Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Targeting the mTOR pathway in hepatocellular carcinoma: Current state and future trends

Matthias S. Matter†, Thomas Decaens†, Jesper B. Andersen, Snorri S. Thorgeirsson*

Laboratory of Experimental Carcinogenesis, National Cancer Institute, Bethesda, MD, USA

Summary

Mechanistic target of rapamycin (mTOR) regulates cell growth, metabolism and aging in response to nutrients, cellular energy stage and growth factors. mTOR is frequently up-regulated in cancer including hepatocellular carcinoma (HCC) and is associated with bad prognosis, poorly differentiated tumors, and earlier recurrence. Blocking mTOR with rapamycin and first generation mTOR inhibitors, called rapalogs, has shown promising reduction of HCC tumor growth in preclinical models. Currently, rapamycin/rapalogs are used in several clinical trials for the treatment of advanced HCC, and as adjuvant therapy in HCC patients after liver transplantation and TACE. A second generation of mTOR pathway inhibitors has been developed recently and is being tested in various clinical trials of solid cancers, and has been used in preclinical HCC models. The results of series of clinical trials using mTOR inhibitors in HCC treatment will emerge in the near future.

Introduction

Target of rapamycin (TOR) is an evolutionary well conserved serine/threonine protein kinase that belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family. Mechanistic TOR (mTOR; originally called mammalian TOR) has a broad range of action and is involved in regulation of cell growth, aging and metabolism [1]. mTOR can be divided into two structurally and functionally distinct complexes named mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [1]. mTORC1 is composed of mTOR, mLST8, DEPTOR, RAPTOR, and PRAS40. mTORC2 consists of mTOR, mLST8, DEPTOR, PROTOR, RICTOR, and mSIN1 [1]. mTORC1 is a nutrient and energy sensor at both cellular and whole-body levels [2]. When nutrients are available, mTORC1 is activated and stimulates anabolic processes such as protein synthesis, lipogenesis, and energy metabolism, whereas autophagy and lysosome biogenesis is inhibited [1] (for more details see Fig. 1). mTORC1 is activated by a myriad of inputs such as growth factors, energy status, proinflammatory cytokines, oxygen levels, amino acids, and the canonical Wnt pathway [1] (Fig. 1). Growth factors, e.g., insulin and insulin-like growth factor 1 (IGF1), exert their action on mTORC1 through receptor tyrosine kinases (RTK) and the well-characterized PI3K-AKT and Ras-Raf-Mek-Erk signaling pathways. These pathways activate mTORC1 by phosphorylating and thereby inhibiting the tumor suppressor TSC1-TSC2 (tuberous sclerosis 1 and 2) complex. The TSC1-TSC2 complex is a key regulator of mTORC1 and functions as a GTPase-activating protein (GAP) that negatively regulates Rheb by converting it into its inactive GDP-bound state [3,4]. In contrast, down-regulation of mTORC1, is accomplished via activation of the TSC1-TSC2 complex by AMPK, LKB1, and REDD1 in situations of low energy (high AMP), low oxygen levels [5], and DNA damage [6].

Much less is known about the later discovered mTORC2 signaling pathway. mTORC2 is insensitive to nutrients but does respond to growth factors such as insulin in association with ribosomes [7]. Besides its initial described role in actin cytoskeleton organization, mTORC2 also activates cell metabolism, survival, and growth. mTORC2-ribosome interaction is a likely conserved mechanism of mTORC2 activation that is physiologically relevant in both normal and cancer cells.

Involvement of mTOR pathway in hepatocellular carcinoma (HCC)

Given its importance in cell growth and metabolism it is not surprising that mTOR plays a pivotal role in HCC. mTORC1 and mTORC2 pathways, including *pRPS6*, *p-AKT*, *IGF-1R*, and *RICTOR* are up-regulated in 40–50% of HCCs [8–10]. A similar upregulation is observed in other common cancer types such as breast, colon, and lung carcinomas [11]. Moreover an up-regulation is frequently observed in cholangiocarcinoma, the second most common primary cancer of the liver [12]. Activation of the mTOR pathway in HCC is associated with less differentiated tumors, bad prognosis, and earlier recurrence independently of the underlying etiology of liver cancer [9,13,14]. Furthermore, it is associated with

Keywords: mTOR; HCC; Rapamycin; Rapalogs; Second generation mTOR inhibitors.
Received 22 July 2013; received in revised form 28 October 2013; accepted 27 November 2013

* Corresponding author. Address: Laboratory of Experimental Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 37 Convent Drive, Room 4146A, Bethesda, MD 20892, USA. Tel.: +1 301 496 1935; fax: +1 301 496 0734.
E-mail address: snorri_thorgeirsson@nih.gov (S.S. Thorgeirsson).
† These authors contributed equally to this work.

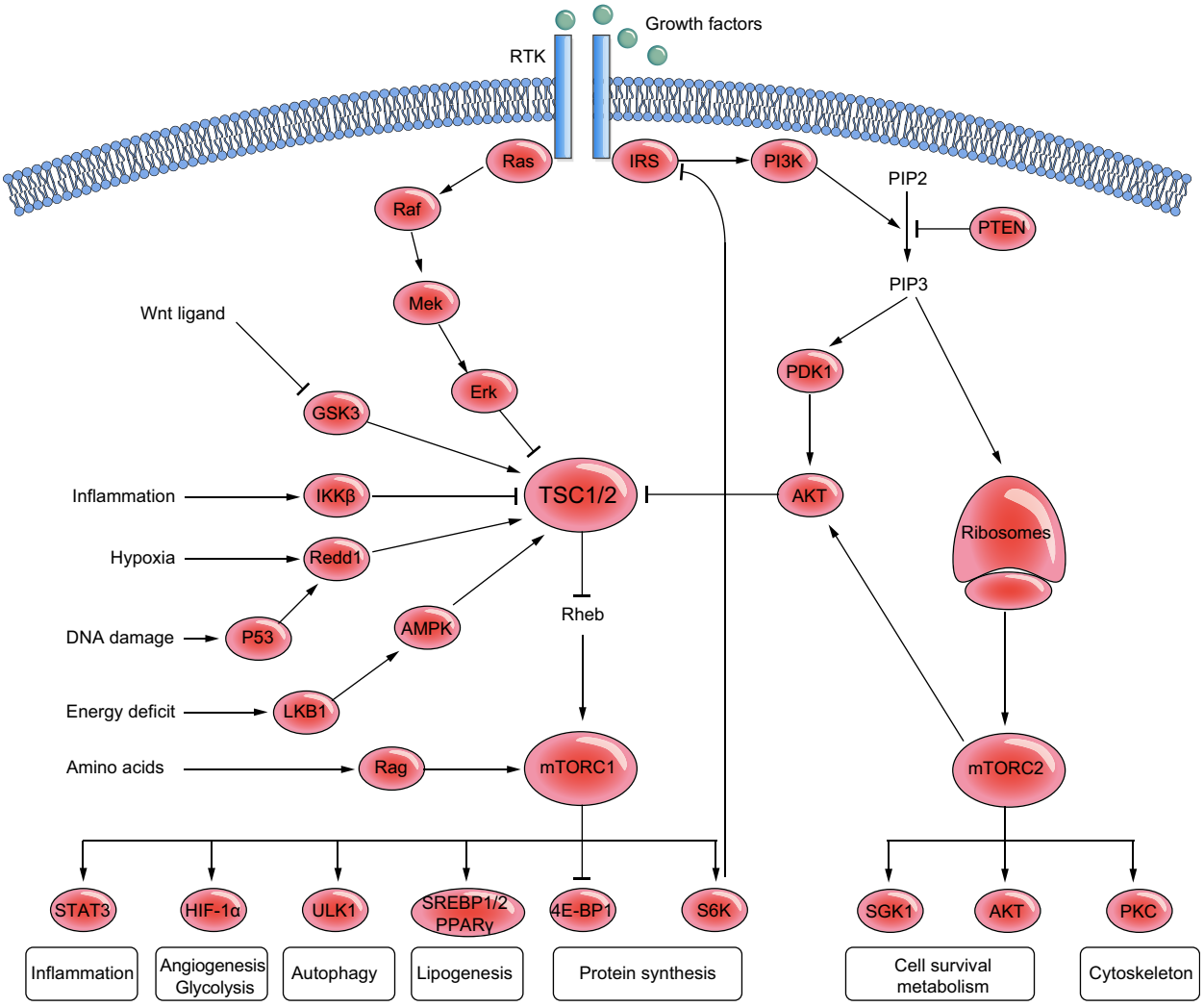

Fig. 1. Schematic overview of the mTOR signaling pathway with the most important factors and their action.

deregulation of EGF, IGF, and PTEN pathways [9] and, as expected, with increased lipogenesis in the tumor [15]. Surprisingly, alterations in copy number or somatic mutations of *PTEN*, *PIK3CA*, and *PIK3B* were not identified as major mechanisms of mTOR pathway deregulation in HCC by PCR [9]. In accordance, more recent studies using next-generation sequencing technique revealed a low frequency of mutations in the mTOR pathway including *mTOR*, *PIK3CA*, and *PTEN* among others [16–18]. The most frequently mutated gene, found in one study in 9.6% of HCC was *RPS6KA3*, a serine/threonine kinase involved in regulating PI3K/RAS signaling [16]. Therefore, mutations in the mTOR pathway is a rare event in HCC and activation of the mTOR pathway appears to result largely from ligand dependent receptor activation.

