



REVIEW

Evaluating Oncogenic Drivers and Therapeutic Potential of the PI3K/AKT/mTOR Pathway in Hepatocellular Carcinoma: An Overview of Clinical Trials

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ABSTRACT: Hepatocellular carcinoma (HCC) is the most common primary liver tumor and the third leading cause of cancer-related mortality globally. The phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway is critically involved in HCC pathogenesis, stimulating uncontrolled cell proliferation, survival, and tumor progression. The overactivation of this pathway is strongly linked to poor prognosis, making it a crucial target for therapeutic intervention. The oncogenic roles of PI3K/AKT/mTOR components in HCC have been highlighted, noting that class I PI3K deregulation, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) upregulation, and mTOR overexpression could be associated with poor HCC outcomes. To the best of our knowledge, this is the first time that the clinical trials investigating PI3K/AKT/mTOR inhibitors in HCC are analyzed. Accordingly, there is a predominance of mTOR inhibitors, with everolimus being the most frequently utilized drug. However, only 10% of studies advanced to phase III or IV, predominantly involving mTOR inhibitors. Challenges such as adverse events like hyperglycemia and bone marrow suppression, as well as the emergence of treatment resistance, have hindered the success of these therapies. Combination therapies, particularly those involving mitogen-activated protein kinase kinase (MEK) inhibitors, chemotherapy, immune checkpoint inhibitors, and vascular endothelial growth factor (VEGF) inhibitors, have shown promise in overcoming these challenges. Recent advances in nanotechnology offer the potential for improving drug delivery and reducing toxicity.

KEYWORDS: HCC; PI3K; AKT; mTOR; clinical trial

1 Introduction

The tumors in the liver can occur in hepatocytes as well as in the bile ducts, leading to hepatocellular carcinoma (HCC) and cholangiocarcinoma, with the former comprising more than 90% of primary liver tumors [1]. According to the World Health Organization (WHO), nearly 900,000 individuals worldwide develop HCC annually, of which 69.8% are male. HCC is the third leading cause of cancer-related death globally, with more than 830,000 recorded deaths each year. Based on reports from the Global Cancer Observatory (GCO), over 72% of HCC cases are identified in Asia, followed by Africa, Oceania, North



America, and Europe [2]. Numerous risk factors contribute to the development of HCC, causing fibrosis and necrosis of hepatocytes, repetitive regeneration, cirrhosis, alterations in genes, chromosomal instability (CIN), DNA damage, and altered protein expression [3]. The most well-studied risk factors include viral hepatitis (hepatitis B and hepatitis C), alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD) and its severe form, non-alcoholic steatohepatitis (NASH), obesity, diabetes, and aflatoxin B1 [4,5].

The key to success for tumors is their capacity to change their metabolic pathways to maintain the demand for energy at the highest level necessary for out-of-control growth [6]. The Warburg effect is one of the prominent events in malignant cells, referring to the generation of excessive energy by elevating the oxygen-dependent glycolysis, followed by lactic acid fermentation with lactate secretion [7]. Several signaling pathways are associated with the Warburg effect; however, the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) axis has been shown to play an imperative role in increasing Warburg effect, leading to out-of-control proliferation [8]. The activation of the PI3K signaling pathway has been demonstrated to be able to turn liver tumors into a more aggressive one as the overactivation of this axis is related to poor prognosis of HCC [9]. Conventional therapeutic strategies such as surgery, radiotherapy, chemotherapy, and liver transplantation have pros and cons and are not a guarantee for HCC treatment. Recent studies have focused on novel therapeutic approaches such as targeting oncogenic signaling pathways [1]. Concerning the oncogenic features of the PI3K/AKT/mTOR signaling pathway, the application of therapeutic inhibitors of the components of this pathway has gained popularity in preclinical and clinical studies [10]. In the present study, we provided an overview of the oncogenic characteristics of the PI3K/AKT/mTOR signaling pathway in HCC development. Furthermore, we, for the very first time, investigated the clinical trials regarding inhibitors of the PI3K axis and discussed the promising traits and shortcomings. In this review, we explore the pivotal role of the PI3K/AKT/mTOR signaling pathway in the pathogenesis and progression of HCC, highlighting its contributions to tumorigenesis and therapeutic shortcomings. Our analysis provides an in-depth overview of the clinical trials involving inhibitors of this pathway, including their efficacy, shortcomings, and the increasing interest in combination regimens to overcome resistance and adverse effects. This comprehensive evaluation aims to illuminate current advancements and inspire innovative approaches for effectively managing HCC.

2 Overview of PI3K/AKT/mTOR Signaling Pathway

The PI3K/AKT/mTOR axis is one of the fundamental signaling pathways necessary for maintaining cell growth and survival in both physiological and pathological conditions. PI3K axis could be particularly activated in cellular stress situations to regulate the survival of the cell [11]; therefore, this signaling pathway plays an imperative role in cancerous conditions as tumors exist in stressful microenvironments such as low amounts of nutrition and oxygen [12]. Three classes of PI3K have been discovered so far [13]. Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), PIK3CB, PIK3CG, and PIK3CD encode four catalytic subunits of class I PI3K, which can catalyze the phosphorylation of the phosphatidylinositol 4,5-bisphosphate (PIP2) and generate phosphatidylinositol 3,4,5-trisphosphate (PIP3) [14]. PI3K-C2 α , PI3K-C2 β , and PI3K-C2 γ are three different isoforms of class II PI3K, with PI3K-C2 α having roles in vesicular trafficking, endocytosis, and mitosis, PI3K-C2 β in mTOR suppression and cell migration, and PI3K-C2 γ in AKT2 activation and glycogen storage [15]. PIK3C3 encodes the third class of PI3K (class III) which plays an essential role in cellular events such as autophagosome formation and autophagy flux [13]. AKT family includes serine and threonine kinases, and the three isoforms are AKT1, AKT2, and AKT3. In the context of the liver, it was shown that the expression of AKT1 and AKT2 could be identified in hepatocytes. In HCC cases, the overactivation of c-MYC is tightly correlated with the expression of AKT1, and high levels of AKT1 are a risk factor for poor prognosis in HCC patients [16]. The crucial roles of

mTOR are remarkably related to linking nutrient sensing to cell growth; thus, it could be a prominent agent in tumorigenesis processes [17]. A combination of some proteins including mTOR, regulatory-associated protein of mTOR (Raptor), PRAS40, mammalian lethal with sec-13 protein 8 (mLST8), telomere maintenance 2 (Tel2), TELO2 interacting protein 1 (Ttil), and DEP domain-containing mTOR-interacting protein (Deptor) compose mTORC1 complex which can influence the stimulation of glycolytic flux and mitochondrial function, synthesize proteins and lipids, block lysosomal biogenesis and autophagy [18]. Furthermore, mTORC2 complex is composed of some proteins including mTOR, rapamycin-insensitive companion of mTOR (Rictor), Deptor, mammalian stress-activated protein kinase interacting protein 1 (mSIN1), mLST8, protein observed with Rictor 1 and 2 (Protor1/2), Tel2, and Ttil. Some of the downstream targets of the mTORC2 complex have been poorly identified; however, the most well-studied targets are from the AGC protein kinase family such as AKT, protein kinase C (PKC), and serum and glucocorticoid-regulated kinase (SGK) [19]. mTORC2 could target these molecules and augment their stability, maturation, and allosteric activation [20]. In hepatocytes, mTORC2 can facilitate hepatosteatosis and induce cancer progression by *de novo* fatty acid and lipid synthesis [21]. The role of phosphatase and tensin homolog (PTEN) is of cardinal importance in regulating the activation of the PI3K/AKT/mTOR signaling pathway. PTEN can dephosphorylate PIP3 and terminate the axis at the initial steps; therefore, the lack of PTEN and/or its loss of function could lead to an uncontrolled activation of the PI3K/AKT/mTOR signaling pathway, contributing to tumorigenesis events [22]. It has been demonstrated that PTEN loss (like PTEN-null mice) is one of the main triggers of carcinogenesis in liver cells [23].

