



## New Drugs

## Targeting the PI3K/AKT/mTOR pathway in biliary tract cancers: A review of current evidences and future perspectives



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## ABSTRACT

Biliary tract cancers (BTCs) are a group of invasive neoplasms, with increasing incidence and dismal prognosis. In advanced disease, the standard of care is represented by first-line chemotherapy with cisplatin and gemcitabine. In subsequent lines, no clear recommendations are currently available, highlighting the need for novel therapeutic approaches.

The PI3K/AKT/mTOR pathway is a core regulator of cell metabolism, growth and survival, and is involved in BTCs carcinogenesis and progression. Mutations, gene copy number alterations and aberrant protein phosphorylation of PI3K, AKT, mTOR and PTEN have been thoroughly described in BTCs and correlate with poor survival outcomes.

Several pre-clinical evidences state the efficacy of PI3K/AKT/mTOR pathway inhibitors in BTCs, both *in vitro* and *in vivo*. In the clinical setting, initial studies with rapamycin analogs have shown interesting activity with an acceptable toxicity profile. Novel strategies evaluating AKT and PI3K inhibitors have risen serious safety concerns, pointing out the need for improved patient selection and increased target specificity for the clinical development of these agents, both alone and in combination with chemotherapy.

This review extensively describes the role of the PI3K/AKT/mTOR pathway in BTCs and examines the rationale of its targeting in these tumors, with particular focus on clinical activity, toxicities and perspectives on further development of PI3K/AKT/mTOR pathway inhibitors.

**Abbreviations:** AEs, adverse events; ALK, anaplastic lymphoma kinase; ARID1A/B, AT-rich interactive domain-containing protein 1; ATP, adenosine triphosphate; BAP1, BRCA1 associated protein-1; Bcl-2, B-cell lymphoma 2; BRAF, rapidly accelerated fibrosarcoma B; BTCs, biliary tract cancers; CBAs, conjugated bile acids; CCA, cholangiocarcinoma; CisGem, cisplatin/gemcitabine; CR, complete response; DCR, disease control rate; DLTs, dose limiting toxicities; eCCA, extrahepatic cholangiocarcinoma; ECM-1, extracellular matrix-1; EGFR, Epidermal Growth Factor Receptor; eIF4E, eukaryotic initiation factor 4E; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal-regulated kinase; FGFR, Fibroblast Growth Factor Receptor; FKBP12, FK506 binding protein; G, grade; GBC, gallbladder carcinoma; GPCRs, G protein-coupled receptors; HIF1 $\alpha$ , Hypoxia Inducible Factor 1  $\alpha$ ; iCCA, intrahepatic colangiocarcinoma; IDH, isocitrate dehydrogenase; IGFR1, Insulin Growth factor Receptor1; IHC, immunohistochemistry; IL, interleukin; KRAS, Kirsten rat sarcoma viral oncogene homolog; LIF, leukemia inhibitory factor; m-FOLFOX, modified Folinic acid/5-Fluorouracil/Oxaliplatin; MAPK, mitogen-activated protein kinase; Mcl-1, myeloid cell leukemia-1; MEK, mitogen-activated ERK Kinase; miRNA, micro RNA; MTD, Maximum Tolerated Dose; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; n, number; NCT, ClinicalTrials.gov identifier number; NTRK, Neurotrophic Tyrosine Receptor Kinase; ORR, Overall Response Rate; OS, overall survival; p-4E-BP1, eukaryotic initiation factor 4E binding protein 1; PBRM1, protein polybromo-1; pCCA, perihilar cholangiocarcinoma; PD, progressive disease; PDKs, phosphoinositide dependent kinases; PFS, progression free survival; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PPAR, peroxisome proliferator-activated receptor; PR, partial response; PTEN, tumor suppressor phosphatase and tensin homolog; Rapalogs, rapamycin and its analogs; Ras, rat sarcoma; RP2D, recommended phase II dose; RTKs, tyrosine kinase receptors; S6K1 or p70S6K, S6 kinase 1; SD, stable disease; SGK1, serum and glucocorticoid-induced protein kinase 1; SREBP 1/2, sterol regulatory element binding protein 1 and 2; TKIs, tyrosine kinase inhibitors; TSC, tuberous sclerosis complex; VEGFR, Vascular Endothelial Growth Factor Receptor

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## Introduction

Biliary tract cancers (BTCs) are a group of relatively rare invasive carcinomas (their incidence varying from 0.1 to 3 cases per 100,000/year in Western countries to 113 cases per 100,000/year in Thailand) arising from the epithelium of gallbladder (gallbladder carcinoma, GBC) and bile ducts (cholangiocarcinoma, CCA) [1,2]. Cholangiocarcinoma is classified into intrahepatic (iCCA, 10–20% of cases), perihilar (pCCA or Klatskin tumor, 50%) and extrahepatic (eCCA, 30–40%), which exhibit different clinical behavior, pathogenesis and molecular profile [1,3].

Nowadays, BTCs prognosis remains poor. Five-year survival rates in patients undergoing surgical resection are 60% for iCCA and 20–30% in pCCA or eCCA, respectively. In advanced disease, defined as inoperable or metastatic, 5-year survival rate is 5–10%, and median overall survival (OS) is  $\leq 12$  months [3,4].

In this setting, systemic treatment options are limited. Combination chemotherapy with cisplatin plus gemcitabine (CisGem) represents the standard first-line treatment [5]. In the second line setting, current guidelines do not support the use of specific regimens due to the lack of adequate evidences from prospective randomized controlled trials [6].

Urgent efforts are therefore needed to identify BTCs-specific molecular targets for novel therapeutic approaches.

## Key pathways and molecular spectra of BTCs

Next generation sequencing and gene expression analyses revealed a complex genomic and transcriptomic landscape of BTCs, defining distinct molecular subtypes and new potential therapeutic targets.

iCCAs, eCCAs and GBCs should be regarded as separate entities due to significant differences in their genomic spectra. Recurrent molecular alterations in iCCAs include Fibroblast Growth Factor Receptor (FGFR) gene fusions (11–45% of cases), Isocitrate Dehydrogenase 1/2 (IDH1/2) mutations (5–36%) and alterations of chromatin-modifying genes (up to 50% of cases). eCCAs are more frequently associated with KRAS (Kirsten rat sarcoma viral oncogene homolog) mutations (42%), while GBCs display the highest frequency of Her2 amplifications (up to 30% of cases). p53 mutations, angiogenesis deregulation and alterations in core intracellular pathways such as the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) have been identified across all subgroups of BTCs [4].

To date, FGFR and IDH1/2 inhibitors appear to be the most promising strategy upcoming in the treatment of molecular selected advanced iCCA patients. FGFR inhibitors reported a 22–25% overall response rate (ORR) and a 75–95% disease control rate (DCR) in phase II clinical trials of pretreated iCCA patients harboring FGFR alterations, while IDH-1 inhibition achieved 60% DCR in a pretreated IDH mutated population [4,7,8]. Histone deacetylase inhibitors may also represent a therapeutic option for iCCAs, due to the high prevalence of mutations of genes involved in chromatin remodeling (ARID1A/B, PBRM1 and BAP1) [4].

Other druggable targets include Epidermal Growth Factor Receptor (EGFR), that is mutated in 0–25% of BTCs and overexpressed in 20–60% of cases, and HER2, whose amplification is observed in 10–30% of BTCs, with higher rates in GBCs and eCCAs compared to iCCAs [4,7]. Despite enhanced ORR, no significant improvements in progression free survival (PFS) and OS have been reported in clinical trials of EGFR tyrosine kinase inhibitor (TKI) erlotinib and anti-EGFR monoclonal antibodies panitumumab and cetuximab in combination with first line chemotherapy [4]. Anti-Her2 targeted agents (lapatinib and trastuzumab) have demonstrated poor activity in CCAs, in the absence of observed objective responses; more promising results have been observed in GBCs, which harbor higher rates of Her2 amplifications [4,7]. Results of the phase II/III randomized controlled trial TreeTopp (ASLAN-001-009) of capecitabine plus varlitinib (EGFR/Her2

co-inhibitor) or placebo in the second line setting in BTCs are awaited to assess the clinical benefit of this class of agents (NCT03093870).

Inhibition of angiogenesis has mostly yielded disappointing results, with only bevacizumab and cediranib demonstrating some clinical activity [4].

Conversely, agents targeting Neurotrophic Tyrosine Receptor Kinase (NTRK)1/2/3, Anaplastic Lymphoma Kinase (ALK) and ROS1 gene rearrangements (3.5%, 2.6% and 8.7–16% of cases respectively) have achieved impressive ORR (57–86%) in early phase clinical trials in solid tumors, eliciting further evaluation of these compounds in BTCs [7].

Targeting of intracellular pathways that regulate cell growth and proliferation is another subject of investigation.