Genomic studies in the past have identified multiple molecular classifications of HCC and demonstrated deregulated signaling pathways unique to subgroups of patients [19–24]. These studies indicated that the mTOR pathway and its upstream pathways PI3K and AKT occupy a central position in the network of deregulated signaling pathways in HCC. With the specific aim to identify driver genes associated with HCC prognosis, we have used an integrative approach combining data obtained from somatic copy number analysis and transcriptomics [25]. Fifty driver genes were recognized and were linked to the mTOR, AMPK or EGFR pathways. In the molecular HCC classification by Boyault *et al.*, 6 robust subgroups (G1-G6) were identified and the G1/G2 subgroup showed AKT activation with overexpression of *IGF2*, *IGF1R*, and *GSK3β* as well as *PIK3CA*, and *AXIN1* mutations [19]. The G1/G2 patient subgroup was further confirmed in a large meta-analysis using integrative transcriptomics of 9 HCC data sets including a total of 603 patients [26]. This analysis assigned the patients into three subclasses (S1-S3), and the G1/G2 subgroup was enriched in the subclass S2, characterized again by activation of the upstream regulator of mTOR, AKT, in combination with MYC.

Taken together, activation of mTOR plays a central role in HCC and blocking this pathway is an attractive strategy for HCC treatment. The main goal of this review is to offer the rationale for the use of mTOR inhibitors in HCC and provide an overview of the current and prospective clinical trials with mTOR inhibitors in HCC.

Rapamycin and first generation mTOR inhibitors

mTOR is targeted by rapamycin, a natural compound discovered from the bacterium *Streptomyces hygroscopius* more than 30 years

ago. The two mTOR-containing complexes have different sensitivities to rapamycin. mTORC1 is inhibited by a complex formed by rapamycin and FKBP12 protein [27]. In contrast, mTORC2 is generally resistant to rapamycin, however, in certain cell types, mTORC2 may show sensitivity after prolonged rapamycin treatment [28]. Rapamycin (sirolimus) was first approved as an immunosuppressant for the prevention of graft rejection in kidney transplant recipients more than a decade ago [2]. A few years later rapamycin obtained approval for its use as an anti-restenosis agent following balloon angioplasty in coronary arterial stents. The early success of rapamycin has encouraged the development of derivative compounds with improved bioavailability, called rapalogs: everolimus (RAD001), temsirolimus (CCI-779), and deforolimus (AP23573). Due to the important role of mTOR in cell growth and metabolism the primary interest shifted to anti-cancer therapy, and in 2007 temsirolimus (CCI-779) was approved for the treatment of renal cell carcinoma and shortly thereafter for mantle cell lymphoma. Meanwhile, everolimus (RAD001), has received approval for treatment of pancreatic neuroendocrine tumours, subependymal giant cell astrocytoma, renal cell carcinoma, and HER2-negative breast cancer in combination with Exemestane.

In general, first generation mTOR inhibitors are well tolerated. The major toxicities include, stomatitis, headache, diarrhea, vomiting, and thrombocytopenia. Due to their role in metabolism they can cause hyperglycemia, hyperlipidemia, and hypophosphatemia. As for every immunosuppressive drug, the risk for infections is increased. Furthermore, reactivation of HBV is a serious complication and has been described in renal cell carcinoma patients under everolimus treatment [29,30]. EASL Clinical practice Guidelines recommend, in which situation patients undergoing immunosuppressive therapy should receive prophylactic treatment against HBV-reactivation with a nucleoside analogue [31].

Currently, neither rapamycin nor rapalogs have gained approval for HCC treatment. However, several clinical trials are ongoing or have recently been completed using rapamycin/rapalogs for the treatment of advanced HCC and as adjuvant therapy after transarterial chemoembolization (TACE) or after liver transplantation of HCC patients. Furthermore, several studies suggest that mTOR inhibition may even prevent HCC development in patients at risk. The application of mTOR inhibitors in these different settings will be discussed in more detail.

Prevention of liver cancer by mTOR inhibition

Several studies indicate that mTOR activation is involved in the initiation of liver cancer and plays a role in the malignant transition of hepatocytes to HCC. A gradual activation of the AKT/mTOR pathway was progressively induced from non-tumorous liver tissue toward the HCC thus supporting this concept [15]. Interestingly, increased mTOR activity conferred a preneoplastic phenotype to HepaRG, considered a terminally differentiated hepatic cell line [32]. Also, treatment with mTOR inhibitor everolimus prevented proliferation of hepatocytes suffering DNA damage in the fumarylacetoacetate hydrolase-deficient mouse model of chronic liver injury and HCC development [33]. In transgenic mice, mTOR activation by itself was shown to be sufficient for HCC development. Two independent studies analyzed liver-specific knockout of TSC1 and the resulting chronic activation of mTOR [34,35]. Both studies demonstrated the development of liver tumors, however, only one was associated with inflammation [34]. Likewise constant activation of mTOR in PTEN-deficient mice induced steatohepatitis and development of liver tumors [36]. mTOR inhibitors may also prevent HCC indirectly by reducing liver fibrosis, a risk factor for the development of HCC. Studies in rats have demonstrated that sirolimus and everolimus attenuated progression of fibrosis, in contrast to cyclosporine A and tacrolimus, two other immunosuppressive drugs [37,38].

Randomized prospective clinical trials have not been conducted to address the question if mTOR inhibitors prevent HCC. The evidence suggesting a potential HCC prevention is solely derived from epidemiological studies with metformin, a widely used anti-diabetic drug that reduces mTOR activity but importantly also ameliorates hyperinsulinemia, which is a risk factor for HCC [39]. Metformin activates AMPK, which in turn suppresses the mTORC1 pathway (Fig. 2) [40]. A recent study showed that metformin controlled gene expression at the level of mRNA translation to an extent comparable to that of canonical mTOR inhibitors and down regulated mRNAs, which encode for proliferation and tumor-promoting proteins via the mTORC1/4E-BP pathway [41]. In addition, it was shown that metformin inhibited mTORC1 signaling independently of AMPK by suppressing Rag GTPase or activating REDD1 [42,43]. A meta-analysis, including 5 clinical studies with more than 100,000 type 2 diabetes patients has been performed lately [44]. This study showed an overall estimated 62% reduction in the risk of liver cancer if metformin was used as an anti-diabetic treatment instead of non-metformin treatment (e.g., sulfonylurea or insulin). Although there was considerable heterogeneity between the studies, the results are encouraging. Preventive clinical trials with metformin are already underway for different cancer types such as breast cancer, colon cancer, and esophagus cancer, however, for HCC they are lacking. Information of the potential preventive mechanism(s) of metformin on liver cancer is also limited. Recently, it was shown *in vitro* and *in vivo,* that metformin inhibited HCC cell growth through AMPK and LKB1 [45,46]. Further elucidation of this mechanism(s) and defining criteria that identify individuals which are likely to benefit are therefore needed. Nevertheless, it has to be kept in mind that a few studies have shown that metformin can have tumor-promoting effects. Metformin enhanced growth of BRAF-mutant melanoma cells and ER-alpha negative breast cancer cell lines *in vivo* [47,48]. Likewise, migration and invasion abilities of human pulmonary adenocarcinoma A549 cell lines increased under metformin treatment *in vitro* [49].