The overactivation of the PI3K/AKT/mTOR pathway has been demonstrated in numerous types of cancers. In the context of HCC, it has been shown that the overactivation of PI3K and AKT could be associated with the aggressiveness of tumors. Indeed, the activation of cascades related to AKT are prominent risk factor related to a poor prognosis and an earlier recurrence of the malignancy in patients with liver cancers [9]. According to positron emission tomography (PET) scans, proteins of the PI3K/AKT/mTOR pathway could be elevated in patients with HCC, implying that this signaling pathway can be activated during the pathogenesis of HCC [24].

Fig. 1 demonstrates the activation of the PI3K axis in liver cells and HCC.

3 Alterations of PI3K/AKT/mTOR Components in HCC

HCC is a highly heterogeneous tumor resulting from the accumulation of various genetic mutations. Several different signaling pathways, including the PI3K/AKT/mTOR, are altered in HCC, and this pathway is actively involved in the initiation, development, tumor progression, and metastasis of HCC. The PI3K/AKT/mTOR signaling pathway regulates several vital cellular processes such as cell cycle, survival, metabolism, motility, and angiogenesis in both physiological and pathological settings [25]. In HCC cases, the PI3K/AKT/mTOR signaling pathway is altered in more than half of the cases [26], with the most frequently mutated genes being PTEN (5%), PIK3CA (4%), mTOR (4%), and AKT2 (2%) [26,27], as stated by several studies.

Deregulation of the class I PI3K is frequent in tumorigenesis of HCC cases according to various studies [28]. Various reports revealed that the PI3K is highly expressed in HCC, and the PIK3CA upregulation is highly associated with HCC proliferation, poor prognosis and negatively correlated with apoptosis [29]. In addition, the PIK3CA mutations are present in HCC cases and they are highly associated with the size of the tumor. Furthermore, the reports also suggest the prognostic value of such mutations in HCC patients [30]. In addition to the PIK3CA, the high mutation rate of PIK3CB was also reported in HCC patients with an early-stage recurrence [31].

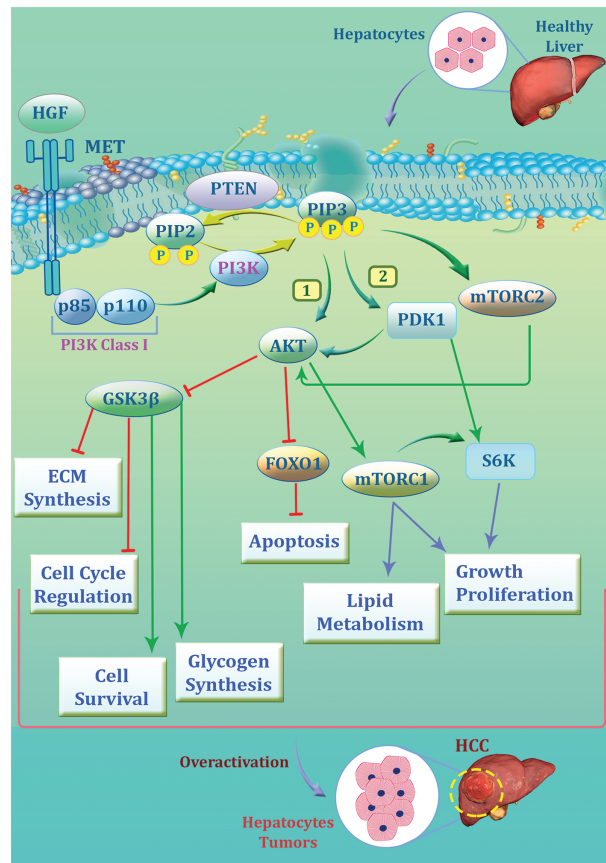


Figure 1: The signaling pathway of phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) in hepatocytes and its role in hepatocellular carcinoma (HCC) development. The pathway begins with hepatocyte growth factor (HGF) binding to its receptor MET, leading to the activation of PI3K (class I), composed of the p85 regulatory and p110 catalytic subunits. PI3K can convert phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3), leading to the activation of AKT through phosphorylation mediated by pyruvate dehydrogenase kinase 1 (PDK1) and mTORC2. Activated AKT plays a pivotal role and promotes cell survival, glycogen synthesis, and lipid metabolism while inhibiting apoptosis via suppression of forkhead box protein O1 (FOXO1). It also regulates cell cycle progression and extracellular matrix (ECM) synthesis through glycogen synthase kinase-3 beta (GSK3 β) blocking. mTORC1, downstream of AKT, drives growth and proliferation by activating S6K. Furthermore, phosphatase and tensin homolog (PTEN) act as tumor suppressors by dephosphorylating PIP3 and counteracting the pathway. It could be concluded that the overactivation of the PI3K/AKT/mTOR axis contributes to the uncontrolled growth of hepatocytes, leading to HCC progression

Class II PI3K also plays various important roles in HCC. According to the reports, the downregulation of PI3K-C2 α in human HCC cells results in degraded vascular endothelial growth factor (VEGF)-mediated signaling and reduced angiogenesis [32]. Furthermore, the PI3K-C2 β plays a vital role in the hepatitis C virus (HCV) spread in human HCC cells [33]. Like two other classes, class III PI3K like PIK3-C3 also has important roles in HCC. According to an article, Vacuolar protein sorting 34 (Vps34), also known as PI3K-C3, is significantly diminished in HCC and could be negatively correlated with the HCC invasiveness *in vitro* and *in vivo*. Furthermore, Vps34 could induce the accumulation of lysosomal juxtanuclear, leading to the reduction of HCC tumor invasiveness via the Rab7-RILP pathway. PIK3C deficiency also affected the endosome-lysosome system, contributing to improved Rab11-mediated endocytic recycling of cell surface receptors and increased invasion of HCC cells [34]. Dysregulation of AKT isoforms is responsible for a wide range

