for liver transplantation, the recent novelties in pharmacologic inhibition of HCC growth mechanisms have opened new interesting opportunities in recipients' immunosuppressive treatment. M-TOR inhibitors belong to the group of rapamycine analogues, are used as second line immunosuppressive drugs and capable to inhibit one of the most active molecular pathways in HCC cells: the m-TOR pathway. In this review, we explain the mechanism and the molecular elements involved, up- and downstream, in m-TOR activation, providing an overview of the other features of HCC tumorigenesis too. Moreover, we describe the effects of m-TOR inhibition on HCC cells *in vitro* and in animal models, as well as on liver transplant recipients at risk of or with a manifest tumor recurrence and on *de novo* post-transplant malignancies. Finally, we discuss the possible efficacy of a proper combined therapy, well tolerated by patients and active against multiple molecular targets, to obtain a synergistic effect on the tumor mass with the greatest benefit for patients' survival and quality of life.

Keywords: m-TOR, m-TOR inhibitors, hepatocellular carcinoma, liver transplantation, immunosuppression.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common tumor among the general population and the most common among cirrhotic patients [1]. Although, in the last few years, the international guidelines have strongly recommended a strict follow-up of cirrhotic patients to recognize tumoral lesions at early stage, a curative therapy is applicable only in 1/3 of cases [2]. Among the available curative treatments, liver transplantation has a better outcome than surgical resection or local ablation [3,4]. The first liver transplant experiences in HCC patients produced scarce results in terms of outcome and disease recurrence; nowadays, since the introduction of the Milan criteria [5], the overall survival rate is of about 60-70% or better at five years after transplantation [6,7]. However, in case of tumors exceeding the Milan criteria, data are contrasting [5]. Unfortunately, despite patients' selection to avoid tumor recurrence, it may be possible and is more frequent within the first two years after liver transplantation, with the feature of a local or a metastatic lesion in lung or bone [8]. At the time of recurrence, patients' survival is significantly reduced [9]. Post-transplantation chemotherapy has reported variable results [10-13], but there is no treatment capable to avoid HCC recurrence yet. Moreover, liver transplant recipients have a three to five fold increased risk to develop a new extrahepatic tumor [14-18]. Virtually, each organ could be involved, even if non-melanotic skin cancers, post-transplant lymphoproliferative disease (PTLD), and Kaposi's sarcoma are the most frequently reported tumors [14-16, 19-21]; about 20% of post-transplantation overall mortality is imputable to *de novo* tumors [22]. The principal explanatory reason could be identified with the immunosuppressive treatment, necessary to avoid graft rejection. Patients' immune system loses its competence in recognizing and fighting tumoral cells and, at the same time, the guard against oncogenic viruses is reduced. Furthermore, immunosuppressive drugs might be responsible for promoting tumor development in solid organ transplant recipients through a non-immunologically-mediated mechanism too [23]. In experimental tumor models, cyclosporine favors the growth of invasive and metastatic tumor cells [24]; a similar effect has been reported for tacrolimus too [25]. This process seems to be related to TGF-b and VEGF overexpression [26]. Clinical studies, such as those from Vivarelli *et al.* [27, 28], reported an increased risk of HCC recurrence in patients overexposed to tacrolimus and cyclosporine, even if these studies were mainly retrospective [29].

THE RECENT DISCOVERY OF M-TOR INHIBITORS

Rapamycin (sirolimus; Rapamune, Wyeth) is a macrolide antibiotic with well-known properties in the regulation of immune system, cellular proliferation and angiogenesis, isolated in the 1970s from a bacterium of the soil (*Streptomyces hydroscopicus)* in the island of Rapa Nui [30, 31]. Rapamycine multiple effects are due to the inhibition of a conserved checkpoint protein-kinase, called mTOR (*mammalian target of rapamycine*), involved in a complex

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Table 1. Principal Characteristics of mTOR Inhibitors [30]

mTOR inhibitor	Absorption	Half-life	Metabolization	Dose adjustment required by age	Dose adjustment required by liver function	Dose adjustment required by renal function
Sirolimus	14% for liquid solution; increased by tablets or lipid meal	62 ± 16 h	CYP3A4 and P-glycoprotein	None	Child A/B increased biodisponibility	None
Temsirolimus	Intravenous	17.7 h (73.3 h for its metabolite sirolimus)	CYP3A4 and P-glycoprotein	None	No data	None
Everolimus	90%; decreased by lipid meal	26-38 h	CYP3A4 and P-glycoprotein	None	2-fold increased biodisponibility	None

pathway of interactions: the m-TOR pathway. Apart from sirolimus, several molecules can inhibit m-TOR activity; at present, everolimus and temsirolimus are the most studied. Generally, m-TOR inhibitors are similar to tacrolimus, but without any anti-calcineurin effect [32].