Prevention of HCC by metformin and mTOR inhibitors may also involve autophagy, an important homeostatic cellular recycling mechanism, which has a dual role in carcinogenesis. Autophagy is important in limiting DNA damage caused by the accumulation of reactive oxygen species and damaged organelles through removal of dysfunctional proteins and organelles [50]. Especially in the context of HCC development, which arises in 80% of the cases in the background of a chronic liver inflammation with constant DNA damage, induction of autophagy by mTOR inhibitors may prevent HCC development and recurrence. In contrast, increased autophagy may promote tumor growth, especially once tumor is established, as autophagy is also a pro-survival mechanism to cellular stress and further enhances chemoresistance [50,51].

Adjuvant therapy with mTOR inhibitors after liver transplantation or TACE

For patients with non-resectable HCC and early stage disease, liver transplantation is recognized as the treatment of choice,

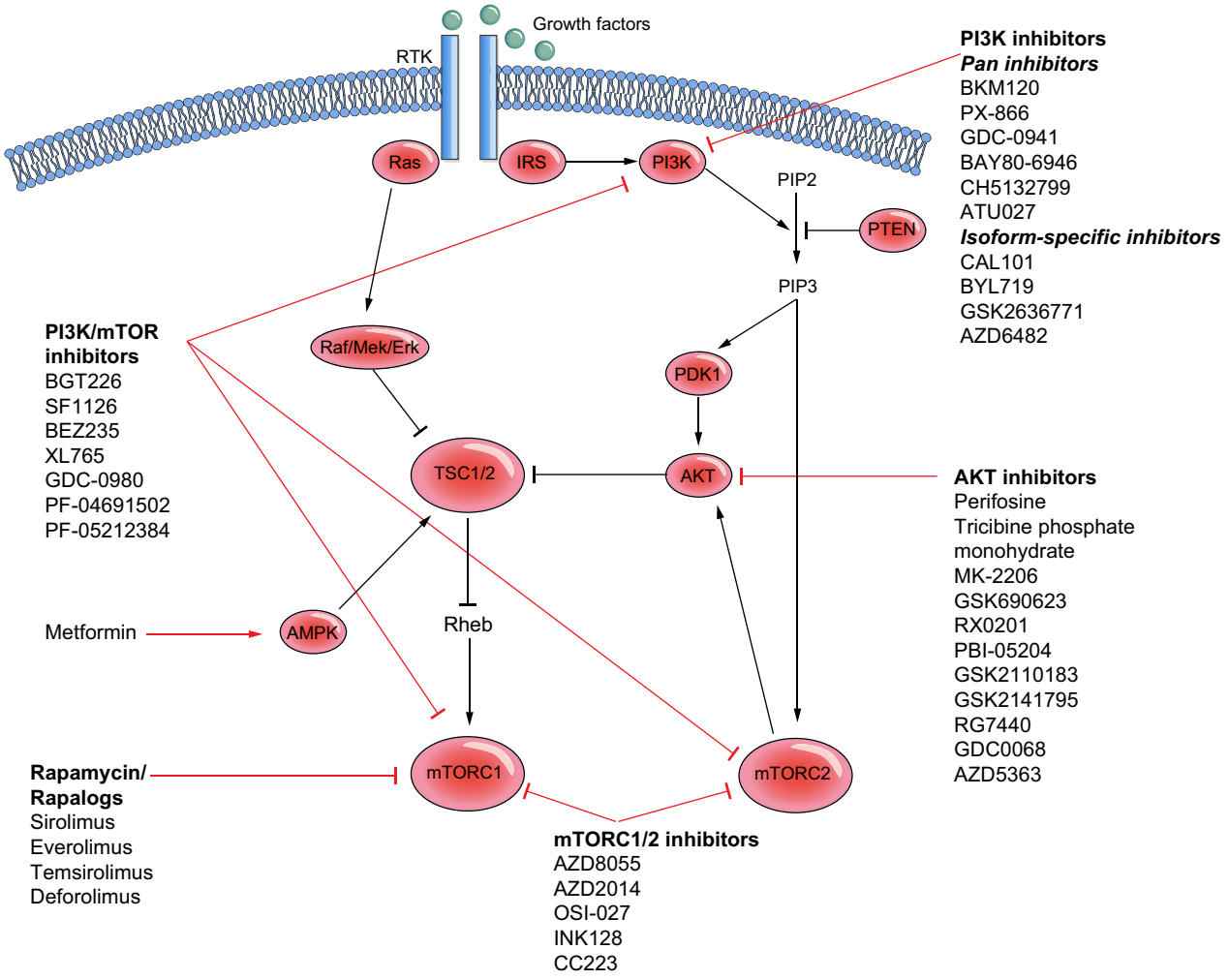

Fig. 2. Schematic overview of the mTOR signaling pathway with the target position of drugs.

with disease-free survival of 60–80% at 5 years [52]. Immunosuppressants, such as cyclosporine A, tacrolimus, and rapamycin are crucial for the prevention of graft rejection after transplantation. However, cyclosporine A and tacrolimus are known to induce tumor growth in preclinical studies [53,54]. On the contrary, rapamycin has additional anticancer function. Accordingly, a meta-analysis of 5 studies with a total of almost 3000 patients indicated that survival is significantly prolonged after liver transplantation of HCC patients if sirolimus is administered instead of non-sirolimus immunosuppressives [55]. Although these results indicate that sirolimus should be used as treatment of choice after liver transplantation of HCC patients, it has to be noted that none of the clinical studies conducted so far were randomized. Additional information regarding the efficacy of sirolimus to improve survival and prevent recurrence should be provided by three ongoing randomized controlled studies (Table 1). The largest trial is a multicenter, phase III study with over 500 patients (SiLVER) comparing sirolimus-based *vs.* sirolimus-free immunosuppression in patients undergoing liver transplantation for HCC. However, results are not expected before 2014.

TACE is the treatment of choice for intermediate HCC patients [52]. Following TACE treatment the levels of vascular endothelial growth factor (VEGF) have been shown to increase. VEGF promotes angiogenesis and is associated with bad prognosis. Therefore, TACE has been combined in clinical trials with other drugs that inhibit angiogenesis such as sorafenib, brivanib, or orantinib. Interestingly, everolimus has also been shown to reduce vessel formation in preclinical HCC models [56] and two ongoing clinical trials are evaluating the effect of TACE in combination with everolimus (Table 1).

mTOR inhibition in advanced HCC

Sorafenib is the only approved drug for HCC treatment [57,58]. However, the treatment effects are small, only selected patients are eligible for therapy, and side effects often limit applicability. Therefore, developing novel and effective therapies are urgently needed. Rapalogs have been shown to inhibit liver tumor growth in a large number of *in vitro* and *in vivo* pre-clinical studies [59] and have encouraged clinical trials in HCC patients (Table 1, information retrieved form www.clinicaltrials.gov).

Table 1. Summary of completed and ongoing clinical trials with first generation mTOR inhibitors in HCC.