of diseases including cancer [35]. The expression of both AKT1 and AKT2 is detected in hepatocytes [36]. AKT plays a significant role in tumorigenesis through apoptosis inhibition and cell proliferation. According to a report, in nearly 70% of the HCC samples, the AKT was phosphorylated at S473, which was highly related to the invasion, metastasis, and vascularization of the tumor [37]. In other studies, phosphorylated AKT was considered a poor prognosis marker as it was highly related to diminished overall survival [38,39]. Reports suggest that in HCC, the liver's altered metabolic state often results in a significant upregulation of aldose reductase, which interacts with the catalytic domain of AKT1, thereby triggering the activation of the AKT/mTOR pathway [40]. In HCC patients, the expression of phosphorylated AKT1 contributed to the activation of c-MYC and unfavorable cellular growth [16]. The other isoform of AKT, AKT2, also has significant roles in HCC and some researchers believe that its role in HCC exceeds AKT1. AKT2 promotes cell proliferation and invasion and influences the prognosis of HCC patients [41]. Studies revealed that 38% of HCC tissues with moderate or lower AKT1 expression exhibited AKT2 overexpression. While no correlation was discovered between AKT1 and clinicopathological attributes, elevated AKT2 expression was associated with histopathological differentiation, tumor nodule count, and portal invasion [41]. In the context of tumor transformation, AKT2 plays a crucial role in metabolic regulation in HCC mice models [42]. Although AKT3 expression is primarily centralized in the brain and testis, it also plays a crucial role in HCC. Studies on miRNA profiles in HCC revealed that miRNA-144 and miRNA-582-5p were downregulated. This downregulation led to sustained expression of their downstream targets, including AKT3, promoting tumor progression and growth. These findings highlight the role of AKT3 in HCC progression [43,44].

The deregulation of mTOR is present in human malignancies and the mTOR signal is frequently altered in approximately 30% of cancers, according to the reports [45,46]. The mTOR pathway can be activated in three different ways. The first way is the activating mutations in mTORC1/2 complexes or mutations in upstream genes which could lead to the hyperactivation of the mTOR signaling. The second one is the overexpression or amplification of the components of mTORC1 and mTORC2, and the last one is the loss of function of negative regulators in the mTOR signaling cascade [47].

According to the articles, both mTORC1 and mTORC2 are upregulated in nearly half of patients with HCC, which signifies their important role as a potential therapeutic target [48,49]. Besides, elevated levels of mTOR expression are reported in poorly differentiated tumors and large tumors with earlier tumor recurrence and lower survival rates and advanced stages [50,51]. Results of a prospective study of HCC patients who underwent liver transplantation depicted that mTOR pathway expression was more intense at the tumor edge where proliferation and cellular growth were more intense [52]. While the mechanisms leading to the mTORC2 activation are not understood, the presence of p-AKT (S473) which is a biomarker for mTORC2 activation is reported in nearly more than half of human HCC samples [53]. Furthermore, no mutations in mTORC2 components have been identified. According to the articles, chromosomal gain of RICTOR was detected in nearly one-fourth of human HCC samples, and elevated levels of RICTOR expression are highly associated with poor HCC prognosis [16]. Articles suggest that mTORC1 and mTORC2 take part in controlling the migration as well as invasion of HCC cells [54], and mTORC2 can promote *de novo* fatty acid and lipid synthesis, causing hepatic steatosis and cancer [21].

The tumor suppressor gene PTEN, which is the negative regulator of the PI3K/AKT/mTOR, also undergoes some changes in HCC. According to the reports, PTEN heterozygosity, which reduces PTEN expression, is present in nearly 32% to 44% of HCC cases; however, PTEN mutations rarely occur in HCC [55]. Some studies revealed the relation between low expression of the PTEN gene and unsatisfactory events in HCC including poor differentiation, advanced TNM stage, intrahepatic metastasis, and decreased survival rates [37,56,57]. In HCC cells, the degradation of the PTEN protein due to miR-221 is reported to be the main reason for the PI3K/AKT/mTOR pathway activation. The overexpression of miR-221 which is an

oncogene, contributes to the downregulation of PTEN expression, and its degradation and is downregulated due to the overexpression of miR-221 [58,59]. Table 1 is an overview of alterations in the PI3K/AKT/mTOR signaling pathway.

Table 1: The alterations of PI3K/AKT/mTOR pathway components in HCC

Gene	Alteration	Frequency (%)	Outcome	Ref.
PIK3CA	Upregulation	NA	It is closely related to proliferation, poor prognosis, and negatively correlated with apoptosis.	[29]
PIK3CA	Mutations	14	There are highly associated with size of the tumor and have prognostic value.	[30]
PIK3CA	Mutations	NA	Although some single nucleotide polymorphisms were detected in HCC cases, their presence was not associated with clinical characteristics.	[60]
PI3KC2 α	Downregulation	NA	It can contribute to decreased angiogenesis caused by degraded VEGFA-mediated signaling in human HCC cells.	[61]
PIK3CB	Mutations	NA	The presence of mutations PIK3CB, KEAP1, TP53, and WRN was higher in early-recurrence HCC patients and patients with microvascular invasion.	[31]
AKT	Overexpression	53	The overexpression of p-AKT was related to tumor differentiation and the number of tumor nodules.	[62]
mTOR	Mutations	50	Aberrant mTOR signaling was related to PTEN dysregulation, epidermal growth factor up-regulation, and insulin-like growth factor pathway activation.	[49]
PTEN	Downregulation	NA	In comparison with PTEN mutations, PTEN promoter methylation, and epigenetic regulation were the major factors responsible for PTEN downregulation	[63]

4 The Induction of Oncogenic Features as a Result of PI3K/AKT/mTOR Alterations in HCC

4.1 Apoptosis, Autophagy and Angiogenesis

Apoptosis, a programmed cell death, involves a series of molecular steps that culminate in cellular demise. Abnormalities in apoptosis are a hallmark of cancer, including HCC. The PI3K/AKT signaling pathway regulates proapoptotic and antiapoptotic downstream molecules, facilitating HCC progression. Consequently, targeting this pathway can induce apoptosis. Autophagy, on the other hand, is a cellular degradation process that eliminates unnecessary or dysfunctional proteins and components via lysosomal degradation. In this mechanism, unwanted cellular components are enclosed within autophagosomes, which then fuse with lysosomes, generating amino acids and free fatty acids that are recycled to maintain cellular

homeostasis [64]. Various autophagy-related proteins (ATGs) that assemble into several complexes can mediate the mentioned mechanism, leading to the formation of autophagosomes, fusion with lysosomes, and eventual degradation [65]. Angiogenesis is defined as the formation of new vessels and it is vital for wound healing, supporting growth, and regenerating tissues [66,67]. In cancer settings including HCC, angiogenesis is important as the cancerous cells can enter the bloodstream and disseminate into different organs by angiogenesis. In the following section, we will review the role of the PI3K/AKT signaling pathway and target it in apoptosis, autophagy, and angiogenesis of the HCC.