The nucleus of m-TOR inhibition is easy to summarize: after binding its intracellular receptor, the FK-binding protein (FKBP12), rapamycin-FKBP complex binds the C-terminal kinase domain of m-TOR inhibiting its action [33]. This is the simple, but, at the same time, complex mechanism of action of rapamycin analogues.

THE M-TOR PATHWAY

What is m-TOR pathway and how does it works? M-TOR is a conserved checkpoint serine-threonine kinase belonging to the phosphatidylinositol 3-kinase (PI3K)-kinase-related kinase superfamily, involved in both cell growth and cell cycle progression [34, 35]. M-TOR functions result from the integration of multiple signals, derived from growth factors and reflecting cellular energy storage and hypoxia (Fig. **1**). In specimen, growth factors may influence m-TOR through the PI3K-Akt pathway, while the intracellular energetic level through LKB1-TSC pathway

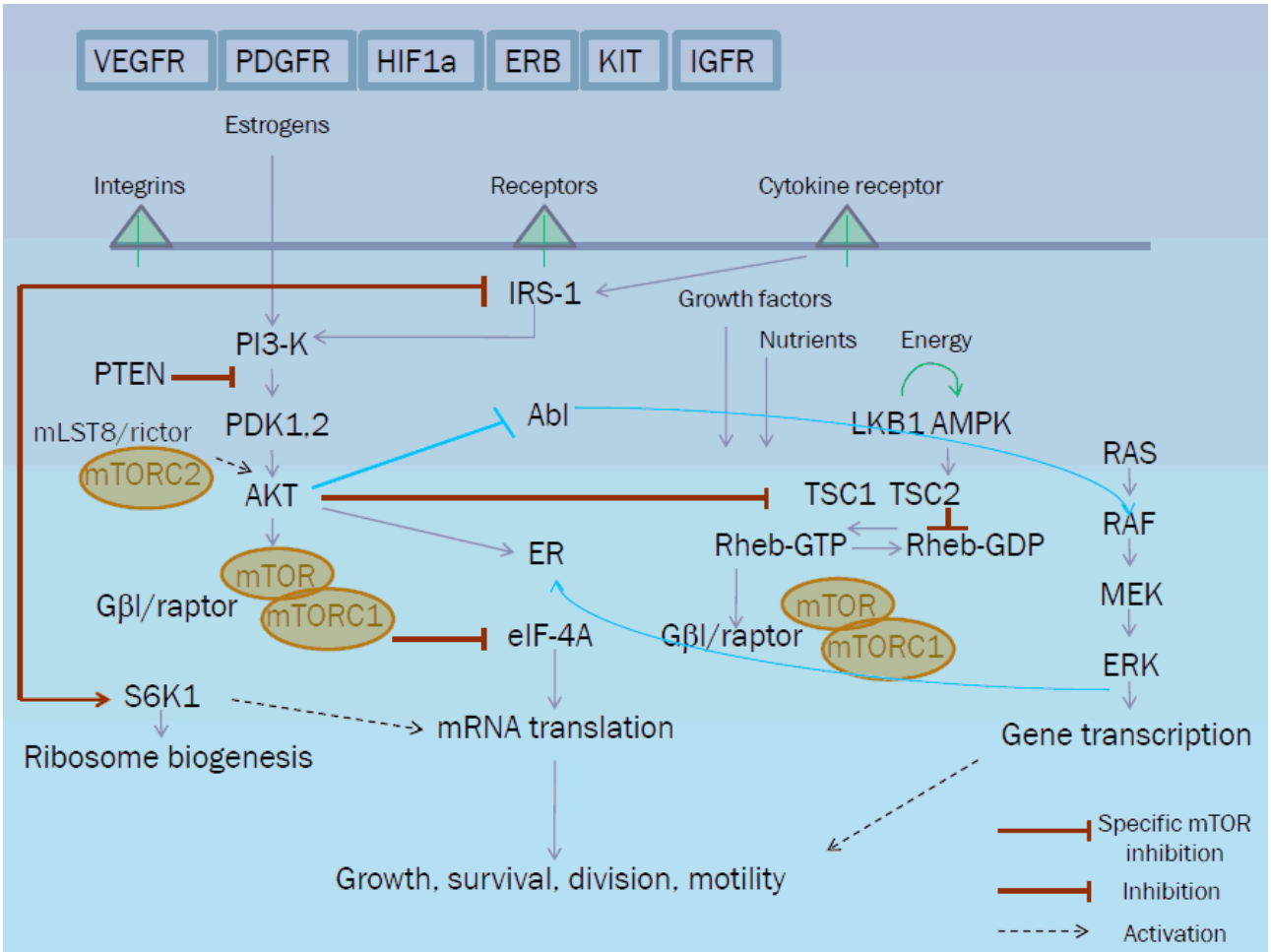

Fig. (1). m-TOR signaling and related pathways [30].

[36]. In response to extracellular growth stimulation, including insulin and insulin-like growth factor 1 (IGF-1), PI3K recruited by the insulin receptor substrate (IRS) generates phosphatidylinositol-3, 4, 5- triphosphate (PIP3), which activates phosphatidylinositol-dependent kinase-1 (PDK1) and Akt [37]. Activated Akt inhibits the tuberous sclerosis complex (TSC) [38, 39], a GTPase-activating protein; the main effect is the activation of GTP-bound protein Rheb, which in turns removes the negative control on mTOR [30, 40, 41]. In case of energy deficiency (reduction of ATP or amino acids intracellular levels), LKB1 activates 5'AMP-activated protein kinase (AMPK), which activates TSC complex; the result is inhibition of Rheb and, consequently, of m-TOR [30, 41-43]. Therefore, energy deficiency leads to m-TOR inhibition stopping the cell cycle in the G1 phase, since the m-TOR pathway initiates the translation of proteins necessary for cell