			Drug	Patient number	HCC stage	Trial phase	Child-Pugh score	Study design	Results (or ID in clinical trials website)
Adjuvant therapy after liver transplantation			Sirolimus *vs.* mTOR inhibitor free	510	Milan criteria and extended	III	n.a.	R	Active, not recruiting (NCT00355862, SiLVER)
			Sirolimus *vs.* FK506	220	Exceeding Milan criteria	III	n.a.	R	Recruiting (NCT00554125)
			Sirolimus *vs.* mTOR inhibitor free	86	Exceeding Milan criteria	II	n.a.	R	Recruiting (NCT01374750)
Adjuvant therapy after TACE			TACE (doxorubicin) ± everolimus	98	B (BCLC)	I/II	A, B (≤7)	R	Recruiting (NCT01009801)
			TACE ± everolimus	90	B (BCLC)	II	A, early B	R	Recruiting (NCT01379521, TRACER)
Treatment of advanced HCC	Single drug	First line therapy	Sirolimus	21	I to IV (TNM)	Pilot	A, B, C	NR	Completed [63]. 1 PR. Dose determined by plasma level. Median dose = 1 mg/d
			Sirolimus	18	B, C, D (BCLC)	Pilot	A, B, C	NR	Completed [62]. No objective response. Administration twice daily. Dose determined by plasma level.
			Sirolimus	25	B or C (BCLC)	II	A, B	NR	Completed [64]. 1 CR and 1 PR. Dose = 30 mg/wk
			Temsirolimus	50	Advanced	II	B	NR	Terminated: toxic events (NCT01079767)
		First and second line	Everolimus	28	B or C (BCLC)	I/II	A, B	NR	Completed [60]. 1 PR. MTD = 10 mg/d
			Everolimus	39	C (BCLC)	I/II	A, B	R	Completed [61]. 1 PR. MTD = 7.5 mg/d or 70 mg/wk. DLT not reached for weekly schedule
		Second line therapy	Everolimus	546	Advanced	III	A	R	Completed, not yet published, failed to demonstrate efficacy compared to placebo (NCT01035229, EVOLVE)
			Temsirolimus	50	Advanced	I/II	A	NR	Active, not recruiting (NCT01251458)
			Temsirolimus	25	Advanced	II	A, B (≤9)	NR	Recruiting (NCT01567930)
	Drug combination	First and second line	Sorafenib and temsirolimus	25	III, IV (TNM)	I	A, B (≤7)	NR	Completed [70]. 2 PR; MTD = temsirolimus 10 mg/wk + sorafenib 200 mg twice daily
		First line therapy	Sorafenib ± everolimus	130	Advanced	I/II	A	NR	Terminated: everolimus MTD too low (NCT00828594)
			Sorafenib ± everolimus	106	B or C (BCLC)	II	A, B (≤7)	R	Active, not recruiting (NCT01005199)
			Sorafenib and everolimus	25	III, IV (TNM)	I	A, B (≤7)	NR	Active, not recruiting (NCT01013519)
			Sorafenib and everolimus	28	II, III, IV (TNM)	II	A, B (≤7)	NR	Recruiting (NCT01687673)
		Second line therapy	Everolimus + bevacizumab	33	B or C (BCLC)	I/II	A, B	NR	Completed. Not yet published (NCT00775073)
			Sirolimus + bevacizumab	24	B or C (BCLC)	I	A, B	NR	Completed [69] 1 CR and 2 PR. MTD = 4 mg/d
			Sorafenib and everolimus	18	Advanced	I/II	A, B (≤8)	NR	Unknown (NCT01335074)
			Everolimus and pasire-otide	30	C (BCLC)	II	A	NR	Recruiting (NCT01488487)

Two phase I/II dose-finding studies of 39 and 28 patients have been completed, using everolimus as a first and second line single agent and resulted in dose recommendations of 7.5 mg/day and 10 mg/day respectively [60,61]. In both studies, complete response was not observed, however, one HCC patient in each study showed a partial response, and stable disease was observed for a short time in 71.4% and 40% of the patients. Three earlier clinical studies with 18 to 25 patients using rapamycin as a first line single agent provided interesting results with partial responses and even one complete response; however, the differences in treatment schedule prevent any firm conclusions [62–64]. Overall, these preliminary results using rapamycin/rapalogs are encouraging; however, further studies with more patients are needed. Besides, one study using temsirolimus as a first line agent had to be terminated due to toxic events. The recent negative outcome of a multicenter randomized, double blind, phase III study dashed the hope to use mTOR inhibitors as a second line therapy for advanced HCC patients (press release). This study investigated the effect of everolimus or placebo in 546 patients with Child-Pugh A cirrhosis, whose disease progressed after treatment with or who were intolerant to sorafenib. It will be interesting to see if two other phase I/II studies using temsirolimus as a second line single agent obtain similar negative results.

Because of resistance and compensatory activation of other signaling pathways the effect of rapalogs can be diminished. For example, after treatment with everolimus, an upregulation of MAPK was shown in tumor samples from breast cancer patients [65]. As anticipated, combination of mTOR inhibition with a MAPK inhibitor (sorafenib) showed enhanced antitumoral effect *in vitro* and *in vivo* in cancer models including HCC [65–68]. A combinatorial approach is currently performed in 9 clinical trials (Table 1). Rapalogs or rapamycin is complemented with multikinase inhibitor sorafenib, with VEGF inhibitor bevacizumab or with pasireotide, a somatostatin analog, which has been shown to inhibit tumor growth. Two phase I combination studies have been published recently [69,70]. A combination of temsirolimus with sorafenib at the maximal tolerated dose (MTD; temsirolimus 10 mg weekly and sorafenib 200 mg twice daily), showed a partial response in 8% of the patients and a stable disease in 60% [70]. Although these results in 25 patients are promising, the median progression free survival was only 5.65 months, which is similar to outcomes from single agent sorafenib in the SHARP trial, though superior to outcomes observed in an Asia-Pacific clinical trial [57,58]. In addition, a phase I study with rapamycin and bevacizumab of 24 patients at MTD (rapamycin 4 mg/day and bevacizumab 5 mg/kg every 14 days), reported a remarkable complete response in one patient that lasted 4.5 months, partial response in two patients, and stable disease in 14 patients [69]. The ongoing clinical trials in phase I or II will reveal if a combinatorial approach improves efficacy in HCC treatment. The combination of drugs is certainly attractive, however, one drawback is that toxicities may increase, especially in cirrhotic patients and prevent that an efficacious dose can be applied. It is therefore important to perform pharmacokinetic studies in future clinical trials with dose escalation of both drugs (e.g., sorafenib and rapalogs), especially in cirrhotic patients.

Second generation mTOR inhibitors

We are currently awaiting the results of several clinical trials using rapalogs in HCC treatment; however, the success of rapalogs in cancer therapy in general has not been as impressive as initially hoped. Several possible reasons may account for the limited action of rapalogs. First, mTORC1 inhibition abrogates the negative feedback loop, which in turn activates PI3K-AKT with MAPK and RAS signaling and therefore may actually increase growth of cancer cells [65]. Second, blocking of mTORC1 primarily leads to inhibition of cell growth and not cell death. Third, mTOR inhibition by rapalogs mainly results in inhibition of S6K, however, the second key substrate, 4E-BP1, is only insufficiently blocked [71]. Finally, rapalogs do not inhibit mTORC2, which is often activated as part of the PI3K-mTORC2-AKT signaling axis [2]. In order to overcome the shortcomings and resistance of rapalogs a second generation of mTOR inhibitors has been developed that functions as ATP-competitive inhibitors of mTOR and has several advantages over rapalogs. Unlike rapalogs, which inhibit only mTORC1, the ATP analogues block the phosphorylation of all known downstream targets of mTORC1 and mTORC2. Furthermore, because of the similarity between the kinase domains of mTOR and PI3Ks, some of these new compounds additionally inhibit PI3K, leading to a broad inhibitory action with blocking of the feedback activation of PI3K-AKT signaling described before. Second generation mTOR inhibitors can therefore be divided into mTORC1/2 inhibitors and mTOR/PI3K inhibitors (Fig. 2). In addition, a series of compounds have been developed that block upstream of the mTOR pathway such as AKT inhibitors and PI3K inhibitors.

Translational genomics and development of second generation mTOR inhibitors

Translational genomic analyses have been used to investigate the resistance to rapamycin. Jimenez *et al.* [72] investigated rapamycin sensitivity or resistance in 13 HCC cell lines using drug-induced growth inhibition as the end point. The authors concluded that promoting or even maintaining effective drug sensitivity might be challenging, because of the molecular heterogeneity observed in the genomic profiles introduced in response to rapamycin, as well as determining (a) specific mechanism(s) of drug resistance. Furthermore, although the sensitivity to rapamycin was variable in all cell lines, the drug inhibited the phosphorylation of RPS6 and 4E-BP1, indicating that S6 and 4E-BP1 phosphorylation is not a useful marker for the antiproliferative effect of mTOR inhibitors. Interestingly, in an attempt to identify rapamycin sensitive genes a later study determined that many of the genes whose expression is altered by rapamycin are E-box containing and their regulation via mTOR was c-MYC independent [73]. Since the mTOR pathway is constitutively activated

Recruiting: The study is currently recruiting participants.
Active not recruiting: The study is ongoing but potential participants are not currently recruited.
Completed: The study has ended normally, and participants are no longer being examined or treated.
Terminated: The study has stopped recruiting participants early and will not start again.
PR, partial response; CR, complete response; n.a., not available; R, randomized; NR, not randomized; MTD, maximal tolerated dose; DLT, dose limiting toxicity.

in a majority of diffuse large B-cell lymphoma this is an interesting disease model to study resistance mechanisms to mTOR inhibitors and to identify drugs, which may compliment the action of rapamycin [74]. The authors compared the genomic signatures of 4 rapamycin sensitive and 4 resistant cell lines, and found that the central mechanism involved in the resistance to rapamycin was controlled by AKT. Using the genomic signatures to explore the Connectivity Map database to identify drugs which may reverse drug resistance, PI3K/AKT and HDAC inhibitors were the most likely candidates to synergize with an mTOR inhibitor. Resistance mechanisms to rapamycin and its derivates also include epigenome based reprogramming (e.g., miRs). Long-term rapamycin treatment results in an increase of the miR-17-92 cluster and inhibition of this change restored the drug sensitivity [75]. Likewise, in HCC the miR-216a/217 cluster is frequently upregulated, resulted in activation of the PI3K/AKT pathway and importantly resistance to sorafenib [76].