The MAPK pathway, consisting of the RAS/RAF/MEK (Mitogen-activated ERK Kinase)/ERK (extracellular signal-regulated kinase) cascade, is frequently deregulated in BTCs, KRAS and BRAF mutations accounting for 8–40% and 0–22% of cases respectively. To date, only preliminary evidence is available about BRAF inhibition in CCA patients, whereas more conspicuous data are available about MEK1/2 inhibitors (selumetinib, binimetinib) [4,7].

The PI3K/AKT/mTOR pathway is a core regulator of cell metabolism, growth and survival [9]. Deregulation of PI3K/AKT/mTOR signaling has been described in multiple human malignancies such as renal cell carcinoma, breast cancer and neuroendocrine tumors, leading to the approval of mTOR inhibitors in these settings [10]. Despite robust preclinical data of activity of PI3K/AKT/mTOR pathway inhibitors in GBCs and CCAs both *in vitro* and *in vivo*, clinical evidences of efficacy of these compounds in BTC patients appear conflicting.

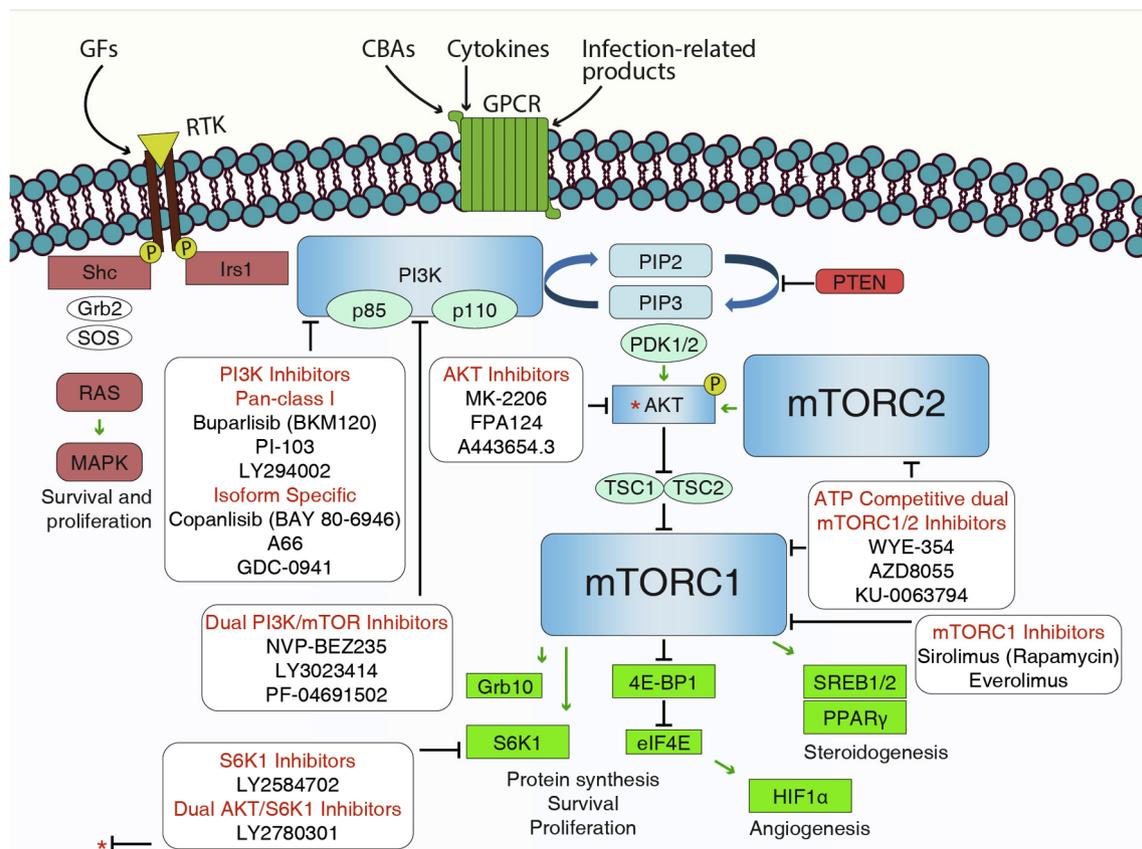
This review aims at clarifying the role of PI3K/AKT/mTOR pathway inhibition in the therapeutic landscape of BTCs. The role of PI3K/AKT/mTOR signaling in BTC carcinogenesis and progression is discussed, along with the prognostic and predictive relevance of PI3K/AKT/mTOR signaling alterations in human BTCs. An analysis of preclinical and clinical evidences of efficacy of PI3K/AKT/mTOR pathway inhibitors, both alone and in combination, is provided. Moreover, strategies to further develop PI3K/AKT/mTOR targeted agents in BTCs are addressed.

## The PI3K/AKT/mTOR pathway in human cancers

The PI3K/AKT/mTOR pathway regulates intracellular metabolism, cell cycle progression, angiogenesis and invasiveness in response to various intracellular and extracellular stimuli, such as metabolites, growth factors and hypoxia.

The signaling cascade is initiated by receptor tyrosine kinases (RTKs-including Insulin Growth factor Receptor1-IGFR1, Vascular Endothelial Growth Factor receptor-VEGFR and EGFR), G protein-coupled receptors (GPCRs) and oncogenic proteins of the RAS family, which trigger PI3K activation [11]. The PI3K protein family comprises three classes (I, II, III) and four isoforms ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) of heterodimers composed of a regulatory (p85) and a catalytic (p110) subunit, with class I<sub>A</sub> being the most frequently involved in human cancers. Activated PI3K converts phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3), that provides a membrane docking site for phosphoinositide dependent kinases (PDKs) 1/2. Once bound to PIP3, PDKs phosphorylate and activate the AKT serine/threonine kinase. AKT signaling promotes mTOR activity through inhibitory phosphorylation of tuberous sclerosis complex (TSC) proteins 1/2, which act as mTOR inhibitors. Negative regulation of the axis is mediated by PTEN (tumor suppressor phosphatase and tensin homolog), a phosphatase that converts PIP3 into inactive PIP2, thus impairing AKT activation [9,11].

mTOR is a 289 kDa serine/threonine kinase that is part of two distinct large multiprotein complexes, mTORC1 (mTOR complex 1) and mTORC2. mTORC1 phosphorylates the eukaryotic initiation factor 4E (eIF4E) binding protein 1 (p-4E-BP1), promoting its dissociation from eIF4E and allowing eIF4E-dependent protein synthesis. Another



**Fig. 1.** PI3K/AKT/mTOR pathway and targeted agents tested in preclinical and clinical studies in BTCs. *Abbreviations:* 4E-BP1: eukaryotic initiation factor 4E binding protein 1; CBAs: Conjugated Bile Acids; eIF4E: eukaryotic initiation factor 4E; GF: Growth Factors; GPCR: G-protein coupled receptor; Irs1: Insulin receptor substrate 1; HIF1 $\alpha$ : hypoxia inducible factor 1  $\alpha$ ; mTORC1/2: mammalian target of rapamycin complex 1/2; PDK1/2: phosphoinositide dependent kinases 1/2; PI3K: phosphatidylinositol 3-kinase; PIP2: phosphatidylinositol-4,5-bisphosphate; PIP3: phosphatidylinositol-3,4,5-trisphosphate; PPAR $\gamma$ : peroxisome proliferator-activated receptor; PTEN: tumor suppressor phosphatase and tensin homolog; RAS: Rat Sarcoma; RTK: Receptor Tyrosine Kinase; S6K1: S6 kinase 1; SREBP  $\frac{1}{2}$ : sterol regulatory element binding protein 1/2; TSC1/2: tuberous sclerosis complex 1/2. *Note:* PI-103, LY294002, A66, GDC-0941, FPA124, A443654.3, NVP-BEZ235, LY3023414, PF-04691502, WYE-354, AZD8055, KU-0063794 have been tested only in preclinical studies. Buparlisib (BKM120), Copanlisib (BAY 80-6946), MK-2206, LY2584702, LY2780301, Sirolimus and everolimus have been tested in clinical trials.

downstream effector of mTORC1 is S6 kinase 1 (S6K1, also known as p70S6K), that participates to mRNA biogenesis and translation. mTORC1 also modulates steroid biogenesis via sterol regulatory element binding protein (SREBP) 1/2 and peroxisome proliferator-activated receptor (PPAR) $\gamma$ , mediates response to hypoxia by enhancing hypoxia inducible factor 1  $\alpha$  (HIF1 $\alpha$ ) transcription, participates to epithelial-to-mesenchymal transition (EMT) and neoangiogenesis. In contrast, the function and downstream effectors of mTORC2 are not fully elucidated. mTORC2 signaling is activated via PI3K in response to growth factors, cytokines and GPCRs and controls various intracellular kinases including AKT and serum and glucocorticoid-induced protein kinase 1 (SGK1) [9] (Fig. 1).