Several different studies review the role of the pathway and targeting it in apoptosis. To begin with, it was reported that inhibition of the PI3K/AKT/mTOR signaling pathway can promote apoptosis in HCC cells by increasing the levels of Caspase-3 and Caspase-9 and decreasing the levels of Bcl-2 [68]. Besides, it was discovered that the inhibition of the signaling pathway contributed to the DNA damage and inhibition of the progression of HCC cells [69]. In another article, using 4-hydroxyderricin to target and decrease the levels of PI3K contributed to the inhibition of the pathway, apoptosis induction, and cell cycle arrest in HCC cells [70]. Furthermore, it was reported that Alpha-fetoprotein (AFP) can enhance the proliferation and invasion of HCC cells through induction of PI3K/AKT signaling by down-regulation of the PTEN. By targeting the PTEN, the pathway induction can contribute to the inhibition of apoptosis and HCC growth [71–74]. There is an evident role of the PI3K/AKT signaling pathway in the regulation of autophagy in HCC. According to an article, suppressing the activity of the PI3K/AKT by Anemoside B4, which is a saponin, leads to elevated levels of Beclin-1 and LC3 expression, inhibition of AKT and mTOR phosphorylation and results in both autophagy and apoptosis of HCC cells [75].

Some anti-cancer drugs can also target the PI3K/AKT/mTOR signaling pathway and suppress angiogenesis. To begin with, it is reported that there is an increase in the levels of both PI3K and mitogen-activated protein kinase (MAPK) in the hypoxia which induces angiogenesis. Asparagus polysaccharide (ASP) can inhibit the angiogenesis and progression of HCC cells by targeting the PI3K/AKT/mTOR signaling, reducing MAPK and PI3K expression levels to suppress the hypoxia-Inducible Factor (HIF)-1 α /VEGF axis. ASP inhibitory effects are mediated by down-regulating the phosphorylation of MAPK and PI3K signaling pathway-related proteins including p-extracellular signal-regulated kinase (ERK), p-AKT, and p-mTOR in HCC cells [76]. Another study revealed that Apatinib, a tyrosine kinase inhibitor that selectively targets VEGFR2, reduced VEGF and PI3K/AKT expression to repress angiogenesis and HCC cell invasion. It inhibited the expression and activity of the pro-angiogenic factors VEGF and VEGFR2, potentially affecting the downstream activation of the PI3K/AKT signaling pathway, and ultimately leading to the suppression of angiogenesis [77]. Furthermore, another study revealed that Saponin D can induce apoptosis and suppress the growth of HCC cells in a dose-dependent manner. It can also diminish the expression of HIF-1 α and VEGF, and diminish tube formation in cells. It was found that Saponin D can inhibit the tumor growth of the HCC xenograft model by inducing apoptosis and increasing the levels of Caspase-3. Additionally, it reduced the expression of VEGF and suppressed angiogenesis. Saponin D played such roles by modulating the PI3K/AKT axis in HCC by suppressing the phosphorylation of AKT, mTOR, and p70S6K both *in vitro* and *in vivo* [78]. All in all, according to the abovementioned justifications targeting the PI3K/AKT/mTOR pathway would be beneficial.

4.2 Metastasis, Invasion, and EMT

Metastasis is considered one of the hallmarks of cancer and elevated levels of tumor invasion and metastasis are significantly associated with cancer mortality. Epithelial-to-mesenchymal transition (EMT) is a phenomenon related to cancer progression, metastasis, invasion, and drug resistance, and various studies have assessed the role of EMT inducing invasiveness and oncogenic features of HCC [79,80]. In the following

section, we will review the role of the PI3K/AKT/mTOR signaling pathway in metastasis, invasion, and EMT of the HCC.

To begin with, the Cartilage oligomeric matrix protein (COMP) is known to play important roles in several cancers including HCC. The serum levels of COMP in high in HCC patients which is in correlation with both malignant clinical characteristics and poor clinical outcomes. A study depicted that recombinant human COMP protein (rCOMP) contributed to increased abilities of proliferation, invasion, migration, and EMT of HCC cells via collaborating with CD36 and PI3K/AKT axis. In other words, COMP-CD36 collaboration can result in the phosphorylation of AKT and ERK, contributing to the upregulation of tumor-progressive genes including EMT markers, matrix metalloproteinases (MMPs)-2/9, Twist, and Slug in HCC cells [81]. In HCC cells, the expression of CD73 is elevated and it is responsible for aggressive clinicopathological aspects of HCC like progression, metastasis, and EMT. The mechanisms of the CD73 activity in HCC are via the PI3K/AKT axis. CD73 can activate Rap1 which recruits P110 β to the plasma membrane triggers PIP3 production, and promotes AKT phosphorylation in HCC cells [82]. The abnormal expression of Protein arginine methyltransferases (PRMT) which catalyzes protein arginine methylation and plays an important role in many biological processes is present in several cancers. In HCC PRMT9 is found to be overexpressed which promotes invasion and metastasis. The overexpression of PRMT9 is significantly correlated with hepatitis B virus antigen (HBsAg) status, vascular invasion, poor tumor differentiation, and advanced TNM stage. In HCC tumor tissues harboring elevated expression of PRMT9, the expression levels of p-AKT, p-GSK-3 β , and Snail were increased which consequently promoted the invasion, EMT, and metastasis of the tumor [83].

PRMT9 promotes EMT by activating the PI3K/AKT/GSK-3 β /Snail signaling pathway. Mammalian sterile-20-like kinase 4 (MST4), a member of the germinal center kinase (GCK) group III, influences various physiological functions. However, its role in cancers remains poorly understood. In HCC cells, MST4 functions as a tumor suppressor, and its low expression serves as a potential biomarker for poor prognosis in HCC. Studies revealed that MST4 inactivation induces the EMT phenotype in cancer cells and enhances their invasive potential through activation of the PI3K/AKT/Snail signaling pathway [84]. Family member with sequence similarity 83 (FAM83A) is part of an 8-member protein family that is upregulated in HCC, and its expression is highly correlation with poor progression, invasion, metastasis, and EMT. It was reported that FAM83A could induce EMT by activating the PI3K/AKT signaling pathway, c-JUN protein, and EMT-relating proteins in HCC. Surprisingly, c-JUN was detected to make a positive feedback loop by binding to the FAM83A promotor region and inducing its expression [85]. Transcription factor activating enhancer binding protein 4 (TFAP4) is known as a regulator of tumor progression. Studies revealed that the expression of TFAP4 is upregulated in HCC, and it is responsible for invasion, metastasis, and EMT via regulating the expression of MMP-9 through activating the PI3K/AKT/mTOR signaling pathway in HCC [86]. Protein kinase D (PKD) has also been shown to contribute to invasion, metastasis, and EMT in several types of cancers including HCC. In HCC, the pyruvate dehydrogenase kinase 1 (PDK1) is overexpressed, and it is activated by the combination of TNFR1 and TRAF2 in the form of a complex. PKD2, then, attached directly to the p110 α and p85 subunits and promoted the signaling cascade of PI3K/AKT/GSK-3 β , leading to the stimulation of invasion and EMT [87].