Preclinical studies of second mTOR, AKT, and PI3K inhibitors in HCC

Several preclinical studies with second generation mTOR, AKT, and PI3K inhibitors were performed in HCC cell lines [77–79], HCC xenograft mouse model [77,79], DEN-mouse HCC model [80] and in a diabetic rat model of HCC [81]. These studies have demonstrated that the second generation inhibitors were able to control HCC proliferation better than everolimus or sirolimus. It has also been demonstrated that combination of a PI3K inhibitor (BKM120) with cisplatin [77] or a PI3K/mTOR dual inhibitor (BEZ235) with everolimus [80] could act synergistically with strong antitumor activity. The two drugs, everolimus and BEZ235 exerted tumor regression via inhibition of mTORC1 and mTORC2. This combined drug-effect was associated with an increase in autophagy independent of 4E-BP1. At low doses both drugs targeted mTORC1, however, inhibition of mTORC2 was enhanced by the drug combination. The cooperative drug-effect was further evident from the microarray analysis identifying a distinct set of genes, suggesting a phenotypic reversal similar to placebo-treated livers. Also, only the drug combination achieved significant inhibition of genes involved in cell cycle. These results have prompted a dose finding and a safety clinical trial of BEZ235 in combination with everolimus in patients with advanced solid tumors.

Moreover, promising results may be expected from the combination of second generation mTOR, AKT or PI3K inhibitors with other drugs. This may be particularly important in cases of sorafenib resistance, which was recently demonstrated in a cancer stem cell subpopulation of HCC cells (i.e., label-retaining cancer cells), demonstrating sustained AKT and MAPK activation [82]. Also, in a study comparing the HCC cell line HuH7 and sorafenib resistance HuH7 derivatives, the molecular alteration related to the acquired drug resistance included upregulation and activation of PI3K/AKT signaling [83]. The sorafenib resistance was overcome by either silencing *AKT* using RNAi or targeting the pathway using the novel allosteric AKT inhibitor (MK-2206). In agreement, the PI3K/mTOR inhibitors (PI103 and PKI-587) also augment the effect of sorafenib [84,85]. Similarly, a recent study suggested that second-generation inhibitors (e.g., mTOR (AZD-8055), PI3K (BKM-120) or mTOR/PI3K (BEZ-235 and GDC-0980)) may be effective in sorafenib resistant HCC [86]. It should be noted that this study unfortunately was performed in SK-HEP1 and its drug-resistant derivative (SK-Sora), which are derived from an HCC patient with ascites but are of endothelial origin. The combination of everolimus together with an AKT inhibitor (MK-2206), mTOR/PI3K inhibitor (BEZ235) or PI3K inhibitor (BKM120) showed improved anti-tumoral activity [77,78,80]. Further, improved efficacy was also achieved *in vitro* and *in vivo* with novel ATP-competitive mTOR kinase inhibitors together with histone deacetylase inhibitors [87].

First results of clinical trials with new mTOR pathway inhibitors

The results of several phase 1 clinical trials in advanced cancer (all types) are now available with this new generation of mTOR inhibitors. A phase 1 clinical trial with patients suffering from solid tumors and lymphoma using pan-mTOR inhibitor AZD8055 [88], demonstrated dose limiting toxicities (DLT) of grade 3 with an increase in transaminases and no RECIST objective response [88]. Accordingly, AZD8055 will not be further tested. It is interesting to note that altered liver function was not described with mTOR inhibitors such as temsirolimus or everolimus. It will be important to see if other pan-mTOR inhibitors such as OSI-027, AZD2014, INK128 or CC223 also alter liver function.

Likewise, several dual mTOR-PI3K inhibitors are under evaluation and for 2 of them the phase 1 trial results have already been published. The first, BGT226 induced grade 3 diarrhea in 46% of patients at 125 mg, however, limiting the dose to 100 mg three times weekly resulted in insufficient inhibition of the PI3K pathway [89]. Modeling based on pharmacokinetic data predicted that BGT226 dose of >4000 mg/day would be required to achieve efficacious plasma exposure, exceeding the safety dose range. The second, SF1126 is composed of the pan-PI3K and mTOR inhibitor LY294002 conjugated to an RGDS-targeting peptide to increase binding to integrins expressed on the tumor vasculature. In phase 1, SF1126 did not reach the maximum tolerated dose with a single dose limiting toxicity grade 3 diarrhea, reduced p-AKT and increased apoptosis [90]. Further studies are planned in combination with rituximab in CD20+ B-cell malignancies. Other compounds such as BEZ235, XL765 (also known as SAR245409), GDC-0980, PF-04691502, and PF-05212384 (also known as PKI-587) have completed phase 1 trials but data are not yet published.

Several new compounds have been designed to selectively inhibit PI3K with pan-PI3K targeting all class IA PI3Ks (BKM120, PX-866, XL147, GDC-0941, BAY80-6946, GSK2126458, CH5132799 and ATU027) or PI3K isoform-specific inhibitors (CAL101, BYL719, GSK2636771, and AZD6482). The phase 1 study with BKM120 [91] has shown hyperglycemia, skin rash, and mood alteration as drug limiting toxicities. Once mood alterations were identified, selective serotonin reuptake inhibitors were prescribed, and no further mood alteration greater than grade 1 was seen. In the expansion study with the MTD (100 mg/d), most frequent grade 3/4 adverse effects (AEs) were increase transaminase (9.1%), asthenia (7.6%), and rash (6.1%). Anti-tumor activity is encouraging with 3 RECIST partial responses. A phase 1 study has also been completed for another compound (PX-866 [92]) showing that dose limiting toxicities consisted of grade 3 diarrhea and elevated AST. No RECIST response was seen in this trial. New trials in association with other drugs are currently ongoing.

Finally, some compounds directly inhibit AKT with different mechanisms of action (plasma membrane disrupting agent, ATP-competitive and allosteric AKT inhibitors). Perifosine, a plasma membrane disrupting agent, has been extensively studied in phase I, II, and even phase III trials [93,94]. Despite promising

first results with good tolerance and interesting anti-tumor activity, the latest phase III trial in metastatic refractory colorectal cancer was disappointing with no difference of overall survival or progression-free survival for perifosine plus capecitabine compared to capecitabine alone. In a small phase 1 trial with patient selection based on p-AKT positive tumor, triciribine phosphate monohydrate [95] has shown no dose-limiting toxicities but a modest decrease of p-AKT after treatment. Response rates were not reported. Finally MK-2206, an allosteric AKT inhibitor, recently showed DLTs as mainly grade 3/4 skin rash [96]. In this trial no objective response was seen, however, tumor shrinkage was noted without reaching the level of the RECIST partial response. Of note, 9 patients have had paired tumor biopsy at baseline and at day 15. All of them demonstrated a decrease of p-AKT. Multiple drug combination and weekly schedule are now being tested to increase anti-tumor activity and minimize drug toxicity. Other compounds such as GSK690693, RX0201 (AKT antisense), PBI-05204, GSK2110183, GSK2141795, RG7440, GDC0068, and AZD5363 are under investigation or have completed phase 1 trials but the results are not yet published.

Perspective on mTOR inhibitors in HCC therapy

HCC is a very complex and heterogenous disease and progress in treatment of advanced HCC will likely occur slowly. One of the advantages of the first generation inhibitors (rapalogs) is the high specificity, the clinical approval and the comparatively few side-effects. Even if single agent therapies do not show the expected efficacy as has been revealed by the recent results from the EVOLVE study, rapalogs are still very attractive for use with other drugs. Moreover, sirolimus will most likely become first line treatment for HCC patients after liver transplantation.