### PI3K-AKT-mTOR pathway deregulation in human BTCs

Deregulation of PI3K/AKT/mTOR pathway and its role in human BTCs carcinogenesis have been thoroughly assessed both *in vitro* and *in vivo*. The most common alterations include activating mutations of the *PIK3CA* and *PIK3R1* genes, which encode respectively for PI3K p110a and p85a subunits, somatic mutations of *AKT*, inactivating mutations or deletion of tumor suppressor *PTEN* and overexpression or aberrant phosphorylation of PI3K, AKT, mTOR and their downstream targets p70S6K, p-4E-BP1 and eIF4E. Moreover, the pathway may be altered through enhanced activity of upstream membrane receptors and their ligands (growth factors, inflammatory cytokines, pro-angiogenic molecules, stromal derived peptides) [9–12].

### PI3K and AKT gene and protein alterations in human BTCs

*PIK3CA* mutations are relatively rare both in GBC (4–16.9% of cases) [12–17], and in CCA (0–9%) [18–21]. About 75% of *PIK3CA* activating mutations cluster in the helical and kinase domains of p110a [13], the most frequent being E542K, E545G, E545K mutations in exon 9 and H1047L and H1047R substitutions in exon 20 [12]. Activating mutations of *PIK3CA* have been associated with poor prognosis, with no correlation with clinical or histologic features [12,14]. Mutated PI3K is probably not predictive of response to rapamycin and its analogs (rapalogs) according to results in BTC cell lines [20]. *PIK3CA* amplification has been recently described, being reported in 6% of cases in a series of 84 BTC specimens [17] and being detected in three patient-derived sarcomatoid GBC cell lines [22]. Only few studies described *PIK3R1* mutations (4–29%) [17,18].

*AKT* gene mutations and copy number variation are uncommon, being detected in 0 and 2.3% of BTC cases, respectively [18,23]. Conversely, protein overexpression and functional activation of PI3K and AKT characterize BTCs at a larger extent. Positive staining for PI3K has been evidenced in about 50% of GBCs, with significantly higher rates in comparison to non-neoplastic epithelium. Moreover, PI3K overexpression has been associated with tumor diameter, differentiation, nodal involvement and reduced OS [24]. Positive immunostaining for AKT and its phosphorylated form (p-AKT) has been reported respectively in 46–100% and 34–100% of human BTC tissue samples, with higher rates in neoplastic cells compared to the surrounding normal and

dysplastic epithelium [19,21,25–30]. Their levels may differ according to BTC site of origin, as significantly higher pAKT expression rates have been described in iCCAs (76.5%) compared to eCCAs (36.8%) and GBCs (46.1%) [19]. No association between pAKT levels and any clinical or pathological parameters has been detected. Its prognostic relevance is debated: two studies did not show correlation of pAKT levels with survival [21,28], while two other series showed improved OS in BTC patients harboring AKT and pAKT overexpression [27,31]. High p-AKT basal levels have been associated with sensitivity to everolimus in KRAS wild type CCA cells *in vitro* [20].

#### *mTOR alterations in BTCs*

Increased *mTOR* gene copy number and p-mTOR protein overexpression have been described in 48–92% of BTC specimens, with a significantly higher staining rate in comparison to the adjacent normal or dysplastic epithelium [30–36]. In one study, mTOR immunohistochemistry (IHC) positivity was associated with well- to moderately-differentiated and non-metastatic tumors [31], whereas other series did not observe correlations between p-mTOR expression and any clinical or pathological variables [34–36]. p-mTOR prognostic value remains controversial: most studies observed shorter relapse-free survival and OS in patients with p-mTOR positive tumors [34,35,37], while Lee et al. showed higher 5-year OS rate for iCCA patients whose tumors harbored p-mTOR overexpression [31].

#### *Downstream mTOR effectors*

Overexpression of downstream mTOR effectors (eIF4-E, p-4E-BP1, p70S6K) is common in BTCs [13,31,36,38,39]. High p70S6K expression is reported both in dysplasia (66.7%) and in BTCs (22–84%) [31,36,38,39] and has been associated with poor tumor differentiation [36]. eIF4-E is expressed in 23–35% of BTC cases [13], while 8–50% of iCCAs, 21% of eCCAs and 44% of GBCs stain positive for p-4E-BP1 [13,31,36]. Wang et al reported the association of high p-4E-BP1 levels with poor prognosis in a series of 77 iCCA patients [36]. Conversely, Lee et al detected a trend towards improved 5-year OS in patients with p-70S6K and p-4E-BP1 positive tumors [31].

#### *Loss of PTEN in BTCs*

Loss of PTEN accounts for PI3K/AKT/mTOR pathway deregulation in 4–71% of BTCs [19,31,40–42]. PTEN inactivation may result from somatic gene mutations (up to 33% of cases) [17], deletions or methylation of the promoter (30%) [43] and post-transcriptional or post-translational deregulation [41]. PTEN IHC staining rates are significantly lower in BTCs and severe dysplasia (33–48%) compared to normal epithelium and gallbladder polyps (93–100%) [31,41–43]. Complete loss of PTEN expression has been detected in 2.8–51.8% of BTC specimens [41–43].

PTEN loss has been related to poor tumor differentiation, depth of invasion, nodal involvement and shorter survival both in GBC and CCA [31,33,41,42]. Again, PTEN status was not predictive of response to everolimus in a study conducted on different BTC cell lines [20].

#### *Growth factor receptors*

EGFR, HER2, IGFR and VEGFR are the main upstream initiators of PI3K/AKT/mTOR pathway involved in BTC carcinogenesis. EGFR/HER2 dependent PI3K/AKT phosphorylation has been demonstrated in several CCA cell lines [14,19,37,44–46] and significant correlation between EGFR and pAKT/p-mTOR IHC levels has been confirmed in human CCA tissue samples [28,37]. HER2 activation via MUC4 has also been associated with AKT hyperphosphorylation in specimens of human GBC [44]. EGFR TKIs [19,37] and anti-HER2 targeted agents [19,45–47] demonstrated anti-tumor activity by suppressing AKT and

p70S6K phosphorylation in BTC cell lines and restoring sensitivity to chemotherapeutic agents in resistant cells [19,48].

The PI3K/AKT/mTOR pathway also acts downstream of IGFR1, whose expression has been identified in about 70% of BTCs [49]. IGFR1 inhibitors NVP-AEW541 [50] and BMS-536924 prevent AKT activation and exert antiproliferative effects in CCA cells. BMS-536924 efficacy *in vitro* was synergistic with 5-Fluorouracil, gemcitabine and cisplatin and the drug also proved effective in reducing tumor burden in a xenograft BTC mouse model [49].

VEGFR is another initiator of PI3K/AKT/mTOR signaling: its inhibitors apatinib and sorafenib reduced p-PI3K, p-AKT and p-mTOR protein levels as well as tumor growth both in CCA cell lines and in a xenograft mouse CCA model [47,51].

#### *Carcinogenic stimuli of the tumor microenvironment promote PI3K/AKT/mTOR pathway activation*

Chronic inflammation is a recognized risk factor for CCA and several evidences support the relationship between inflammatory stimuli and mTOR aberrant activation in BTCs. Inflammatory cytokines such as interleukin (IL)-6, IL-33, Leukemia inhibitory factor (LIF), CCL5 and Stromal Cell Derived Factor-1 trigger PI3K/AKT activation in CCA cell lines, leading to proliferation, invasion and resistance to chemotherapeutic agents [25,52–57]. In particular, IL-6 and LIF exert anti-apoptotic effects on CCA cells through AKT-mediated upregulation of myeloid cell leukemia-1 (Mcl-1), an anti-apoptotic protein part of the Bcl-2 (B-cell lymphoma 2) family [25,53]. The AKT inhibitor A443654.3 impairs AKT phosphorylation and Mcl-1 expression and sensitizes CCA cells to apoptosis [25]. Moreover, IL-33 expression promotes CCA formation and liver metastatization in mice engineered to constitutively express active AKT and Yes-associated protein [58].

Prostaglandins are also involved in CCA carcinogenesis through PI3K/AKT/mTOR pathway deregulation. Cyclo-Oxygenase 2 inhibitor celecoxib has been evaluated as an antineoplastic agent in BTC cell lines due to its ability to reduce Prostaglandin E synthesis, thus affecting AKT phosphorylation and BTC cell survival [59].

Chronic parasite hepatic infections and exposure to conjugated bile acids (CBAs) are other recognized risk factors for CCA. Infection-associated products, such as *Opisthorchis viverrini*-derived glutathione S-transferase [60], *Clonorchis Sinensis* granulin [61] as well as CBA taurocholate [62] induce aberrant PI3K/AKT/mTOR signaling activation and enhanced proliferation in CCA cells.

PI3K/AKT/mTOR pathway deregulation in BTCs occurs also through  $\alpha 6\beta 4$  and  $\alpha 5\beta 1$  integrin signaling, that promote cell invasiveness through interaction with extracellular matrix components [63]. Elevated levels of Integrin  $\alpha 6$  in iCCA cells correlate with advanced T and N stage and poor prognosis [64]. Similarly, stroma-derived mediators like extracellular matrix-1 (ECM-1) [65] and nectin 4 [66] are able to enhance cancer progression via the mTOR pathway; elevated levels of these factors in CCA correlate with poor differentiation, positive nodal status and poor prognosis [65,66].