cycle progression; in contrast, the response to growth stimulation is m-TOR activation [44]. Activated m-TOR may form many complexes with other proteins [45-49]. The complex m-TOR-Raptor (m-TORC1) leads to the phosphorylation of eukaryotic initiation factor 4E binding protein-1 (4E-BP1) and protein S6 kinase 1 (S6K1), both regulators of protein translation [37]; m-TORC1 is rapamycine sensitive and used in laboratory to quantify m-TOR activity. The complex m-TOR-Rictor (m-TORC2), instead, is not influenced by rapamycine and determines the full activation of Akt. In conclusion, the S6K1 pathway leads to the translation of mRNAs encoding ribosomal proteins, elongation factors and insulin growth factor-II, while the 4E-BP1 to the translation of mRNAs encoding cell cycle regulators, such as cyclin D1 or ornithine decarboxylase, or growth factors [44, 50]. Rapamycin has also some effects on tumor angiogenesis [23], related to the reduction of VEGF production and of endothelial response to VEGF [51]. The hypoxia inducible factor (HIF-1) plays a pivotal role in regulating this response. Indeed, when the oxygen tension is low, angiogenesis is promoted by HIF-1 through VEGF production. Since HIF-1 is molecularly located downstream of m-TOR, it is consequently inhibited by rapamycin [52-54]. Moreover, m-TOR pathway is the scenario of multiple interactions and feedbacks. Indeed, m-TORC1 may inhibit m-TOR activation by insulin through S6K1. M-TORC1 may compete with m-TORC2 too, through the activation of Akt. M-TORC2 activates Akt, which inhibits TSC2 complex, stimulating the formation of m-TORC1; on the other hand, m-TORC1 may antagonize mTORC2 and reduce Akt activity [55]. Hyperactive mTORC1 may also inhibit insulin receptor substrate (IRS) upstream in the m-TOR pathway. Interestingly the anti-oncogen PTEN (phosphatase and tensin homologue deleted on chromosome 10), modulating PIP3 formation, may reduce Akt activity [41].