With regard to current available second generation mTOR inhibitors we can anticipate limitations in their use in HCC patients, although they are more effective than first generation inhibitors in preclinical studies. First, for patients with impaired liver function, the increase in transaminase is troublesome, as observed in several clinical trials with the mTOR inhibitor AZD8055 but also with PI3K/mTOR dual inhibitor BGT226. Second, the performed phase 1 clinical trials demonstrated modest monotherapy efficacy, which was related to adverse events limiting dose escalation, but also related to a cytostatic effect more than a cytolytic effect of these drugs. Therefore, improved efficacy may mainly be reached in combination with other drugs. Third, new generation mTOR inhibitors may stimulate autophagy more than first generation inhibitors, and may consequently cause tumor cell protection against chemotherapy-induced death.

A strong focus should also be directed towards the discovery and validation of biomarkers, which predict tumor-response after therapy. Biomarkers for mTOR inhibitor efficacy have been evaluated in preclinical, but also in clinical studies [97,98]. Among others, these biomarkers include inactivation of PTEN, activating mutations of PI3KCA, expression levels of pS6, pS6K, S6K, and pAkt. Interestingly, a study demonstrated that human cancer cell lines carrying PI3KCA mutations were responsive to everolimus, except when KRAS mutations occurred concomitantly [99]. Similar results were obtained in a study with colon cancer cell lines [100], and mTOR resistance was also shown in ovarian cancer cell lines, which overexpressed the apoptosis-inhibitory protein Bcl2 [101]. Novel technologies such as next-generation sequencing may further path the way for the identification of new biomarkers, as has been shown in a recent study with bladder cancer patients, in which response to everolimus was clearly more effective in patients with a somatic mutation in the TSC1 complex [102]. However, none of these markers have been confirmed in clinical trials. It is thus currently difficult to recommend any of these biomarkers for patient selection in clinical trials. In addition, it has to be emphasized, that substantial progress in the identification of biomarkers for HCC treatment is unlikely to occur, if tumor tissue is not taken before and after chemotherapy by means of a liver biopsy. Acquiring tumor tissue should therefore be mandatory for any phase II or phase III clinical study and will further help to develop novel therapies.

In spite of these drawbacks, mTOR inhibitors together with AKT and PI3K inhibitors still remain an attractive and promising therapeutic option for the treatment of HCC and ongoing as well as future clinical studies will reveal if they can be used for the therapy of this devastating disease.

Key Points

- The mTOR pathway is upregulated in 40-50% of HCC cases, is related to bad prognosis, and contributes to sorafenib resistance in HCC. Preclinical *in vitro* and *in vivo* studies showed a reduction of HCC tumor growth by mTOR inhibitors
- Rapamycin and first generation mTOR inhibitors (rapalogs) are used in clinical trials for advanced HCC but also for adjuvant therapy in HCC patients after transplantation and TACE
- Preclinical studies in HCC have shown that second generation mTOR inhibitors are more efficacious than first generation mTOR inhibitors and could synergize with them
- Phase 1 clinical trials with second generation mTOR inhibitors in various solid cancers revealed dose limiting toxicities such as diarrhea, skin rash, and increase of transaminase
- Predictive biomarkers for efficacy of mTOR inhibitors have been identified in preclinical studies but not yet confirmed in clinic studies

Financial support

This work was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. M.S.M. is supported by "Schweizerische Stifung für Medizinisch-Biologische Stipendien" (PASMP-3_140071). T.D. is supported by the "Fondation Monahan" and "Prix Amgen pour la Recherche et l'Innovation en oncologie digestive 2011".

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012;149:274–293.
[2] Benjamin D, Colombi M, Moroni C, Hall MN. Rapamycin passes the torch: a new generation of mTOR inhibitors. Nat Rev Drug Discov 2011;10:868–880.
[3] Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev 2003;17:1829–1834.
[4] Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC, Blenis J. Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. Proc Natl Acad Sci U S A 2002;99:13571–13576.
[5] Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell 2003;115:577–590.
[6] DeYoung MP, Horak P, Sofer A, Sgroi D, Ellisen LW. Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. Genes Dev 2008;22:239–251.
[7] Zinzalla V, Stracka D, Oppliger W, Hall MN. Activation of mTORC2 by association with the ribosome. Cell 2011;144:757–768.
[8] Sieghart W, Fuereder T, Schmid K, Cejka D, Werzowa J, Wrba F, et al. Mammalian target of rapamycin pathway activity in hepatocellular carcinomas of patients undergoing liver transplantation. Transplantation 2007;83:425–432.
[9] Villanueva A, Chiang DY, Newell P, Peix J, Thung S, Alsinet C, et al. Pivotal role of mTOR signaling in hepatocellular carcinoma. Gastroenterology 2008;135:1972–1983, [e1971–1911].
[10] Sahin F, Kannangai R, Adegbola O, Wang J, Su G, Torbenson M. mTOR and P70 S6 kinase expression in primary liver neoplasms. Clin Cancer Res 2004;10:8421–8425.
[11] Menon S, Manning BD. Common corruption of the mTOR signaling network in human tumors. Oncogene 2008;27:S43–S51.
[12] Andersen JB, Spee B, Blechacz BR, Avital I, Komuta M, Barbour A, et al. Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. Gastroenterology 2012;142:e1015.
[13] Baba HA, Wohlschlaeger J, Cicinnati VR, Hilgard P, Lang H, Sotiropoulos GC, et al. Phosphorylation of p70S6 kinase predicts overall survival in patients with clear margin-resected hepatocellular carcinoma. Liver Int 2009;29:399–405.
[14] Zhou L, Huang Y, Li J, Wang Z. The mTOR pathway is associated with the poor prognosis of human hepatocellular carcinoma. Med Oncol 2010;27:255–261.
[15] Calvisi DF, Wang C, Ho C, Ladu S, Lee SA, Mattu S, et al. Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling, promotes development of human hepatocellular carcinoma. Gastroenterology 2011;140:1071–1083.
[16] Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012;44:694–698.
[17] Cleary SP, Jeck WR, Zhao X, Chen K, Selitsky SR, Savich GL, et al. Identification of driver genes in hepatocellular carcinoma by exome sequencing. Hepatology 2013;58:1693–1702.
[18] Kan Z, Zheng H, Liu X, Li S, Barber TD, Gong Z, et al. Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma. Genome Res 2013;23:1422–1433.
[19] Boyault S, Rickman DS, de Reynies A, Balabaud C, Rebouissou S, Jeannot E, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology 2007;45:42–52.
[20] Chiang DY, Villanueva A, Hoshida Y, Peix J, Newell P, Minguez B, et al. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. Cancer Res 2008;68:6779–6788.
[21] Lee JS, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, et al. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. Hepatology 2004;40:667–676.
[22] Lee JS, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. Nat Med 2006;12:410–416.
[23] Lee JS, Thorgeirsson SS. Genome-scale profiling of gene expression in hepatocellular carcinoma: classification, survival prediction, and identification of therapeutic targets. Gastroenterology 2004;127:S51–S55.
[24] Nault JC, De Reynies A, Villanueva A, Calderaro J, Rebouissou S, Couchy G, et al. A hepatocellular carcinoma 5-gene score associated with survival of patients after liver resection. Gastroenterology 2013;145:176–187.
[25] Woo HG, Park ES, Lee JS, Lee YH, Ishikawa T, Kim YJ, et al. Identification of potential driver genes in human liver carcinoma by genome wide screening. Cancer Res 2009;69:4059–4066.
[26] Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. Cancer Res 2009;69:7385–7392.
[27] Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science 1991;253:905–909.
[28] Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell 2006;22:159–168.
[29] Sezgin Goksu S, Bilal S, Coskun HS. Hepatitis B reactivation related to everolimus. World J Hepatol 2013;5:43–45.
[30] Mizuno S, Yamagishi Y, Ebinuma H, Nakamoto N, Katahira M, Sasaki A, et al. Progressive liver failure induced by everolimus for renal cell carcinoma in a 58-year-old male hepatitis B virus carrier. Clin J Gastroenterol 2013;6:188–192.
[31] EASL clinical practice guidelines. Management of chronic hepatitis B virus infection. J Hepatol 2012;57:167–185.
[32] Parent R, Kolippakkam D, Booth G, Beretta L. Mammalian target of rapamycin activation impairs hepatocytic differentiation and targets genes moderating lipid homeostasis and hepatocellular growth. Cancer Res 2007;67:4337–4345.
[33] Buitrago-Molina LE, Pothiraju D, Lamle J, Marhenke S, Kossatz U, Breuhahn K, et al. Rapamycin delays tumor development in murine livers by inhibiting proliferation of hepatocytes with DNA damage. Hepatology 2009;50:500–509.
[34] Menon S, Yecies JL, Zhang HH, Howell JJ, Nicholatos J, Harputlugil E, et al. Chronic activation of mTOR complex 1 is sufficient to cause hepatocellular carcinoma in mice. Sci Signal 2012;5:ra24.
[35] Kenerson HL, Yeh MM, Kazami M, Jiang X, Riehle KJ, McIntyre RL, et al. Akt and mTORC1 have different roles during liver tumorigenesis in mice. Gastroenterology 2013;144:1055–1065.
[36] Horie Y, Suzuki A, Kataoka E, Sasaki T, Hamada K, Sasaki J, et al. Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. J Clin Invest 2004;113:1774–1783.
[37] Neef M, Ledermann M, Saegesser H, Schneider V, Reichen J. Low-dose oral rapamycin treatment reduces fibrogenesis, improves liver function, and prolongs survival in rats with established liver cirrhosis. J Hepatol 2006;45:786–796.
[38] Patsenker E, Schneider V, Ledermann M, Saegesser H, Dorn C, Hellerbrand C, et al. Potent antifibrotic activity of mTOR inhibitors sirolimus and everolimus but not of cyclosporine A and tacrolimus in experimental liver fibrosis. J Hepatol 2011;55:388–398.
[39] Siegel AB, Zhu AX. Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. Cancer 2009;115:5651–5661.
[40] Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. Cancer Res 2006;66:10269–10273.
[41] Larsson O, Morita M, Topisirovic I, Alain T, Blouin MJ, Pollak M, et al. Distinct perturbation of the translatome by the antidiabetic drug metformin. Proc Natl Acad Sci U S A 2012;109:8977–8982.
[42] Ben Sahra I, Regazzetti C, Robert G, Laurent K, Le Marchand-Brustel Y, Auberger P. Metformin, independent of AMPK, induces mTOR inhibition and cell-cycle arrest through REDD1. Cancer Res 2011;71:4366–4372.
[43] Kalender A, Selvaraj A, Kim SY, Gulati P, Brule S, Viollet B, et al. Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. Cell Metab 2010;11:390–401.
[44] Zhang ZJ, Zheng ZJ, Shi R, Su Q, Jiang Q, Kip KE. Metformin for liver cancer prevention in patients with type 2 diabetes: a systematic review and meta-analysis. J Clin Endocrinol Metab 2012;97:2347–2353.
[45] Chen HP, Shieh JJ, Chang CC, Chen TT, Lin JT, Wu MS, et al. Metformin decreases hepatocellular carcinoma risk in a dose-dependent manner: population-based and in vitro studies. Gut 2013;62:606–615.
[46] Zheng L, Yang W, Wu F, Wang C, Yu L, Tang L, et al. Prognostic significance of AMPK activation and therapeutic effects of metformin in hepatocellular carcinoma. Clin Cancer Res 2013;19:5372–5380.
[47] Phoenix KN, Vumbaca F, Claffey KP. Therapeutic metformin/AMPK activation promotes the angiogenic phenotype in the ERalpha negative MDA-MB-435 breast cancer model. Breast Cancer Res Treat 2009;113:101–111.
[48] Martin MJ, Hayward R, Viros A, Marais R. Metformin accelerates the growth of BRAF V600E-driven melanoma by upregulating VEGF-A. Cancer Discov 2012;2:344–355.
[49] Wu N, Gu HJ, Li Q. Effects of antidiabetic drug metformin on the migration and invasion abilities of human pulmonary adenocarcinoma A549 cell line in vitro. J Thorac Dis 2010;2:76–80.