#### **PI3K/AKT/mTOR pathway inhibitors in the pre-clinical and clinical setting**

Evidences of PI3K/AKT/mTOR pathway deregulation in BTCs provide the rationale for investigating targeted therapies in this setting. Several agents have been developed, with most pre-clinical and clinical evidences regarding mTOR, PI3K and AKT inhibitors. Tables 1 and 2 provide details about published and ongoing clinical trials evaluating PI3K/AKT/mTOR inhibitors in BTCs.

#### *mTOR inhibitors*

Rapamycin (Rapamune, Sirolimus) is a macrolide fungicide produced by *Streptomyces hygroscopicus* that forms a complex with the

**Table 1**  
Completed clinical trials testing PI3K/AKT/mTOR pathway inhibitors in BTCs.

Compound	Dosing (MTD)	Phase	Condition	Pts Enrolled <sup>a</sup>	Response <sup>**</sup>	Survival <sup>**</sup>
<i>mTOR inhibitors</i>						
Sirolimus [70]	Variable dose to obtain target sirolimus serum levels (4–15 ng/ml)	II	Advanced HCC and iCCA	9	PR 0 (0%) SD 3 (33%) PD 6 (67%)	mOS 7 mts (2.6–35 mts)
Sirolimus [71]	1 mg/d	II	PIK3CA mutant/amplified refractory solid tumors (gastric cancer; pCCA)	1	DCR 0 (0%)	PFS 0.9 mts
Everolimus [74]	5–10 mg/d	I	metastatic Renal Cell Carcinoma, pancreatic neuroendocrine tumors, BTCs	22	DCR 11 (50%)	NA
Everolimus + CisGem [75]	5 mg 3 d/w + Cis 12.5 mg/m <sup>2</sup> + Gem 600 mg/m <sup>2</sup> d1, 8q21	I	Cohort III: advanced, not previously treated CCA and GBC	10	PR 0 (0%) SD 6 (60%) PD 4 (40%)	NA
Everolimus [76]	10 mg/d	II	unresectable BTCs refractory to first line chemotherapy	39	ORR 5.1% DCR 44.7%	mPFS 3.2 mts (1.8–4.0 mts) mOS 7.7 mts (5.5–13.2 mts)
Everolimus [77]	10 mg/d	II	advanced BTCs, not previously treated for advanced disease	27	DCR 48% ORR 12% PR 3 (12%) SD 15 (60%) of the 15 patients evaluable for response	mPFS 5.5 mts (2.0–10.0 mts) mOS 9.5 mts (5.5–16.6 mts)
Everolimus [78]	10 mg/d	II	PIK3CA amplified or mutated and/or PTEN loss advanced refractory solid tumors (gastric, CRC, pancreatic, CCA)	1	No specific data for CCA pts; in the overall study population PR 0% SD 40%	No specific data for CCA pts; in the overall study population mPFS 1.6 mts (0.8–2.4 mts)
<i>PI3K inhibitors</i>						
BKM120 [83] (Buparlisib) + mFOLFOX6	40 mg/d + Oxaliplatin: 85 mg/m <sup>2</sup> Leucovorin: 400 mg/m <sup>2</sup> 5-FU bolus: 400 mg/m <sup>2</sup> 5-FU infusion: 2400 mg/m <sup>2</sup> q2w	I	advanced refractory solid tumors (CRC, CCA, breast, pancreatic, squamous esophageal, GEJ, ocular melanoma)	4	PR 0 (0%) SD 1 (25%) PD 3 (75%)	NA
BKM120 [84] (Buparlisib)	100 mg/d	I	advanced unresectable refractory solid tumors (breast, rectum, lung, ovary, head and neck, pancreas, prostate, liver, gastric, renal, GBC, other)	1	No specific data for GBC pts; in the overall study population DCR 41%	No specific data for GBC pts; in the overall study population treated ≥ MTD mPFS 57 ds (50–106 ds) NA
BAY 80-6946 [86] (Copanlisib) ± Gem or CisGem	0.8 mg/kg ± Gem 1000 mg/m <sup>2</sup> weekly 3 ws on/1 w off Or Cis 25 mg/m <sup>2</sup> + Gem 1000 mg/m <sup>2</sup> d1, 8q21	I	advanced or refractory solid tumors (breast, GBC, iCCA, NSCLC, pancreas, SCLC, other)	23	ORR: 17% PR 4 (17%) SD NA PD NA All responders received copanlisib + CisGem	NA
<i>AKT inhibitors</i>						
MK2206 [88]	200 mg/w	II	Advanced unresectable BTCs refractory to first line therapy: iCCA, eCCA	8	PR 0 (0%) SD 2 (25%) PD 6 (75%)	mPFS 1.7 mts mOS 3.5 mts
<i>Combination of other mTOR /PI3K/AKT pathway inhibitors</i>						
LY2584702 [92] + Erlotinib vs Everolimus	50–200 mg/d + 150 mg/d vs 10 mg/d	Ib	advanced solid tumors refractory to standard therapy (CRC, breast, cervix, sarcoma, melanoma, other)	29 (total not selected)	No specific data for BTC pts; in the overall study population DCR 28%	NA
LY2780301 [93]	100–500 mg/d	I	advanced refractory solid tumors (CRC, breast, mesothelioma, sarcoma, other)	32 (total not selected)	No specific data for BTC pts; in the overall study population DCR 29%	NA
LY2780301 + Gem [94]	MTD 500 mg/d + 750–1000 mg/m <sup>2</sup> d1, 8q21	Ib	advanced refractory solid tumors harboring PI3K mutations, PTEN loss (breast, cervical/ endometrial, head and neck, ovary, stomach, kidney, BTCs)	4	No specific data for BTC pts; in the overall study population PR 5% SD 72% PD 23%	NA

**Abbreviations:** BTC biliary tract cancer; CCA: cholangiocarcinoma; Cis: Cisplatin; CisGem: Cisplatin/Gemcitabine; CRC: colorectal cancer; CT: chemotherapy; d(s): day (s); w(s): week(s); d/w days per week; DCR disease control rate; eCCA: extrahepatic cholangiocarcinoma; FU: fluorouracil; GBC gallbladder carcinoma; GEJ gastroesophageal junction carcinoma; Gem: Gemcitabine; HCC: hepatocarcinoma; iCCA: intrahepatic cholangiocarcinoma; m-FOLFOX: modified Folinic acid/5-Fluorouracil/Oxaliplatin; mts: months; MTD: maximum tolerated dose; NA: not available; NSCLC: non small cell lung cancer; ORR overall response rate; (m)OS: (median) overall survival; pCCA perihilar cholangiocarcinoma; PD: progressive disease; (m)PFS: (median) progression free survival; PI3K phosphatidylinositol 3-kinase; PR: partial response; PTEN: tumor suppressor phosphatase and tensin homolog; Pts: patients; SCLC: small cell lung cancer; SD: stable disease.

<sup>a</sup> Number of patients enrolled, considering trials with more than one disease-specific cohort, refers exclusively to BTC patients, as evidenced in bold for each study, unless otherwise specified.

<sup>\*\*</sup> Response rate and survival data are referred to only patients with BTCs.

**Table 2**  
Ongoing clinical trials or clinical trials with completed recruitment without results.

Agent	Phase	Randomized	Condition	Primary Outcomes	Status	ClinicalTrials.gov Identifier
Sirolimus + CisGem	I	no	Resected CCA at high risk for recurrence after liver transplant or surgery	Therapy completion rate at 4 and 6 mts	Recruitment completed	NCT01888302
Everolimus	II	no	Advanced unresectable CCA with no prior systemic treatment	PFS	Ongoing, not recruiting	NCT01525719
Everolimus + Gem (cohort I) or CisGem (cohort II/III)	I	no	Unresectable solid tumors refractory to standard therapy (cohort I/II), metastatic CCA or GBC (cohort III)	MTD and toxicity of treatment in cohort I and II, toxicity and best response in cohort III	Recruitment completed	NCT00949949
Copanlisib + CisGem	II	no	Advanced unresectable CCA with no prior systemic treatment for advanced disease	PFS	Active recruiting	NCT02631590
GEMOX vs GEMOX + targeted agents according to tumor genomic profile among which Everolimus	II	yes	Advanced or recurrent metastatic eCCA and GBC, unresectable or progressed after surgery, with no prior systemic treatment	PFS	Active recruiting	NCT02836847
Target agents according to tumor profile, among which I: PIK3CA mut, no RAS mut or PTEN loss: taselisib L: mTOR mut: sapanisertib N: PTEN mut or deleted or expressed: GSK2636771 P: PTEN loss GSK2636771 Y: AKT mut: AZD5363	II	no	Advanced solid tumors (including intrahepatic BTCs), lymphoma, multiple myeloma, refractory to first line treatment or with no standard therapy	ORR	Active recruiting	NCT02465060
Capecitabine + varlitinib/placebo	II	yes	Advanced BTCs in the second line setting	Part 1: ORR + PFS (co-primary endpoint) Part 2: OS	Active recruiting	NCT03093870