THE M-TOR PATHWAY, TUMOR DEVELOPMENT AND HEPATOCELLULAR CARCINOMA

How could m-TOR pathway be responsible for tumor development? As discussed above, m-TOR pathway involves several components, complexly linked each to the other. Mutations of PTEN, Akt, TSC1and 2, PI3K-dependent signaling and aberrant protein translation can lead to m-TOR hyperactivation and promote oncogenesis. It has recently been shown that inflammation is linked to the m-TOR pathway via tumor necrosis factor signaling [56, 57]. Moreover, alterations of m-TOR pathway have been demonstrated in several tumor- predisposing syndromes, such as tuberous sclerosis (TSC1/2), Peutz-Jeghers syndrome (LKB1), and Cowden's syndrome (PTEN) [58]. As regards HCC, some studies have assessed the expression of m-TOR related markers in tumor cells and liver tissue. Sahin *et al.* [59] reported that cells strongly positive for m-TOR or p-(phosphorilated) m-TOR did not show any difference in Ki-67 proliferation index, while the normal liver tissue was always weakly positive. Hepatocellular carcinoma cells were also highly positive for S6K1 expression. Sieghart *et al.* [60] concluded that 40% of transplanted patients with HCC present an hyperactivation of m-TOR pathway; however, no correlation was found with the expression any single protein (i.e. Akt, S6K1, 4EBP1, PTEN). Baba *et al.* [61] highlighted the increased expression of p-S6K1in the cytoplasm of HCC cells, while in the normal liver tissue it was absolutely less represented. Moreover, p-S6K1 hyperexpression was inversely related to cellular differentiation, and directly related to the expression of cyclin D1 in HCC cells. Apparently, there was no relation between p-S6K1 expression and the etiology of liver disease. Finally, Villanueva *et al.* [62] reported the hyperexpression of m-TOR-Rictor (m-TORC2) complex in those cells with an increased number of DNA copy. Furthermore, increased levels of m-TOR activation products were found in 48% of HCC while in only 19% of the normal liver tissue, and were associated to less differentiated tumors, advanced BCLC stage, higher levels of alpha-fetoprotein and larger tumor volume. In addition, protein localization in hepatocellular carcinoma cells was almost cytoplasmatic. The presence or the absence of these alterations may, obviously, condition cancer cell sensitivity to m-TOR inhibitors.

Another interesting aspect is that m-TOR pathway is expressed in a wide number of cells of the human body, and its stimulation may lead to protean manifestations of different biological significance [63-65]. The most investigated one is T-cells response to proliferative and survival stimuli through m-TOR activation [66-68]; m-TOR inhibition can block effector T-cell expansion, producing immunosuppression [69]. In contrast, regulatory T cells may respond to mitogen-dependent activation through m-TOR but, also, through other pathways of signaling; thus, they are less influenced by rapamycine and its analogues and could be selected for expansion if stimulated.15 It is only one of the multiple features of m-TOR pathway, which highlights a complex network of interactions with local microenvironment and systemic perturbations, influencing the activity of a great number of cells in different tissues.

M-TOR INHIBITION AND TUMOR PREVENTION: THE GREAT IMPACT ON LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA

Based on the mechanisms discussed in this article, m-TOR pathway inhibition has a great impact in the prevention of tumor development. The mTORC1complex is the only FKBP12-rapamycin sensitive target. Since this interaction is irreversible, m-TOR availability and, consequently, the formation of m-TORC2 are sensibly reduced [70].

Several studies confirmed that rapamycine reduces tumoral cells' proliferation *in vitro* [48, 71, 72] and the expression of molecules associated to tumor progression and metastatic potential in mice [73]. Koehl *et al.* [74] reported that sirolimus prevents rejection, inhibits tumor growth and prolongs survival in models of cardiac allograft with tumor grafts, while, in the same conditions, cyclosporine is only able to prevent rejection. The efficacy of sirolimus as anti-cancer agent depends on the dose, so that low nanogram per milliliter concentrations might produce a potent anti-angiogenic and a potential antiproliferative effect, whereas higher doses might have a direct cytotoxic effect on tumor cells [75].