4.3 Tumor Microenvironment Reprogramming

The tumor microenvironment (TME) is a critical contributor to cancerous cell growth, invasion, and metastasis [88], and according to the reports the metabolism of cell types in the TME, like immune cells, can modulate tumor progression [89]. Immune cells such as NK cells, macrophages, and T cells are an essential part of the TME and play significant roles as both prognostic and therapeutic markers [90]. In the TME,

the great metabolic competition between tumor cells and immune cells could influence the growth of tumor cells and the loss of immune cell functions [91]. Notably, metabolic reprogramming following the immune cell activation or immunometabolism relies on the crosstalk between the liver kinase B1 (LKB1)/AMP-activated protein kinase (AMPK) signaling pathway and the PI3K/AKT/mTOR which is vital for regulating the metabolism of immune and nonimmune cell [92].

Macrophages deliver nutritional supplements to the cancerous cells and help both tumor progression and resistance to therapy [93]. In HCC, the AKT signaling pathway plays a vital role in the M1/M2 polarization of macrophages. To begin with, in TME of HCC tumors, the glycolysis of the cancerous cells contributes to the elevated levels of lactic acid and M2 polarization of macrophages through the AKT pathway [94–96]. Nevertheless, M1 polarized macrophages have enhanced glycolytic metabolism and damaged oxidative phosphorylation via AKT/mTOR/HIF-1 α pathway [97].

T cells, particularly CD8⁺ T cells, are key effectors of adaptive immunity against cancers, including HCC. However, the tumor microenvironment (TME) reprograms these cells. In HCC, tumor tissues exhibit a lower density of CD8⁺ T cells and a higher density of Tregs, creating conditions favorable for cancer growth and intrahepatic metastasis. AKT plays a critical role in T cell reprogramming, as its inhibition reduces fatty acid oxidation and enhances the mitochondrial spare respiratory capacity of tumor-infiltrating T cells [98–100]. Interestingly, programmed death ligand 1 (PD-L1) as the immune checkpoint affects the Treg cells by elevating PTEN and inhibiting the AKT/mTOR/S6 signaling pathway [101]. Furthermore, the PD-1/PD-L1 interaction may inhibit PI3K and AKT contributing to the mTOR signaling blocking thereby decreasing the glycolysis rate of T-infiltrating cells in HCC [102].

NK cells are the main elements of innate lymphoid cells (ILCs) that have a major role in the immunity against various viral and transformed infected cells [103,104], and the PI3K/AKT/mTOR pathway is vital for promoting the development, differentiation, and activation of NK cells [105]. According to an article, PD-1/PD-L1 blockade can augment the phosphorylation of AKT, in HCC, thus PD-1 exhibits its inhibitory roles on NK cells by diminishing PI3K/AKT signaling in HCC [106].

5 Regulation of the PI3K/AKT/mTOR Signaling Pathway by Long Noncoding RNAs in HCC

Noncoding RNAs are transcription products that are not able to be translated into proteins. They normally featured with a high density of terminators and lacking effective open reading frames. Several different kinds of noncoding RNAs such as miRNA, lncRNA, piRNA, and snoRNA could have functions in many transcriptional and post-transcriptional events [107]. It has been reported that lncRNAs, whose length is generally more than 200 nucleotides, have the highest frequency among all ncRNAs. Their open reading frames are normally around 50~100 nucleotides, and RNA polymerase II mainly conducts their transcription and splicing [108,109]. By interacting with DNA, RNA, and protein, lncRNAs can serve as regulatory factors. LncRNAs control gene expression by DNA methylation, chromosomal remodeling, and histone modifications. Besides, they can also influence the transcription and translation of DNA by a series of cis- and trans-regulations. Compared with target mRNA sequences, lncRNAs can affect the editing, splicing, transport, translation, and degradation of mRNA [110–112]. lncRNAs are responsible for the initiation, development, and clinical prognosis of several different tumors including HCC. In the following table, we will review the most important lncRNAs in HCC. Table 2 represents an overview of studies assessing noncoding RNAs in the regulation of the PI3K axis in HCC.

Table 2: Studies evaluating the roles of noncoding RNAs in the regulation of PI3K/AKT/mTOR pathway in HCC

Non coding RNA	Role	Outcome	Ref.
HEIH	Oncogene	By sponging miR-98-5p and activating the PI3K/AKT pathway, HEIH overexpression could contribute to sorafenib resistance.	[113]
MALAT1	Oncogene	It increases the expression of the splicing factor serine/arginine-rich splicing factor 1 (SRSF1), leading to the alternative splicing of ribosomal Protein S6 Kinase B1 (RPS6KB1) and subsequent activation of mTORC1, a mechanism that contributes to the transformation of liver progenitor cells.	[114]
HULC	Oncogene	It diminishes miR-15a in Hep3B cells, resulting in the inhibition of PTEN via P62, and activation of PI3K/AKT/mTOR pathway.	[115]
NEAT1	Oncogene	Rapamycin exerts its anti-cancer influences by affecting the mTOR/NEAT1 pathway.	[116]
TUG1	Oncogene	It contributes to mTOR activation and hexokinase 2 (HK2) induction, leading to increased cell growth, glycolysis, and metastasis.	[117]
CASC9	Oncogene	It interacts with heterogeneous nuclear ribonucleoprotein L (HNRNPL) contributing to the activation of the AKT signaling pathway and further tumorigenesis of HCC cells.	[118]
CASC11	oncogene	It interacts with the enhancer of zeste homolog 2 (EZH2) and epigenetically silences PTEN and activates the PI3K/AKT/mTOR pathway.	[119]
RP11-295G20.2	Oncogene	It degrades PTEN by facilitating its interaction with p62 contributing to the activation of the AKT pathway.	[120]
NR027113	Oncogene	It is up-regulated in HCC samples, and it promotes the proliferation and invasion of HCC through the PI3K/AKT signaling pathway.	[121]
PTTG3P	Oncogene	It activates the PI3K/AKT signaling pathway and promotes the proliferation of HCC cells.	[122]
HAGLROS	Oncogene	It is highly expressed in HCC, with its elevated levels potentially contributing to HCC progression and development via the PI3K/AKT/mTOR signaling pathway and the miR-5095/ATG12 axis.	[123]
LINC00963	Oncogene	It can activate the PI3K/AKT/mTOR pathway and consequently extend the G0/G1 phase of HCC cells and improve proliferative ability.	[124]

(Continued)

Table 2 (continued)

Non coding RNA	Role	Outcome	Ref.
OR3A4	Oncogene	It promotes angiogenesis through modulating angiogenic factors with G patch and FHA domains 1 (AGGF1)/AKT/mTOR pathway.	[125]
Linc01296	Oncogene	It is highly expressed in HCC and is involved in HCC progression via regulating miR-26a/PTEN.	[126]
THOR	Oncogene	It promotes HCC cell proliferation and metastasis by PI3k/AKT/mTOR signaling cascade.	[127]
AK023948	Oncogene	High expression can promote both proliferation and invasion of HCC cells by regulating the PI3K/AKT/mTOR signaling.	[128]
Meg3	Tumor suppressor	Its overexpression activated PTEN and suppressed AKT signaling, leading to the upregulation of the pro-apoptotic protein Bax and the downregulation of the anti-apoptotic protein Bcl-2.	[129]
FER1L4	Tumor suppressor	It suppresses both the proliferation and migration of the HCC by regulating the PI3k/AKT/mTOR signaling cascade.	[130]
CADM1-AS1	Tumor suppressor	It inhibits cell-cycle progression and invasion by PTEN/AKT/GSK-3 β signaling pathway.	[131]
F11-AS1	Tumor suppressor	It suppresses HCC progression by regulating PTEN expression.	[132]
USP16	Tumor suppressor	It regulates PTEN expression.	[133]