*Abbreviations:* AEs: adverse events; BTC: biliary tract cancer; CCA: cholangiocarcinoma; CisGem: cisplatin + gemcitabine; CT: chemotherapeutic; eCCA: extrahepatic cholangiocarcinoma; GBC: gallbladder carcinoma; Gem: Gemcitabine; GEMOX: gemcitabine + oxaliplatin; MTD: maximum tolerated dose; mTOR: mammalian target of rapamycin; mts: months; mut: mutation; ORR: overall response rate; OS: Overall Survival; PFS: progression free survival; PTEN: tumor suppressor phosphatase and tensin homolog; RAS: Rat Sarcoma.

immunophilin FK506 binding protein (FKBP12), becoming able to inhibit the activity of mTOR when part of mTORC1 (with a significantly lower inhibitory effect on mTORC2) [9]. Everolimus (RAD001/Afinitor), Temsirolimus (Torisel) and Ridaforolimus (MK-8669), collectively known as rapalogs, are semi-synthetic rapamycin analogues with improved pharmacokinetic profiles. A strong body of preclinical evidence assessed the antineoplastic effect of rapamycin and its analogs in BTCs, both *in vitro* and *in vivo*. Treatment with rapamycin significantly inhibited proliferation and invasion and induced apoptosis in several human CCA and GBC cell lines in a dose-dependent manner [39,67–69]. Moreover, significant antitumor activity of rapamycin *in vivo* has been assessed in different GBC xenograft models [30,68,69].

In the clinical setting, a single arm prospective study evaluated sirolimus in 30 patients with advanced hepatocarcinoma (n = 21) and iCCA (n = 9). In the CCA cohort, median OS was 7 (2.6–35) months, with 3 out of 9 patients experiencing stable disease (SD). There were no specific safety concerns, aphthous ulcers being the only relevant toxicity [70].

A pilot study testing sirolimus in patients with mutant/amplified *PIK3CA* refractory tumors included three patients with gastric cancer and one patient with pCCA, with no reported benefit [71].

A Phase I clinical trial testing sirolimus, gemcitabine and cisplatin in patients at high risk of CCA recurrence after liver transplant is ongoing (NCT01888302).

Preclinical evidences confirmed that also everolimus may be effective in BTC treatment. Yeung et al. tested everolimus in a panel of 20 BTC cell lines, showing mostly a cytostatic effect. Apoptosis was observed in only one (*TGBC2TKB*) of the three most sensitive lines and only at high concentrations [20]. Conversely, Heits *et al* proved that everolimus inhibits proliferation of two CCA cell lines (*HuCC1*, *TFK1*) even at very low concentrations (1 nM) [72]. Other studies confirmed the activity of everolimus both in CCA [26,73] and GBC cells [38,39].

Everolimus has been evaluated both as a single agent and in combination with chemotherapy in BTC patients, showing modest but significant activity.

A Phase I trial assessing the safety profile of everolimus in different solid tumors enrolled 22 BTC patients. In this subgroup, DCR was 50% and one patient (5%) experienced a complete response (CR). Tolerability in BTC patients seemed to be worse when compared to other neoplasms such as renal cell carcinoma and neuroendocrine tumors, with higher rates of neutropenia and thrombocytopenia [74]. Another Phase I trial testing the combination of everolimus, gemcitabine and cisplatin in solid tumors included an expansion cohort of 10 patients with CCA or GBC, who received everolimus 5 mg three days a week. No objective responses were observed. Six patients (60%) had SD and 4 (40%) reported progressive disease (PD) as best response. Two BTC patients experienced dose limiting toxicities (DLTs), namely grade (G) 3 neutropenia and thrombocytopenia [75].

A Phase II study (EUDRACT 2008-007152-94) evaluated everolimus as single agent in 39 patients with unresectable BTC refractory to first line chemotherapy. The primary endpoints, DCR and ORR, were 44.7% and 5.1%, respectively. PFS was 3.2 (1.8–4.0) months and median OS was 7.7 (5.5–13.2) months. The most common toxicities were asthenia (43.6%), thrombocytopenia (35.9%) and pyrexia (30.8%). Twenty-one G4 adverse events (AEs) were reported, six of which were considered drug-related (thrombocytopenia, pneumonia, anorexia, stomatitis, nausea and vomiting). Despite the limited activity documented, the Authors highlighted that everolimus showed longer OS compared to that achieved in second line trials of chemotherapy in BTCs [76].

The Phase II RADiChol trial assessed single-agent everolimus activity in 27 previously untreated BTC patients, reporting a DCR of 48% at 12 weeks and an ORR of 12%. Among the 15 patients evaluable for response, 3 (12%) achieved partial response (PR) and 15 (60%) SD. No CRs were observed. Median PFS was 5.5 (2.0–10.0) months and median OS was 9.5 (5.5–16.6) months. GBC patients experienced significantly worse DCR compared to CCA patients (25% vs 71%,  $p = 0.047$ ), as well

as a non-significant trend towards worse PFS (2.1 vs 8.4 months,  $p = 0.395$ ) and OS (5.6 vs 15.5 months,  $p = 0.092$ ). The most common G3/4 AEs were infection (26%), pain (15%), hyperglycemia (11%) and anemia (11%) [77]. An exploratory assessment of predictive biomarkers identified a trend towards worse OS in patients with high tumor p-4E-BP1 IHC staining ( $p = 0.052$ ). No correlation was observed between efficacy outcomes and staining intensity of p-AKT, p-mTOR, p-S6 or *KRAS* mutations [77].

Finally, a recent Phase II trial of everolimus in patients with advanced solid tumors refractory to standard therapy with *PIK3CA* amplification/mutation and/or PTEN loss included one patient with cholangiocarcinoma with PTEN loss. No specific data on this patient were reported, but no responses were observed in any of the enrolled patients [78].

No *in vitro* nor *in vivo* studies testing temsirolimus and ridaforolimus in BTCs have been published so far to our knowledge.

### *PI3K inhibitors in BTCs*

PI3K inhibitors encompass both Pan-class I inhibitors, targeting all four isoforms of p110 catalytic subunits ( $\alpha$ - $\delta$ ), and isoform-specific molecules [79]. Among pre-clinically tested pan-class I PI3K inhibitors, LY294002, buparlisib (BKM120) and PI-103 demonstrated significant *in vitro* activity against BTCs cells. LY294002 inhibited BTC cell proliferation, invasiveness and EMT [57,64–66] by decreasing p-AKT and p70S6K levels [39,57,65,66] and restored chemosensitivity in cell lines resistant to cisplatin, 5-fluorouracil or gemcitabine [80]. Buparlisib demonstrated important anti-proliferative activity in either mutant or wild-type *KRAS* BTC cells [81], while PI-103 showed anti proliferative activity in a xenograft CCA mouse model [82].

Among isoform-selective PI3K inhibitors, p110 $\alpha$ -selective agent A66 proved effective in impairing proliferation of PI3K wild type and mutated GBC cells, both *in vitro* and in engrafted mice [14], while the PI3K $\alpha$ / $\delta$  selective GDC-0941 effectively inhibited cell growth of sarcomatoid GBC cell lines [22].

Clinical reports on the activity of PI3K inhibitors in BTCs are limited.

The rationale of combining chemotherapy with PI3K inhibitors in BTC was explored in two studies. A Phase I trial evaluated the activity of mFOLFOX6 (modified Folinic acid/5-Fluorouracil/Oxaliplatin) combined with buparlisib in 17 patients with advanced refractory gastrointestinal tumors (4 of whom were CCAs). The Maximum Tolerated Dose (MTD) (primary endpoint) of buparlisib was established at 40 mg. Only 8 patients out of 17 were treated for at least two cycles (8 weeks) and were evaluable for response. One patient (12.5%) experienced unconfirmed PR, 3 (one of whom with CCA) had SD (37.5%), being on treatment for 9, 15 and 26 weeks respectively. The remaining 4 patients (50%) showed PD as best response [83]. The treatment was severely toxic: 76% of the evaluable patients (n = 17) experienced treatment-related G3/4 AEs, the most common being neutropenia, hyperglycemia and thrombocytopenia. Seventy-one percent of patients experienced buparlisib-related hyperglycemia. No toxic deaths were reported. The Authors concluded that the combination of mFOLFOX6 with buparlisib resulted in increased toxicity compared to either treatment alone and did not prove efficacious in treatment of advanced refractory gastrointestinal malignancies [83]. In contrast with these results, in a previous phase I dose escalation study of single-agent buparlisib in solid tumors (including GBC) the MTD was established at 100 mg daily, with 20% of enrolled patients remaining on treatment for over 8 months [84]. Moreover, buparlisib proved effective and tolerable at the daily dose of 100 mg in combination with paclitaxel/carboplatin in a Phase I study in solid tumors [85].