Several clinical trials and case reports confirmed data from *in vitro* and animal experimentation. A prospective, randomized trial reported the efficacy of temsirolimus in the treatment of advanced renal adenocarcinoma [76]; other studies underlined the same anti-tumor activity in the transplant setting [77-79]. In liver transplant recipients too, *de novo* or recurrent malignancies are an important cause of morbidity and mortality [80, 81], and m-TOR inhibitors might open new perspectives in the management of recipients with tumors or with a high risk of tumor development. In specimen, the experience of Kneteman *et al.* [82] has been precious to understand rapamycine analogues utility in the liver transplant setting. In this study, HCC patients undergoing liver transplantation received sirolimus-based immunosuppression reducing CNIs and steroids administration; 19 patients were transplanted within the Milan criteria, 21 beyond. As a result, tumor-free 4-year survival was of 81.1%, with less than 5.3% recurrence (1 patient), for recipients transplanted within the Milan criteria, while it was of 76.8% with about 19% of recurrence (4 patients) for patients responding to the extended criteria. The rate of rejection was of 30%, but no case of graft loss occurred. Yao *et al.* [5] reported an overall 11% rate of recurrence (8 patients) in 70 liver transplant recipients meeting or not the Milan criteria, on treatment with cyclosporine or tacrolimus; 3 of them, about 4% of the entire population, met the Milan criteria. As noted in an interesting editorial by Wall [83], in patients meeting the Milan criteria, Kneteman results where almost the same of those obtained by Yao in the same group of patients receiving CNI-based immunosuppression; on the other hand, there was a consistent gain in tumor-free survival in patients beyond the Milan criteria. Subsequently, Kneteman's group [84] reported data from the follow-up of 70 OLT recipients treated with a sirolimus-based protocol; tumor recurrence was observed in about 6% of patients meeting the Milan criteria, while it was more frequent (about 17%) in those beyond the Milan criteria. In the former group, 1 and 4-year tumor-free survival was of 83% and 73% respectively, in the latter of 83% and 75%. Graft rejection occurred in half of patients.

m-TOR inhibitors seem to be able to delay tumor recurrence and prolong patients' survival after recurrence, with some effects on metastases spreading too. In a large series of kidney transplant recipients [85] presenting *de novo* post-transplant tumors, the medium survival of patients not eligible for surgery and converted to sirolimus was of 14.5 months, *versus* 3 months of those receiving a standard immunosuppressive regimen. Moreover, 12- and 20 months-survival in the non-sirolimus group was significantly lower (6.7% and 0%, respectively). After the conversion to a sirolimus-based immunosuppressive regimen, regression of pulmonary metastases or a long recurrence-free interval after surgery of ovarian metastases have also been observed [86, 87]. By the way, in the previously mentioned study by Sieghart *et al.* [60], m-TOR hyperexpression seems to have no influence on disease-free interval or overall survival; furthermore, Zimmerman *et al.* [88] excluded an effective survival benefit on about 37% of patients receiving sirolimus.

Finally, another mechanism advocated to explain the effects of rapamycin analogues on HCC cells is pro-apoptotic stimulation, through the activation of caspase-3, disruption of mitochondrial membrane potential, downregulation of the anti-apoptotic protein Bcl-2 and upregulation of the pro-apoptotic protein Bcl-xl [89].

M-TOR INHIBITION PREVENTS *DE NOVO* TUMORS DEVELOPMENT IN TRANSPLANT RECIPIENTS

Since the m-TOR pathway is abnormally active and altered in some tumoral cells, m-TOR inhibition may not only prevent the recurrence of pre-existing tumors in transplant recipients, but also the development of *de novo* tumors. In animal models, rapamycine prevents tumor development in p53-mutated mice [90] and the clinical data available so far suggest a pivotal role of m-TOR inhibition in preventing *de novo* tumors development after transplantation. A wide number of retrospective studies reported, in renal transplant recipients receiving m-TOR inhibitors, a significantly lower incidence of *de novo* post-transplant tumors, in respect to those receiving CNIs [91-97].