6 Evaluating Clinical Trials in the Context of PI3K/AKT/mTOR Pathway Inhibitors in HCC

6.1 Trends of Trials Investigating PI3K/AKT/mTOR Pathway Inhibitors in HCC

In the present study, we specify over 223 keywords, which include the names of 106 PI3K inhibitors, 45 dual PI3K/mTOR inhibitors, 42 AKT inhibitors, 26 mTOR, and more than 4 other keywords to inhibitors to identify relevant clinical trials. Following a multi-step screening process, 60 studies related to HCC were included. In this section, through a conceptual analysis of these trials, we aim to present a clearer understanding of the progress made so far and identify the areas that still need attention in the future. According to our analysis, 58% of trials were completed, 7% were recruiting, and only 5% were active, not recruiting, while 13% were terminated and 7% were withdrawn.

Although there has been a reduction in the number of clinical trials concerning mTOR and AKT inhibitors in recent years, PI3K inhibitors have attracted more attention from researchers since 2017. Indeed, the trend of mTOR inhibitors and AKT inhibitors has been the same, except for the sharp rise of mTOR inhibitors in 2009–2012. In almost all investigated periods, the number of Phase I trials exceeded other phases; however, between 2009 and 2012, which was the most-studied period, the number of Phase II trials was the highest. It could be observed that the sharp rise in the trend of inhibitors in 2009–2012 was majorly related to mTOR inhibitors, while PI3K inhibitors were the least at that period. Furthermore, mTOR inhibitors have always been the most utilized drug in each phase, while in Phase I PI3K inhibitors were

used more than AKT inhibitors and *vice versa* in Phase II. Fig. 2A–F provides an overview of the general characteristics of 60 clinical trials investigating PI3K/AKT/mTOR pathway inhibitors in HCC.

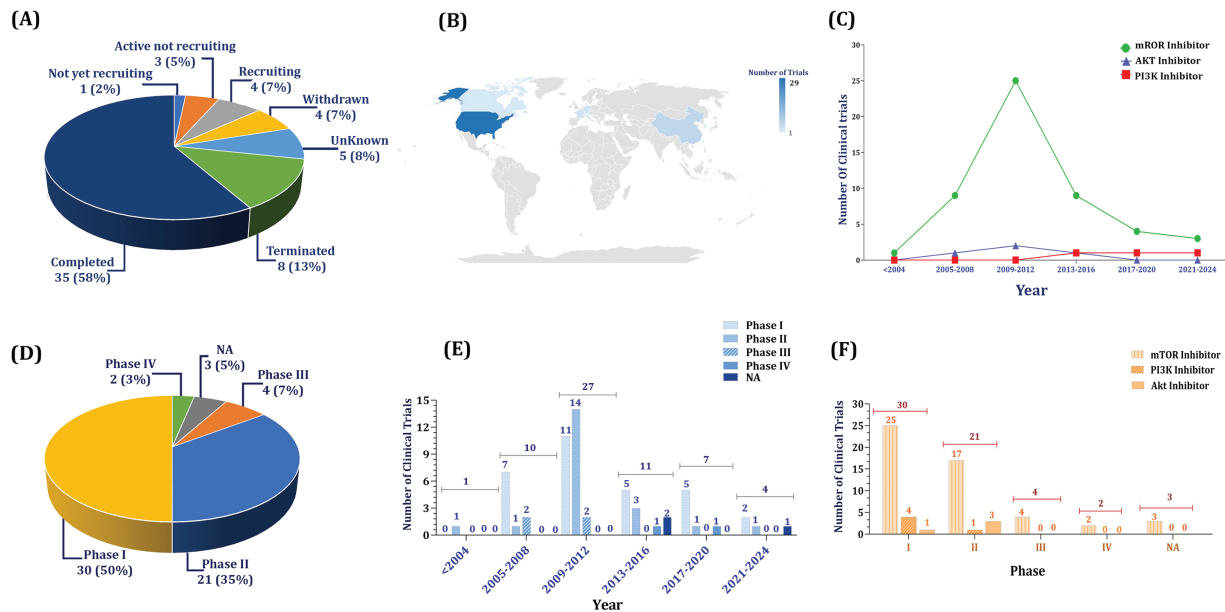


Figure 2: An overview of clinical trials investigating PI3K/AKT/mTOR pathway inhibitors in HCC. A. The status of clinical trials shows that nearly 60% of trials were completed and 7% are recruiting. B. According to the world distribution, most of the studies have been performed in the USA (29 numbers) followed by China (six numbers), and Germany and France (three numbers each). C. Generally, mammalian targets of rapamycin (mTOR) inhibitors have drawn more attention compared with phosphatidylinositol-3 kinase (PI3K) and protein kinase B (AKT) inhibitors. D. Ratio of phases. The rate of trials in phase I is higher than others (50%), while only 10% of trials have entered Phases III and IV. E. The highest numbers of clinical trials were recorded between 2009 and 2012, when, particularly, the rate of Phase II trials exceeded Phase I. F. Most of the trials have been performed in Phase I, and all phases, mTOR inhibitors have drawn more attention compared with the others, indicating their better effectiveness and tolerability

6.2 Promising Inhibitors of PI3K Axis; Analysis of Trials Entered Phases III and IV

Comparing 51 studies in Phases I and II (85%) to six in Phases III and IV (10%) demonstrates that PI3K and AKT inhibitors do not advance to Phases III and IV; however, there have been clinical trials for mTOR inhibitors in Phases III and IV. In this regard, four trials in Phase III and two trials in phase IV were recorded as mTOR inhibitors, while their trends were 25 trials in Phase I and 17 in Phase II, implying that mTOR inhibitors have been the center of attention regarding their more promising outcomes. Fig. 2A–F gives information regarding the proportion and the trends of utilized drugs in Phases III and IV compared with other phases.

6.3 The Most Promising Drugs and Therapeutic Strategies

As mentioned, the most utilized category has been mTOR inhibitors followed by PI3K inhibitors and AKT inhibitors. Among mTOR inhibitors, everolimus, rapamycin, and temsirolimus have been majorly utilized, while the tendency toward sapanisertib and onatasertib has been lower. Our assessment shows that pan-class I PI3K inhibitors have been used more, in which copanlisib has demonstrated a higher tendency. Furthermore, MK2206 was the most used AKT inhibitor followed by perifosine. Overall, it could be inferred

that everolimus has been the most utilized drug regarding all PI3K/AKT/mTOR pathway inhibitors in HCC, indicating its promising clinical benefits.