[50] Janku F, McConkey DJ, Hong DS, Kurzrock R. Autophagy as a target for anticancer therapy. Nat Rev Clin Oncol 2011;8:528–539.
[51] Cui J, Gong Z, Shen HM. The role of autophagy in liver cancer: molecular mechanisms and potential therapeutic targets. Biochim Biophys Acta 2013;1836:15–26.
[52] Colombo M, Raoul JL, Lencioni R, Galle PR, Zucman-Rossi J, Banares R, et al. Multidisciplinary strategies to improve treatment outcomes in hepatocellular carcinoma: a European perspective. Eur J Gastroenterol Hepatol 2013;25:639–651.
[53] Freise CE, Ferrell L, Liu T, Ascher NL, Roberts JP. Effect of systemic cyclosporine on tumor recurrence after liver transplantation in a model of hepatocellular carcinoma. Transplantation 1999;67:510–513.
[54] Hojo M, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 1999;397:530–534.
[55] Liang W, Wang D, Ling X, Kao AA, Kong Y, Shang Y, et al. Sirolimus-based immunosuppression in liver transplantation for hepatocellular carcinoma: a meta-analysis. Liver Transpl 2012;18:62–69.
[56] Semela D, Piguet AC, Kolev M, Schmitter K, Hlushchuk R, Djonov V, et al. Vascular remodeling and antitumoral effects of mTOR inhibition in a rat model of hepatocellular carcinoma. J Hepatol 2007;46:840–848.
[57] Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–390.
[58] Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25–34.
[59] Buitrago-Molina LE, Vogel A. mTor as a potential target for the prevention and treatment of hepatocellular carcinoma. Curr Cancer Drug Targets 2012;12:1045–1061.
[60] Zhu AX, Abrams TA, Miksad R, Blaszkowsky LS, Meyerhardt JA, Zheng H, et al. Phase 1/2 study of everolimus in advanced hepatocellular carcinoma. Cancer 2011;117:5094–5102.
[61] Shiah HS, Chen CY, Dai CY, Hsiao CF, Lin YJ, Su WC, et al. Randomised clinical trial: comparison of two everolimus dosing schedules in patients with advanced hepatocellular carcinoma. Aliment Pharmacol Ther 2013;37:62–73.
[62] Schoniger-Hekele M, Muller C. Pilot study: rapamycin in advanced hepatocellular carcinoma. Aliment Pharmacol Ther 2010;32:763–768.
[63] Rizell M, Andersson M, Cahlin C, Hafstrom L, Olausson M, Lindner P. Effects of the mTOR inhibitor sirolimus in patients with hepatocellular and cholangiocellular cancer. Int J Clin Oncol 2008;13:66–70.
[64] Decaens T, Luciani A, Itti E, Hulin A, Roudot-Thoraval F, Laurent A, et al. Phase II study of sirolimus in treatment-naive patients with advanced hepatocellular carcinoma. Dig Liver Dis 2012;44:610–616.
[65] Carracedo A, Ma L, Teruya-Feldstein J, Rojo F, Salmena L, Alimonti A, et al. Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Invest 2008;118:3065–3074.
[66] Piguet AC, Saar B, Hlushchuk R, St-Pierre MV, McSheehy PM, Radojevic V, et al. Everolimus augments the effects of sorafenib in a syngeneic orthotopic model of hepatocellular carcinoma. Mol Cancer Ther 2011;10:1007–1017.
[67] Newell P, Toffanin S, Villanueva A, Chiang DY, Minguez B, Cabellos L, et al. Ras pathway activation in hepatocellular carcinoma and anti-tumoral effect of combined sorafenib and rapamycin in vivo. J Hepatol 2009;51:725–733.
[68] Wang Z, Zhou J, Fan J, Qiu SJ, Yu Y, Huang XW, et al. Effect of rapamycin alone and in combination with sorafenib in an orthotopic model of human hepatocellular carcinoma. Clin Cancer Res 2008;14:5124–5130.
[69] Choo SP, Chowbay B, Ng QS, Thng CH, Lim C, Hartono S, et al. A phase 1 dose-finding and pharmacodynamic study of rapamycin in combination with bevacizumab in patients with unresectable hepatocellular carcinoma. Eur J Cancer 2013;49:999–1008.
[70] Kelley RK, Nimeiri HS, Munster PN, Vergo MT, Huang Y, Li CM, et al. Temsirolimus combined with sorafenib in hepatocellular carcinoma: a phase I dose-finding trial with pharmacokinetic and biomarker correlates. Ann Oncol 2013;24:1900–1907.
[71] Choo AY, Yoon SO, Kim SG, Roux PP, Blenis J. Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. Proc Natl Acad Sci U S A 2008;105:17414–17419.
[72] Jimenez RH, Boylan JM, Lee JS, Francesconi M, Castellani G, Sanders JA, et al. Rapamycin response in tumorigenic and non-tumorigenic hepatic cell lines. PLoS One 2009;4:e7373.
[73] Jimenez RH, Lee JS, Francesconi M, Castellani G, Neretti N, Sanders JA, et al. Regulation of gene expression in hepatic cells by the mammalian Target of Rapamycin (mTOR). PLoS One 2010;5:e9084.
[74] Petrich AM, Leshchenko V, Kuo PY, Xia B, Thirukonda VK, Ulahannan N, et al. Akt inhibitors MK-2206 and nelfinavir overcome mTOR inhibitor resistance in diffuse large B-cell lymphoma. Clin Cancer Res 2012;18:2534–2544.
[75] Totary-Jain H, Sanoudou D, Ben-Dov IZ, Dautriche CN, Guarnieri P, Marx SO, et al. Reprogramming of the microRNA transcriptome mediates resistance to rapamycin. J Biol Chem 2013;288:6034–6044.
[76] Xia H, Ooi LL, Hui KM. MicroRNA-216a/217-induced epithelial-mesenchymal transition targets PTEN and SMAD7 to promote drug resistance and recurrence of liver cancer. Hepatology 2013;58:629–641.
[77] Kirstein MM, Boukouris AE, Pothiraju D, Buitrago-Molina LE, Marhenke S, Schutt J, et al. Activity of the mTOR inhibitor RAD001, the dual mTOR and PI3-kinase inhibitor BEZ235 and the PI3-kinase inhibitor BKM120 in hepatocellular carcinoma. Liver Int 2013;33:780–793.
[78] Grabinski N, Ewald F, Hofmann BT, Staufer K, Schumacher U, Nashan B, et al. Combined targeting of AKT and mTOR synergistically inhibits proliferation of hepatocellular carcinoma cells. Mol Cancer 2012;11:85.
[79] Masuda M, Shimomura M, Kobayashi K, Kojima S, Nakatsura T. Growth inhibition by NVP-BEZ235, a dual PI3K/mTOR inhibitor, in hepatocellular carcinoma cell lines. Oncol Rep 2011;26:1273–1279.
[80] Thomas HE, Mercer CA, Carnevalli LS, Park J, Andersen JB, Conner EA, et al. mTOR inhibitors synergize on regression, reversal of gene expression, and autophagy in hepatocellular carcinoma. Sci Transl Med 2012;4:139ra184.
[81] Evert M, Calvisi DF, Evert K, De Murtas V, Gasparetti G, Mattu S, et al. V-AKT murine thymoma viral oncogene homolog/mammalian target of rapamycin activation induces a module of metabolic changes contributing to growth in insulin-induced hepatocarcinogenesis. Hepatology 2012;55:1473–1484.
[82] Xin HW, Ambe CM, Hari DM, Wiegand GW, Miller TC, Chen JQ, et al. Label-retaining liver cancer cells are relatively resistant to sorafenib. Gut 2013;62:1777–1786.
[83] Chen KF, Chen HL, Tai WT, Feng WC, Hsu CH, Chen PJ, et al. Activation of phosphatidylinositol 3-kinase/Akt signaling pathway mediates acquired resistance to sorafenib in hepatocellular carcinoma cells. J Pharmacol Exp Ther 2011;337:155–161.
[84] Gedaly R, Angulo P, Chen C, Creasy KT, Spear BT, Hundley J, et al. The role of PI3K/mTOR inhibition in combination with sorafenib in hepatocellular carcinoma treatment. Anticancer Res 2012;32:2531–2536.
[85] Gedaly R, Angulo P, Hundley J, Daily MF, Chen C, Evers BM. PKI-587 and sorafenib targeting PI3K/AKT/mTOR and Ras/Raf/MAPK pathways synergistically inhibit HCC cell proliferation. J Surg Res 2012;176:542–548.
[86] Serova M, de Gramont A, Tijeras-Raballand A, Dos Santos C, Riveiro ME, Slimane K, et al. Benchmarking effects of mTOR, PI3K, and dual PI3K/mTOR inhibitors in hepatocellular and renal cell carcinoma models developing resistance to sunitinib and sorafenib. Cancer Chemother Pharmacol 2013;71:1297–1307.
[87] Shao H, Gao C, Tang H, Zhang H, Roberts LR, Hylander BL, et al. Dual targeting of mTORC1/C2 complexes enhances histone deacetylase inhibitor-mediated anti-tumor efficacy in primary HCC cancer in vitro and in vivo. J Hepatol 2012;56:176–183.
[88] Naing A, Aghajanian C, Raymond E, Olmos D, Schwartz G, Oelmann E, et al. Safety, tolerability, pharmacokinetics and pharmacodynamics of AZD8055 in advanced solid tumours and lymphoma. Br J Cancer 2012;107:1093–1099.
[89] Markman B, Tabernero J, Krop I, Shapiro GI, Siu L, Chen LC, et al. Phase I safety, pharmacokinetic, and pharmacodynamic study of the oral phosphatidylinositol-3-kinase and mTOR inhibitor BGT226 in patients with advanced solid tumors. Ann Oncol 2012;23:2399–2408.
[90] Mahadevan D, Chiorean EG, Harris WB, Von Hoff DD, Stejskal-Barnett A, Qi W, et al. Phase I pharmacokinetic and pharmacodynamic study of the pan-PI3K/mTORC vascular targeted pro-drug SF1126 in patients with advanced solid tumours and B-cell malignancies. Eur J Cancer 2012;48:3319–3327.
[91] Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birle D, et al. Phase I, dose-escalation study of BKM120, an oral pan-Class I PI3K inhibitor, in patients with advanced solid tumors. J Clin Oncol 2012;30:282–290.
[92] Hong DS, Bowles DW, Falchook GS, Messersmith WA, George GC, O'Bryant CL, et al. A multicenter phase I trial of PX-866, an oral irreversible phosphatidylinositol 3-kinase inhibitor, in patients with advanced solid tumors. Clin Cancer Res 2012;18:4173–4182.
[93] Van Ummersen L, Binger K, Volkman J, Marnocha R, Tutsch K, Kolesar J, et al. A phase I trial of perifosine (NSC 639966) on a loading dose/