In this regard, the treatment of Kaposi sarcoma, whose incidence is 500 times higher among transplant recipients, is the most convincing experience. In renal transplant recipients who developed Kaposi sarcoma receiving CNI-based immunosuppression, the introduction of sirolimus with or without CNI withdrawal produced sarcoma complete regression [23]. Regression or stabilization of Kaposi metastatic and cutaneous lesions has also been reported [98-101]. However, sirolimus benefits are not so evident in patients with extensive cutaneous or visceral Kaposi disease and cases of failure have also been reported [81, 101-104]. Whether the regression of Kaposi lesions could be due to sirolimus introduction rather than to CNI withdrawal [105, 106], is still to be demonstrated.

Apart from Kaposi sarcoma, rapamycin immunosuppressive treatment may also reduce the incidence of skin tumors in transplant recipients [99, 107-112]. Moreover, several data show the complete regression or remission of PTLD after introducing m-TOR inhibitors-based immunosuppression [113-121]. Two studies [122, 123] reported that patients with various post-transplant malignancies (lung, prostate, colon, stomach, esophagus, breast, larynx etc.) might benefit of sirolimus administration. In both these studies, a conspicuous number of patients had a complete remission (about 65% and 50%, respectively), with an increased survival in respect to the control group. However, other reports are contrasting [124-126], and all these evidences need to be confirmed.

THE NEW ERA OF MOLECULAR TARGETING IN HCC MANAGEMENT: A COMBINED APPROACH

In addition to the previously discussed m-TOR pathway, it is now clear that HCC proliferation is strongly dependent by the mitogen-activated protein kinase pathway too. Four kinases are involved: Ras, Raf, the mitogen-activated protein extracellular kinase (MEK), and extracellular signal-regulated kinase (ERK) [127]. This pathway is crucial for cell proliferation and could be activated in HBV and HCV infection or in presence of growth factors as well as in hepatocellular tumors, in specimen those with an aggressive phenotype [128]. However, mutations of Ras pathway are relatively rare in HCC cells [129, 130]; furthermore, Ras-binding proteins, such as RASSF1A and NORE1A, may counterbalance Ras pathway activation by inhibiting Ras-dependent mitogenic stimulation and promoting apoptosis [131, 132].

Among human tumors, HCC is one of the most hypervascularized, overexpressing VEGF in proportion to microvascular density and in relation to the invasive and metastatic potential and PDGF-B, which is directly involved in tumor angiogenesis [133].

Taking in account the different and complex modalities of signaling responsible for tumor growth, invasion and spreading, and referring in particular to HCC, different pharmacological agents should be combined to obtain a synergistic therapeutic effect and the highest efficacy. Sorafenib is an oral multikinase inhibitor used as standard of care in advanced cases of HCC, capable to block Raf/MEK/ERK pathway VEGFR-2, VEGFR-3, PDGFR-b, Ret and c-Kit [134]. The inhibition of other pathways of intracellular signaling may contribute to sorafenib efficacy [135].

A study by Wang *et al.* [136], successively confirmed by Newell *et al.* [133] and Huynh *et al.* [128, 135], reported that rapamycin (or its analogues), alone and in combination with sorafenib, inhibits primary and metastatic tumor growth in human HCC xenografts; moreover, combined therapy seems to be more effective in reducing tumor size and angiogenesis than a single agent therapy. The combination of antiproliferative, proapoptotic and antiangiogenetic effects may explain these results. This attractive, orally administered and well-tolerated new modality of combined treatment should be seriously considered to design clinical trials, to assess its efficacy on disease progression and, consequently, the benefits on patients' quality of life.

Finally, it could be interesting to evaluate whether the administration of rapamycine analogues may lead to a specific resistance. As previously discussed in this article, the inhibition of m-TOR pathway may produce the overexpression of such molecules, as Akt, upstream of m-TOR, leading to a potential mean of resistance. Therefore, a combined therapeutic approach might prevent the occurrence of drug resistance, with a more complete regulation of the complex network of m-TOR pathway interactions [137].