Another point to be mentioned is the therapeutic strategies i.e., monotherapy and combination therapy. The promising outcomes of pre-clinical studies paved the way for clinical studies to evaluate the effectiveness of combination regimens in HCC cases [134]. For instance, the combination of everolimus plus sorafenib was assessed in patients with unresectable or metastatic HCC in a randomized Phase II clinical trial. They showed that progression-free survival at 12 weeks was 68% in patients receiving the combination regimen. Furthermore, response evaluation criteria in solid tumors (RECIST) and modified RECIST (mRECIST) response rates were 10% and 35%, respectively. While 86% of patients demonstrated grade 3/4 adverse events, the median progression-free survival (PFS) was 5.7 months and the median overall survival (OS) was 12 months [135]. Our analysis exhibits that the proportion of combination therapy has been higher in almost all categories of PI3K/AKT/mTOR pathway inhibitors. Concerning mTOR inhibitors, MEK inhibitors have been combined more followed by chemotherapy, VEGF inhibitors, and hormone therapy. Combining PI3K inhibitors has been majorly conducted using anti-PD-L1 immune checkpoint inhibitors (ICI) followed by tyrosine kinase inhibitors (TKI). Moreover, MEK inhibitors have been chiefly combined with AKT inhibitors. The contribution of PI3K/AKT/mTOR pathway inhibitors in HCC clinical trials and their therapeutic strategies are depicted in Fig. 3A,B.

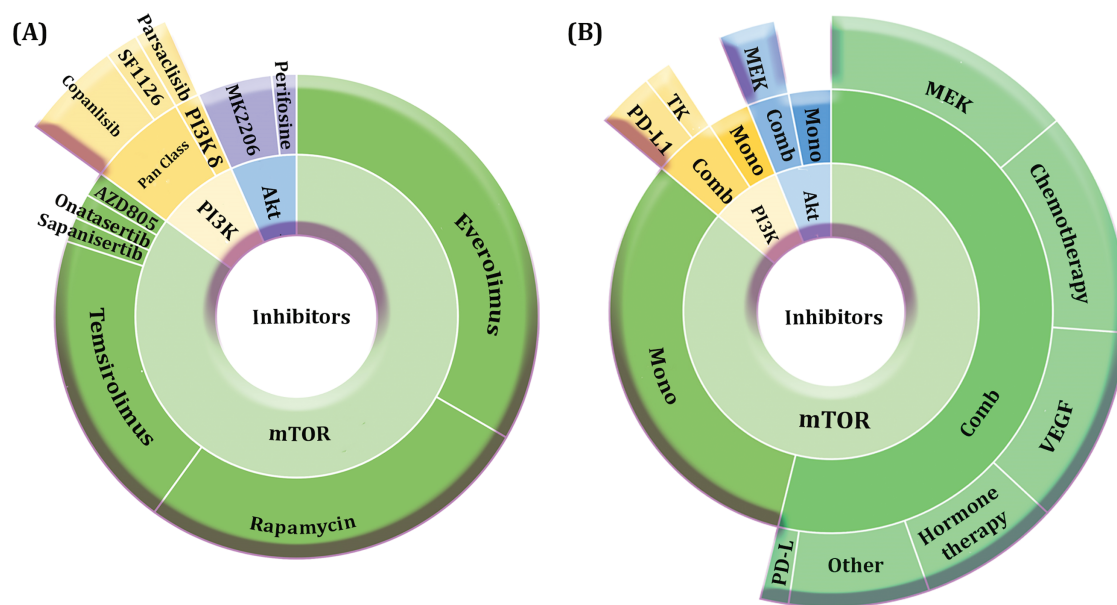


Figure 3: Contribution of PI3K/AKT/mTOR pathway inhibitors in HCC clinical trials. A. The proportion of mTOR inhibitors exceeds PI3K and AKT inhibitors. Everolimus, rapamycin, and temsirolimus are among the most utilized mTOR inhibitors, while copanlisib and MK2206 have been the most used phosphatidylinositol-3 kinase (PI3K) and protein kinase B (AKT) inhibitors, respectively. B. The contribution of combination therapy is higher compared with monotherapy when investigating all inhibitors. The combination of regimens mostly consists of MEK inhibitors, chemotherapy, and vascular endothelial growth factor (VEGF) inhibitors regarding mTOR inhibitors, anti-PD-L1 and tyrosine kinase inhibitors (TKI) regarding PI3K inhibitors, and MEK inhibitors regarding AKT inhibitors

7 Pros and Cons of Targeting PI3K/AKT/mTOR Pathway in HCC

Although targeting the PI3K/AKT/mTOR axis has been shown to be a promising therapeutic strategy to diminish the growth of liver tumors, targeting the component of this signaling pathway could be associated with the induction of severe adverse events which could terminate the conducting of the studies [10]. Indeed, one of the main causes contributing to the discontinuation of clinical trials has been the presence of uncontrollable adverse events, leading to the failure of trials. A vast range of clinical toxicities could be overserved as a result of PI3K/AKT/mTOR axis inhibition. In this regard, the mentioned therapies could induce adverse events such as hyperglycemia, stomatitis, bone marrow suppression, hyperlipidemia, pneumonitis, and hepatotoxicity [136]. Studies demonstrated that the inhibition of PI3K α could majorly cause hyperglycemia; however, hyperlipidemia is a common side effect when mTOR is suppressed. Accordingly, pan-PI3K inhibitors, which are able to block all isoforms of PI3K, exhibit a broadened range of adverse events [137].

Another imperative challenge related to the inhibition of the PI3K/AKT/mTOR signaling pathway in HCC is the induction of treatment resistance. The induction of resistance seemed to be the main obstacle to low efficacy in clinical trials [138]. Generally, the induction of resistance to drugs in cancers could be stratified into two categories, primary and acquired resistances [139], with the former referring to the genetic heterogeneity of tumor cells and the latter referring to the activation of compensatory signaling pathways [140]. The genetic profiling of some tumors could induce the phenotype of drug resistance. Accordingly, it has been demonstrated that PDK1 overactivation could drive resistance to treatments such as radiotherapy along with the induction of EMT and cancer stemness; therefore, therapeutic inhibitors targeting PDK1 demonstrate promise in reversing these influences [141,142]. Furthermore, the bioinformatics analyses and advancements in genetic profiling pave the way for identifying molecular biomarkers related to therapy resistance. For instance, acquiring resistance to radiotherapy due to PDK1 alteration was shown to be associated with elevated flow cytometry-based Aldehyde Dehydrogenase (ALDH) activity and side-population enrichment as well as direct ALDH1-PDK1 interaction in Huh7 human HCC cell line. They exhibited that PDK1-driven resistance to radiotherapy could positively correlate with the upregulation of some biomarkers such as ALDH1A1, SOX2, PROM1, KLF4, and POU5F1 [142]. When the components of the PI3K/AKT/mTOR axis are suppressed, the tumors can activate parallel or compensatory pathways like the MAPK pathway to maintain survival and proliferation despite inhibition of the axis. Furthermore, some pathways have cross-talk between the PI3K axis; therefore, by blocking the PI3K signaling, those pathways could compensate for the survival of tumors [11,143]. As a result, understanding the cross-talk of other signaling pathways to establish methods to target them in combination regimens is of cardinal importance.