Another Phase I study evaluated the combination of copanlisib (BAY 80-6946), an intravenous pan-class I PI3K inhibitor with predominant PI3K $\alpha$ / $\delta$  activity, with gemcitabine or CisGem in 50 patients with advanced solid malignancies [83]. The study included an expansion

cohort of 20 patients with BTC who received the recommended phase II dose (RP2D) of copanlisib plus CisGem, with a total of 23 BTC patients enrolled. The MTD and RP2D of copanlisib was 0.8 mg/kg. Response rate was 6.3% in the copanlisib plus gemcitabine group (1/16 patients) and 12% (4/34 patients) in the copanlisib plus CisGem group, accounting for one CR and three PRs that occurred all in patients with BTC. Overall, among the 23 patients with BTC the response rate was 17% (4/23 patients). All responders were treatment naïve. Ninety-four percent of patients experienced at least one copanlisib-related AE, most commonly hyperglycemia (76%), nausea (74%) and fatigue (66%). Rates of copanlisib-related G3 neutropenia (48%), thrombocytopenia (28%) and hypertension (38%) were significant. Interruption or dose reduction of study drugs was necessary in 45 patients (90%). No deaths were considered related to study treatment. Among explored biomarkers (*PIK3CA*, *KRAS* and *BRAF* mutations, PTEN protein loss), none was significantly associated with outcome, possibly due to the small numbers of patients included in the biomarker analysis [86].

#### AKT inhibitors in BTCs

AKT inhibitors include either allosteric inhibitors (MK-2206) or adenosine triphosphate (ATP)-competitive molecules [79]. AKT inhibition with small molecules MK-2206, FPA124 and A443654.3 proved effective to impair cell proliferation, survival and migration downstream of CCL5, progranulin and IL-6 induced signaling in different BTC cell lines [25,52,54,87].

Among these compounds, the most promising AKT inhibitor so far is MK2206. Its anti-proliferative activity was confirmed *in vitro* in primary cultures of iCCA where this drug exhibited a strong pro-apoptotic effect [47]. MK2206 as an orally-administered single agent was evaluated at the weekly dose of 200 mg in a Phase II study enrolling 8 patients affected by advanced, pretreated BTCs [85]. None of the 8 patients had an objective response. Two patients achieved SD (25%) at 12 weeks, whereas 75% of patients experienced PD as best response. Median PFS was 1.7 months and median OS was 3.5 months. Common toxicities were lymphopenia (75%), skin rash (63%), fatigue (50%), fever (50%), vomiting (50%) and diarrhea (50%), mostly G1 and G2. Three patients experienced G3 skin rash that required dose reduction. The study was stopped prematurely due to loss of funding. However, preliminary findings did not demonstrate a meaningful clinical activity of MK2206 as a single agent in advanced refractory CCA [88].

#### Resistance to single-agent mTOR pathway inhibitors

Knowledge of pharmacologic resistance mechanisms may be useful to potentiate treatment with PI3K/AKT/mTOR pathway targeted agents and prevent therapeutic failure in BTC patients. mTOR activity is strictly regulated by negative feedback mechanisms, mainly mediated by p70S6K and growth factor receptor bound protein 10, that impair further RTK-dependent PI3K activation. Disruption of mTOR signaling affects these physiologic feedbacks with subsequent PI3K-AKT rebound activity. Moreover, rapalogs efficiently inhibit mTORC1 but not mTORC2, that in turn is able to continuously promote AKT phosphorylation even upon mTORC1 blockade [9,79]. Increased feedback AKT phosphorylation has been confirmed in various BTC cell lines after treatment with everolimus [26].

A first strategy to counteract rapalogs-induced AKT phosphorylation is the use of double PI3K-mTOR inhibitors or dual AKT-mTOR blockade. Based on the evidence that AKT activity is quickly restored after mTOR blockade, Ewald *et al* investigated combined targeting of MK-2206 with everolimus or the ATP competitive mTOR kinase inhibitor AZD8055, observing strong synergistic effects on BTC cell survival and proliferation [26,89]. These results were confirmed by Yeung and colleagues [20]. As expected, MK2206 significantly attenuated both basal and everolimus-induced AKT phosphorylation as well as downstream p70S6K and 4E-BP1 phosphorylation [20]. Overall, these compounds

showed antiproliferative rather than pro-apoptotic activity [20,89].

NVP-BEZ235, a dual PI3K/mTOR targeted agent, showed a strong inhibitory effect at nanomolar concentration on iCCA primary cultures and in a xenograft mouse model with a significantly superior anti-proliferative activity in comparison to single agent AKT inhibitor MK2206 [47]. Also, the dual PI3K/mTOR inhibitors LY3023414 and PF-04691502 showed significant antiproliferative effects in patient-derived BTC cell lines, including gemcitabine-resistant ones [22,90].

A second strategy encompasses combined targeting of both mTORC1 and mTORC2 with ATP competitive catalytic mTOR inhibitors, in order to potentiate pathway inhibition and prevent development of mTORC2-mediated AKT phosphorylation. Dual mTORC1/2 inhibitors WYE-354 and KU-0063794 exerted anti-proliferative (rather than pro-apoptotic) effects on BTC cells *in vitro*, efficaciously decreasing phosphorylation of mTOR, 4EBP-1 and P70S6K [20,68]. Moreover, WYE-354 induced anti-angiogenic effects in a GBC mouse xenograft [68]. Another dual mTORC1/2 inhibitor, OSI-027, showed a synergistic anti-proliferative effect with 5-Fluorouracil on GBC cell lines [91].

Lastly, mTOR blockade enhances compensatory activation of complementary pathways such as the RAS-MEK-ERK cascade and growth factor receptor signaling. The presence of *KRAS* mutations or amplification confers primary resistance to everolimus in BTC cell lines [20]; inhibition of mTOR and parallel RAS-MEK-ERK and EGFR cascades results in synergistic BTC cell growth inhibition [37,38,81,89].

Clinical experiences of combined pathway inhibition are limited.

The combination of p70S6K inhibitor LY2584702 tosylate and everolimus exhibited unacceptable toxicity (coagulation disorders, fatigue and weight loss) without evidence of objective responses in a Phase I study of patients with advanced refractory solid tumors [92].

The dual p70S6K and AKT inhibitor LY2780301 proved to be tolerable in a Phase I study of patients with advanced solid tumors [93], and was subsequently tested in combination with gemcitabine in a Phase Ib dose escalation study of 50 patients with advanced refractory solid tumors harboring *PI3K* mutations or loss of PTEN. Four enrolled patients had BTCs [94]. The MTD of LY2780301 (combined with gemcitabine) was 500 mg daily. Again, treatment was poorly tolerable, with 90% of patients experiencing G3/4 AEs (mostly hematological and hepatic toxicities). Five patients (10%) exhibited  $\geq$  G3 AEs related to study drug (abdominal pain, urinary tract disorders, thrombotic microangiopathy, hyperglycemia and increased transaminases). Of the 39 patients evaluable for response, 2 (5%) exhibited PR, 28 (72%) had SD and 9 (23%) showed PD. No specific information on BTC patients is available [94].

#### Other drugs affecting the PI3K-AKT-mTOR pathway

Other drugs interfere with the PI3K/AKT/mTOR pathway, both directly or indirectly.

Histone Deacetylase Inhibitors trichostatin-A and suberoylanilide hydroxamic acid induced apoptosis in GBC cells by suppressing AKT/mTOR signaling and decreasing phosphorylation of AKT, mTOR, p70S6K, 4E-BP1 [95].

HSP90 is an intracellular chaperone that facilitates the post-translational maturation of a variety of proteins, including EGFR, AKT, p-ERK. HSP90 inhibitors (17-AAG, NVP-AUY922) impaired AKT maturation and GBC cells viability *in vitro* and reduced tumor burden in GBC mouse models [96,97]. These effects were synergistic when HSP90 inhibitors were administered in combination with the dual PI3K/mTOR inhibitor NVP-BEZ235 [97].

Also, metformin exhibited a dose- and time-dependent anti-proliferative effect on CCA cells associated with decreased phosphorylation of mTOR, 4E-BP1, p70S6K and enhanced sensitivity to chemotherapeutic agents (5-fluorouracil, cisplatin) [98,99]. Moreover, an epidemiological analysis associated metformin use with a 60% reduction in iCCA risk in diabetic patients [100].

## Conclusions

The pathogenetic relevance of the PI3K/AKT/mTOR pathway in human BTCs has been extensively investigated in preclinical studies, providing a strong rationale for its targeting in this setting.

Functional deregulation of the pathway in response to growth factors, proangiogenic agents, inflammatory cytokines and other carcinogenic stimuli has been broadly characterized *in vitro*, and several genomic and proteomic alterations have been identified both in cell lines and in patient-derived tissue samples [12–66]. On these bases, remarkable efficacy of PI3K/AKT/mTOR inhibitors in BTCs has been shown both *in vitro* and *in vivo* [14,20,22,25,26,30,37–39,47,52,54,57,64–69,80–82,87,89–91].

Despite this strong body of preclinical evidence, including over 160 *in vitro* and 20 *in vivo* studies, available results of Phase I and II trials highlight only modest activity of PI3K/AKT/mTOR inhibitors in the clinical setting, with low rates of objective responses both in treatment naïve and in refractory BTC patients (0–17%) [70,71,74–78,83,86,88,94].