Table 2. Adverse Effects of mTOR Inhibitors [138]

Adverse Effect	Clinical Onset and Characteristics	Incidence
Mucositis	Clinical onset: rapid onset (within 5 days), usually not severe (grade 1-2) reversible after withholding treatment. Characteristics: 1-3 oval ulcers surrounded by erythema on the mucosa of the lips, lateral tongue, buccal mucosa and soft palate	75% temsirolimus 78% deforolimus 41% everolimus
Pulmonary Toxicity	Clinical onset: after 6 months to 1 year from the onset of therapy. Characteristics: dyspnea and dry cough, fatigue and fever, ground glass opacities or lung parenchymal consolidation at chest x-ray, restrictive pulmonary disease pattern. Frequent in patients with previous pulmonary disease	5-50% incidence
Skin toxicity	Clinical onset: during the first few weeks of treatment, usually not severe (grade 1-2), spontaneous resolution or after topic treatment. Characteristics: maculopapular or acneform rash on the face and neck, dryness, eczema, skin discoloration, nail dystrophy	>50% incidence
Bone Marrow toxicity	Bone marrow suppression, predominant for megakaryocytes, reversible, dose and concentration dependent	Thrombocytopenia was reported about 29–33% with temsirolimus, 10% for everolimus, and 20–25% for deforolimus. Leucopenia has also been reported up to 27% for temsirolimus, 38% for deforolimus, neutropenia is less common
Renal toxicity	Discordant data. Acute or chronic renal failure, thrombotic microangiopathy, glomerulonephritis, tubular toxicity have been reported	Variable
Metabolic toxicity	Hyperlipidemia (increased HDL, LDL, cholesterol and triglycerids levels). Hyperglicemia	Hyperlipidemia: 21–37% for temsirolimus, 8–44% for everolimus, 28–41% for deforolimus. Hyperglicemia: 8–22%
Infections	No evidence of significant immune suppression	Variable
Malignancies	No evidence of significant increase in secondary malignancies	Variable

CONCLUSION

The recent discovery of the molecular mechanisms at the basis of tumorigenesis has permitted a more specific approach in the battle against malignancies. The m-TOR pathway has been recognized as one of the principle regulator of cellular growth, proliferation and angiogenesis in different tissues, as well as in tumoral lesions. The recognition of rapamycin and its analogues properties has opened the possibility to exercise an inhibition on tumoral development and spreading, as well as, at the same time, the modulation of the immune system activity. For these protean features, the so called "m-TOR inhibitors" have a primary importance in preventing tumor recurrence or the development of new tumors in transplanted patients, whose immune system must also be suppressed to avoid graft rejection. In the particular case of HCC, m-TOR pathway is only one of the complex and multiphasic processes involved in tumor progression. Therefore, it is intuitive that a "future" therapeutic approach should target the inhibition of the most part of these processes. However, since the creation of an ideal drug capable by itself to fulfill this proposal seems distant, to combine more therapeutic weapons could be the best way to, at least, control or delay tumor progression, or prevent tumor development.

ABBREVIATIONS

HCC = Hepatocellular carcinoma

PTLD = Post-transplant lymphoproliferative disease

TGF-b = Transforming Growth Factor beta

VEGF = Vascular Endothelial Growth Factor

FKBP12 = FK-binding protein

PI3K = Phosphatidylinositol 3-kinase

ATP = Adenosine triphosphate

IGF-1 = Insulin-like growth factor 1

IRS = Insulin receptor substrate

PIP3 = Phosphatidylinositol-3, 4, 5- triphosphate

PDK1 = Phosphatidylinositol-dependent kinase-1

GTP = Guanidine-5'-triphosphate

AMPK = 5'AMP-activated protein kinase

m-TORC1/2 = m-TOR-Complex 1/2

4E-BP1 = Eukaryotic initiation factor 4E binding protein-1

S6K1 = Protein S6 kinase 1

HIF-1 = Hypoxia inducible factor

PTEN = Phosphatase and tensin homologue deleted on chromosome 10

IRS = Insulin receptor substrate

BCLC = Barcelona Clinic Liver Cancer

CNI = Calcineurin Inhibitor

MEK = Mitogen-activated protein extracellular kinase

ERK = Extracellular signal-regulated kinase

VEGFR-2/3 = Vascular Endothelial Growth Factor Receptor 2/3

PDGFR-b = Platelet-Derived Growth Factor beta

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