maintenance dose schedule in patients with advanced cancer. Clin Cancer Res 2004;10:7450–7456.
[94] Unger C, Berdel W, Hanauske AR, Sindermann H, Engel J, Mross K. First-time-in-man and pharmacokinetic study of weekly oral perifosine in patients with solid tumours. Eur J Cancer 2010;46:920–925.
[95] Garrett CR, Coppola D, Wenham RM, Cubitt CL, Neuger AM, Frost TJ, et al. Phase I pharmacokinetic and pharmacodynamic study of triciribine phosphate monohydrate, a small-molecule inhibitor of AKT phosphorylation, in adult subjects with solid tumors containing activated AKT. Invest New Drugs 2011;29:1381–1389.
[96] Yap TA, Yan L, Patnaik A, Fearen I, Olmos D, Papadopoulos K, et al. First-in-man clinical trial of the oral pan-AKT inhibitor MK-2206 in patients with advanced solid tumors. J Clin Oncol 2011;29:4688–4695.
[97] Delbaldo C, Albert S, Dreyer C, Sablin MP, Serova M, Raymond E, et al. Predictive biomarkers for the activity of mammalian target of rapamycin (mTOR) inhibitors. Targeted Oncol 2011;6:119–124.
[98] Liao YM, Sy A, Yen Y. Markers for efficacy of mammalian target of rapamycin inhibitor. Anticancer Res 2012;32:4235–4244.
[99] Di Nicolantonio F, Arena S, Tabernero J, Grosso S, Molinari F, Macarulla T, et al. Deregulation of the PI3K and KRAS signaling pathways in human cancer cells determines their response to everolimus. J Clin Invest 2010;120:2858–2866.
[100] Ducker GS, Atreya CE, Simko JP, Hom YK, Matli MR, Benes CH, et al. Incomplete inhibition of phosphorylation of 4E-BP1 as a mechanism of primary resistance to ATP-competitive mTOR inhibitors. Oncogene 2013, [advanced online publication].
[101] Aguirre D, Boya P, Bellet D, Faivre S, Troalen F, Benard J, et al. Bcl-2 and CCND1/CDK4 expression levels predict the cellular effects of mTOR inhibitors in human ovarian carcinoma. Apoptosis 2004;9:797–805.
[102] Iyer G, Hanrahan AJ, Milowsky MI, Al-Ahmadie H, Scott SN, Janakiraman M, et al. Genome sequencing identifies a basis for everolimus sensitivity. Science 2012;338:221.