In our opinion, several factors may contribute to the poor clinical achievements obtained by PI3K/AKT/mTOR pathway inhibitors in BTCs so far.

First, demonstration of clinical benefit may be undermined in the absence of enrichment for molecularly selected patients. Preclinical data show reduced sensitivity to everolimus in *KRAS* mutated or amplified BTC cell lines even in the presence of concurrent *PI3K* activating mutations/PTEN loss, suggesting that *KRAS* status prevails over PI3K/AKT/mTOR signaling activation in predicting response to mTOR inhibitors. In the absence of *KRAS* mutations, elevated pAKT levels, *PI3K* mutations and PTEN inactivation have been associated with enhanced cell growth inhibition in BTC cells treated with everolimus [20]. To date, no predictive biomarkers of efficacy of mTOR inhibitors have been clinically validated; exploratory tissue biomarker analyses conducted in two recent Phase I clinical trials were inconclusive due to small sample sizes and lack of statistical power [77,86]. Since incorporation of predictive biomarkers may improve the effectiveness of drug development, future studies may consider the investigation of PI3K/AKT/mTOR inhibitors in patients selected for *KRAS* status, *PI3K* mutations, PTEN loss or elevated levels of phosphorylated PI3K, AKT and mTOR proteins. Multicentric collaborative approaches are essential, due to the difficulty of identifying adequate subsets of patients with specific molecular abnormalities and the small number of patients with suitable performance status to be included in clinical trials.

Development of early resistance to single-agent PI3K/AKT/mTOR pathway inhibitors is another topic of concern. Based on the identified mechanisms of resistance to single-agent PI3K/AKT/mTOR pathway inhibitors [20,26,89], attempts to improve therapeutic efficacy through combined pathway blockade or addition of chemotherapy have been carried out. However, important toxicity issues have emerged. Whereas single agent rapalogs display an acceptable toxicity profile [70,74], most PI3K and AKT inhibitors result severely toxic both alone and combined with chemotherapy, in the absence of relevant clinical benefit [83,88,92,94]. One exception is represented by copanlisib, a class I PI3K inhibitor with predominant PI3K $\alpha$ / $\delta$  activity, that has shown promising results in combination with CisGem in a Phase I clinical trial [86], displaying an ORR of 17%, with significant but manageable adverse events. Since this study was not designed to assess PFS and OS outcomes, results of a Phase II trial investigating the activity of copanlisib plus CisGem in patients with advanced CCA (NCT02631590) are awaited to determine the clinical benefit of this combination. Development of other isoform-selective PI3K/AKT inhibitors could improve toxicity profile, helping to enhance therapeutic outcomes.

Furthermore, given the extensive cross-talk involving the PI3K/AKT/mTOR pathway, single level-targeting may still result unsuccessful as has been for other targeted therapies in BTCs so far, like anti-EGFR and anti-VEGFR [4]. New combinations based on a strong preclinical rationale should be designed to optimize the effectiveness of treatment and reduce toxicity. For example, given the connection between this

pathway and IGF1R signaling and the possible anti-mTOR action of metformin, a combination with everolimus may be tested in future trials.

The main limitation of our work certainly relies in the modest activity documented in clinical trials with PI3K and mTOR inhibitors in BTCs, if compared to more promising strategies like FGFR2 and IDH inhibitors. Nevertheless, given the limited number of therapeutic options in BTCs, this class of agents may still deserve further investigation in this setting. Furthermore, as IDH and FGFR2 inhibitors may enter clinical practice in the near future [4], the PI3K/AKT/mTOR pathway may be studied as a possible resistance mechanism to these agents and targeted with combination or sequential treatment approaches.

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## Author contributions

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

## Declarations of interest

We wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest.

Dr. Sara Pusceddu received honoraria from Novartis, Ipsen, Italfarmaco, Pfizer, and Advanced Accelerator Applications outside the submitted work.

Professor Filippo de Braud received honoraria from Novartis, Roche, Merck Serono, Bristol Myers Squibb, GlaxoSmithKline, BMS, Celgene, Servier, Ignyta, Pfizer, MSD, Philogen, Astra Zeneca, Boehringer Ingelheim, Sanofi Aventis, GSK, Giscad, Italfarmaco, AboutPharma, Eli Lilly, Amgen, Nadirex, Genentech outside the submitted work.

The remaining authors disclose no conflicts.

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## Appendix A. Supplementary material

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## References

- [1] Valle JW, Borbath I, Khan SA, Huguet F, Gruenberger T, Arnold D, et al. Biliary cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2016;27:v28–37.
- [2] Rizvi S, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. *Gastroenterology* 2013;145:1215–29.
- [3] Razumilava N, Gores GJ. Classification, diagnosis, and management of cholangiocarcinoma. *Clin Gastroenterol Hepatol* 2013;11:13–21. e1; quiz e3–4.
- [4] Valle JW, Lamarca A, Goyal L, Barriuso J, Zhu AX. New horizons for precision medicine in biliary tract cancers. *Cancer Discov* 2017;7:943–62.
- [5] Valle J, Wasan H, Palmer DH, Cunningham D, Anthony A, Maraveyas A, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010;362:1273–81.
- [6] Lamarca A, Hubner RA, David Ryder W, Valle JW. Second-line chemotherapy in advanced biliary cancer: a systematic review. *Ann Oncol* 2014;25:2328–38.
- [7] Lombardi P, Marino D, Fenocchio E, Chila G, Aglietta M, Leone F. Emerging molecular target antagonists for the treatment of biliary tract cancer. *Expert Opin Emerg Drugs* 2018;23:63–75.
- [8] Lowery MAAAG, Burris HA, Janku F, Shroff RT, Cleary JM, Azad NS, et al. Phase I study of AG-120, an IDH1 mutant enzyme inhibitor: results from the cholangiocarcinoma dose escalation and expansion cohorts. *J Clin Oncol* 2017;35;15\_suppl:4015.
- [9] Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012;149:274–93.