It could be inferred that there are need to develop novel drugs and/or combination regimens to increase the accuracy of treatment and overcome the drug resistance issue [11]. It has been demonstrated that the pathogenesis of HCC is derived from a complex system of signaling pathways involving PI3K/AKT/mTOR, RAS/RAF/MEK/ERK, c-MET, VEGF, as well as histone deacetylases (HDACs). Targeting the key components of these pathways could enhance the therapeutic dilemma of HCC cases [140]. It is worth mentioning that by combining inhibitors a sizable number of tumor proliferation systems could be blocked simultaneously; thus, the growth of tumors could be hindered notably; nonetheless, these methods could lead to more toxicity concerns; therefore, their utilization ought to be managed and considered [144]. As we evaluated and according to some preclinical studies, the combination of mTOR inhibitors with AKT or PI3K inhibitors as dual targeting strategies has shown promising potential in the induction of clinical benefits for HCC patients [25]. Moreover, combining mTOR inhibitors with other classes of targeted therapies such as MEK inhibitors, VEGF inhibitors, and hormone therapies as well as chemotherapy have been of great interest among studies, exhibiting their promising therapeutic potency in HCC treatment. Interestingly,

novel selective mTOR inhibitors have been developed with the capacity to elevate the radiosensitization of HCC tumors [145], implying the effectiveness of combination therapies.

8 Conclusion and Prospects

HCC remains a challenging concern in oncology regarding its high incidence and mortality rates worldwide. The PI3K/AKT/mTOR signaling pathway has been identified as a crucial player in the pathogenesis of HCC, primarily by promoting uncontrolled cellular proliferation. The overactivation of this pathway is strongly correlated with poor prognosis, making it a critical target for therapeutic intervention. Our comprehensive review underscores the oncogenic characteristics of the PI3K/AKT/mTOR axis in HCC. A sizable number of studies have shown the deregulation of class I PI3K in the tumorigenesis events of HCC. Indeed, the upregulation of PIK3CA is related to a poor prognosis and liver tumor proliferation. The oncogenic features are not just associated with class I PI3K, but class II and III PI3Ks have been linked with HCV spread and HCC invasiveness. Furthermore, the presence of overexpression in AKT, upregulation in mTOR, and downregulation in PTEN are related to invasiveness, metastasis, tumor recurrence, and poor survival of patients. Overall, the out-of-control activation of the PI3K axis is associated with the inhibition of apoptosis and autophagy, the stimulation of angiogenesis, and the elevation of EMT and metastasis. In addition, non-coding RNAs such as lncRNAs could play an imperative role in HCC development. On the one hand, some lncRNAs like HEIH, MALAT1, HULC, and CASC9 serve as oncogenes by activating the PI3K, AKT, and mTORC1, and suppressing PTEN. On the other hand, Meg3, FER1L4, CADM1-AS1, and F11-AS1 are sorts of tumor suppressor lncRNA able to regulate PI3K/AKT/mTOR axis and activate PTEN.

According to our analysis, 58% of clinical trials in the context of inhibiting PI3K/AKT/mTOR signaling pathway in HCC are completed, while 7% and 5% are recruiting and active, not recruiting. The tendency toward using AKT and mTOR inhibitors reduced in recent years; however, the application of PI3K inhibitors increased. In the investigated period, the number of studies between 2009 and 2012 was the highest, of which mTOR inhibitors dedicated the majority proportion. Regarding the phases, it could be concluded that mTOR inhibitors have been the most utilized drugs in each phase. Moreover, we demonstrated that only 10% of studies advanced to Phases III and IV, all of which were mTOR inhibitors, indicating the promising outcomes of these drugs compared with PI3K and AKT inhibitors. The most used drugs have been copanlisib (a pan-class I PI3K inhibitor), MK2206 (an AKT inhibitor), and everolimus (an mTOR inhibitor); nonetheless, everolimus has been the most utilized drug among all categories.

Although there are trends toward using PI3K/AKT/mTOR inhibitors, their application could be associated with challenges and shortcomings. In this regard, these drugs have been shown to stimulate out-of-control adverse events like hyperglycemia, stomatitis, and bone marrow suppression, which are one of the main causes of trial failures. The induction of resistance to these drugs is another imperative challenge affecting the outcomes of therapies in HCC. The nature of treatment resistance could be due to tumor genetic heterogeneity (the primary) and/or the activation of compensatory pathways (the secondary). In the presence of PI3K/AKT/mTOR inhibitors, some parallel signaling such as the MAPK pathway could be overactivated to compensate for the survival and proliferation of tumors.

The application of combination therapy is of cardinal importance in overcoming the mentioned challenges. Accordingly, we exhibited that combination therapy has had more proportion in almost all categories of PI3K/AKT/mTOR inhibitors compared with monotherapy, of which MEK inhibitors, chemotherapy, ICI, and VEGF inhibitors have been the most used drugs in combination regimens. Therefore, it could be inferred that combining novel approaches to target other compensatory pathways simultaneously could lead to a more successful control of HCC growth in the future. It is worth mentioning that some therapeutic agents have been particularly studied for their specific inhibitory roles on PI3K/AKT/mTOR signaling pathways

such as everolimus or MK2206. Nonetheless, the fundamental functions of the PI3K/AKT/mTOR signaling pathway make it to be targeted by a variety of drugs whose main responsibility is focused on another domain. Metformin, which is commonly known for its blood glucose-lowering functions, was discussed to have an anti-tumor effect by modulating some key signaling pathways in cells including the PI3K axis [146]. Therefore, utilizing these kinds of drugs in combination regimens would be a potential strategy in future therapeutic studies.

It has been demonstrated that combining molecular inhibitors with nanocarriers enables localized delivery of the therapeutic payload and controlled drug release, potentially minimizing toxicities associated with systemic exposure to the inhibitors [147]. Nanoparticles can overcome various biological barriers, deliver drugs directly to the target site, and enable the simultaneous delivery of multiple drugs [148]. Although the application of nanoparticle-based drug delivery methods has been turned into a desirable topic in scientific studies, the use of nanoparticles in combination with inhibitors for HCC has been explored in only a limited number of studies [149–151]. Therefore, the application of nanoscience to conjugate PI3K/AKT/mTOR inhibitors into nanoparticles can pave the way for future studies to increase the accuracy of drugs and reduce their doses as well as toxicities. In a study of HCC tumor-bearing nude mice, the conjugation of miR-199a-3p, a miRNA targeting mTOR, with arginine α , β -dehydrophenylalanine (R Δ F) nanoparticles resulted in the elevation of miR-199a-3p concentration over 500 fold. This event led to a remarkable downregulation of mTOR *in vitro* and a considerable reduction in tumor growth *in vivo* [152]. All in all, it could be inferred that the encapsulation of PI3K/AKT/mTOR inhibitors in nanoparticles could not only increase the local concentration of drugs but also reduce the off-target toxicities, leading to an improvement in the outcomes of therapy. Nonetheless, the application of nanoscience in the context of PI3K/AKT/mTOR inhibitors for HCC seems to be in its early stages.

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