- [10] Ocana A, Vera-Badillo F, Al-Mubarak M, Templeton AJ, Corrales-Sanchez V, Diez-Gonzalez L, et al. Activation of the PI3K/mTOR/AKT pathway and survival in solid tumors: systematic review and meta-analysis. *PLoS ONE* 2014;9:e95219.
- [11] Ersahin T, Tuncbag N, Cetin-Atalay R. The PI3K/AKT/mTOR interactive pathway. *Mol Biosyst* 2015;11:1946–54.
- [12] Roa I, Garcia H, Game A, de Toro G, de Aretxabala X, Javle M. Somatic mutations of PI3K in early and advanced gallbladder cancer: additional options for an orphan cancer. *J Mol Diagn* 2016;18:388–94.
- [13] Riener MO, Bawohl M, Clavien PA, Jochum W. Rare PIK3CA hotspot mutations in carcinomas of the biliary tract. *Genes Chromosomes Cancer* 2008;47:363–7.
- [14] Zhao S, Cao Y, Liu SB, Wang XA, Bao RF, Shu YJ, et al. The E545K mutation of PIK3CA promotes gallbladder carcinoma progression through enhanced binding to EGFR. *J Exp Clin Cancer Res* 2016;35:97.
- [15] Javle M, Rashid A, Churi C, Kar S, Zuo M, Eterovic AK, et al. Molecular characterization of gallbladder cancer using somatic mutation profiling. *Hum Pathol* 2014;45:701–8.
- [16] Deshpande V, Nuaguba A, Zimmerman SM, Kehoe SM, Macconail LE, Lauwers GY, et al. Mutational profiling reveals PIK3CA mutations in gallbladder carcinoma. *BMC Cancer* 2011;11:60.
- [17] Papadopoulou K, Murray S, Manousou K, Tikas I, Dervenis C, Sgourous J, et al. Genotyping and mRNA profiling reveal actionable molecular targets in biliary tract cancers. *Am J Cancer Res* 2018;8:2–15.
- [18] Nakamura H, Arai Y, Totoki Y, Hirota T, Elzawahry A, Kato M, et al. Genomic spectra of biliary tract cancer. *Nat Genet* 2015;47:1003–10.
- [19] Pignocchino Y, Sarotto I, Peraldo-Neia C, Penachioni JY, Cavalloni G, Migliardi G, et al. Targeting EGFR/HER2 pathways enhances the antiproliferative effect of gemcitabine in biliary tract and gallbladder carcinomas. *BMC Cancer* 2010;10:631.
- [20] Yeung Y, Lau DK, Chionh F, Tran H, Tse JWT, Weickhardt AJ, et al. K-Ras mutation and amplification status is predictive of resistance and high basal p-AKT is predictive of sensitivity to everolimus in biliary tract cancer cell lines. *Mol Oncol* 2017;11:1130–42.
- [21] Moon A, Chin S, Kim HK, Kwak JJ, Koh ES, Kim YW, et al. EGFR, COX2, p-AKT expression and PIK3CA mutation in distal extrahepatic bile duct carcinoma. *Pathology* 2016;48:35–40.
- [22] Feng F, Cheng Q, Yang L, Zhang D, Ji S, Zhang Q, et al. Guidance to rational use of pharmaceuticals in gallbladder sarcomatoid carcinoma using patient-derived cancer cells and whole exome sequencing. *Oncotarget* 2017;8:5349–60.
- [23] Riener MO, Bawohl M, Clavien PA, Jochum W. Analysis of oncogenic AKT1 p.E17K mutation in carcinomas of the biliary tract and liver. *Br J Cancer* 2008;99:836.
- [24] Li Q, Yang Z. Expression of phospho-ERK1/2 and PI3-K in benign and malignant gallbladder lesions and its clinical and pathological correlations. *J Exp Clin Cancer Res* 2009;28:65.
- [25] Kobayashi S, Werneburg NW, Bronk SF, Kaufmann SH, Gores GJ. Interleukin-6 contributes to Mcl-1 up-regulation and TRAIL resistance via an Akt-signaling pathway in cholangiocarcinoma cells. *Gastroenterology* 2005;128:2054–65.
- [26] Ewald F, Grabinski N, Grottko A, Windhorst S, Norz D, Carstensen L, et al. Combined targeting of AKT and mTOR using MK-2206 and RAD001 is synergistic in the treatment of cholangiocarcinoma. *Int J Cancer* 2013;133:2065–76.
- [27] Javle MM, Yu J, Khoury T, Chadha KS, Iyer RV, Foster J, et al. Akt expression may predict favorable prognosis in cholangiocarcinoma. *J Gastroenterol Hepatol* 2006;21:1744–51.
- [28] Schmitz KJ, Lang H, Wohlschlaeger J, Sotiropoulos GC, Reis H, Schmid KW, et al. AKT and ERK1/2 signaling in intrahepatic cholangiocarcinoma. *World J Gastroenterol* 2007;13:6470–7.
- [29] Hori H, Ajiki T, Mita Y, Horiuchi H, Hirata K, Matsumoto T, et al. Frequent activation of mitogen-activated protein kinase relative to Akt in extrahepatic biliary tract cancer. *J Gastroenterol* 2007;42:567–72.
- [30] Wu Q, Kiguchi K, Kawamoto T, Ajiki T, Traag J, Carbajal S, et al. Therapeutic effect of rapamycin on gallbladder cancer in a transgenic mouse model. *Cancer Res* 2007;67:3794–800.
- [31] Lee D, Do IG, Choi K, Sung CO, Jang KT, Choi D, et al. The expression of phospho-AKT1 and phospho-mTOR is associated with a favorable prognosis independent of PTEN expression in intrahepatic cholangiocarcinomas. *Mod Pathol* 2012;25:131–9.
- [32] McKay SC, Unger K, Pericleous S, Stamp G, Thomas G, Hutchins RR, et al. Array comparative genomic hybridization identifies novel potential therapeutic targets in cholangiocarcinoma. *HPB (Oxford)* 2011;13:309–19.
- [33] Chung JY, Hong SM, Choi BY, Cho H, Yu E, Hewitt SM. The expression of phospho-AKT, phospho-mTOR, and PTEN in extrahepatic cholangiocarcinoma. *Clin Cancer Res* 2009;15:660–7.
- [34] Herberger B, Puhalla H, Lehnert M, Wrba F, Novak S, Brandstetter A, et al. Activated mammalian target of rapamycin is an adverse prognostic factor in patients with biliary tract adenocarcinoma. *Clin Cancer Res* 2007;13:4795–9.
- [35] Leal P, Garcia P, Sandoval A, Letelier P, Brebi P, Ili C, et al. Immunohistochemical expression of phospho-mTOR is associated with poor prognosis in patients with gallbladder adenocarcinoma. *Arch Pathol Lab Med* 2013;137:552–7.
- [36] Wang Z, Zheng T, Wu Q, Wang J, Wu C, Wang J. Immunohistochemical analysis of the mTOR pathway in intrahepatic cholangiocarcinoma. *Neoplasma* 2012;59:137–41.
- [37] Herberger B, Berger W, Puhalla H, Schmid K, Novak S, Brandstetter A, et al. Simultaneous blockade of the epidermal growth factor receptor/mammalian target of rapamycin pathway by epidermal growth factor receptor inhibitors and rapamycin results in reduced cell growth and survival in biliary tract cancer cells. *Mol Cancer Ther* 2009;8:1547–56.
- [38] Mohri D, Iijchi H, Miyabayashi K, Takahashi R, Kudo Y, Sasaki T, et al. A potent therapeutics for gallbladder cancer by combinatorial inhibition of the MAPK and mTOR signaling networks. *J Gastroenterol* 2016;51:711–21.
- [39] Leal P, Garcia P, Sandoval A, Buchegger K, Weber H, Tapia O, et al. AKT/mTOR substrate P70S6K is frequently phosphorylated in gallbladder cancer tissue and cell lines. *Onco Targets Ther* 2013;6:1373–84.
- [40] Xu X, Kobayashi S, Qiao W, Li C, Xiao C, Radaeva S, et al. Induction of intrahepatic cholangiocellular carcinoma by liver-specific disruption of Smad4 and Pten in mice. *J Clin Invest* 2006;116:1843–52.
- [41] Roa I, de Toro G, Fernandez F, Game A, Munoz S, de Aretxabala X, et al. Inactivation of tumor suppressor gene pten in early and advanced gallbladder cancer. *Diagn Pathol* 2015;10:148.
- [42] Liu DC, Yang ZL. Overexpression of EZH2 and loss of expression of PTEN is associated with invasion, metastasis, and poor progression of gallbladder adenocarcinoma. *Pathol Res Pract* 2011;207:472–8.
- [43] Tekcham DS, Gupta S, Shrivastav BR, Tiwari PK. Epigenetic downregulation of PTEN in gallbladder cancer. *J Gastrointest Cancer* 2017;48:110–6.
- [44] Miyahara N, Shoda J, Ishige K, Kawamoto T, Ueda T, Taki R, et al. MUC4 interacts with ErbB2 in human gallbladder carcinoma: potential pathobiological implications. *Eur J Cancer* 2008;44:1048–56.
- [45] Kawamoto T, Ishige K, Thomas M, Yamashita-Kashima Y, Shu S, Ishikura N, et al. Overexpression and gene amplification of EGFR, HER2, and HER3 in biliary tract carcinomas, and the possibility for therapy with the HER2-targeting antibody pertuzumab. *J Gastroenterol* 2015;50:467–79.
- [46] Treekitkarnmongkol W, Suthiphongchai T. High expression of ErbB2 contributes to cholangiocarcinoma cell invasion and proliferation through Akt/p70S6K. *World J Gastroenterol* 2010;16:4047–54.
- [47] Fraveto A, Cardinale V, Bragazzi MC, Giuliante F, De Rose AM, Grazi GL, et al. Sensitivity of human intrahepatic cholangiocarcinoma subtypes to chemotherapeutics and molecular targeted agents: a study on primary cell cultures. *PLoS ONE* 2015;10:e0142124.
- [48] Yoon H, Min JK, Lee DG, Kim DG, Koh SS, Hong HJ. L1 cell adhesion molecule and epidermal growth factor receptor activation confer cisplatin resistance in intrahepatic cholangiocarcinoma cells. *Cancer Lett* 2012;316:70–6.
- [49] Ohashi H, Adachi Y, Yamamoto H, Taniguchi H, Noshio K, Suzuki H, et al. Insulin-like growth factor receptor expression is associated with aggressive phenotypes and has therapeutic activity in biliary tract cancers. *Cancer Sci* 2012;103:252–61.
- [50] Wolf S, Lorenz J, Mossner J, Wiedmann M. Treatment of biliary tract cancer with NVP-AEW541: mechanisms of action and resistance. *World J Gastroenterol* 2010;16:156–66.
- [51] Peng H, Zhang Q, Li J, Zhang N, Hua Y, Xu L, et al. Apatinib inhibits VEGF signaling and promotes apoptosis in intrahepatic cholangiocarcinoma. *Oncotarget* 2016;7:17220–9.
- [52] Frampton G, Invernizzi P, Bernuzzi F, Pae HY, Quinn M, Horvat D, et al. Interleukin-6-driven progranulin expression increases cholangiocarcinoma growth by an Akt-dependent mechanism. *Gut* 2012;61:268–77.
- [53] Morton SD, Cadamuro M, Brivio S, Vismara M, Stecca T, Massani M, et al. Leukemia inhibitory factor protects cholangiocarcinoma cells from drug-induced apoptosis via a PI3K/AKT-dependent Mcl-1 activation. *Oncotarget* 2015;6:26052–64.
- [54] Zhong W, Tong Y, Li Y, Yuan J, Hu S, Hu T, et al. Mesenchymal stem cells in inflammatory microenvironment potentially promote metastatic growth of cholangiocarcinoma via activating Akt/NF-kappaB signaling by paracrine CCL5. *Oncotarget* 2017;8:73693–704.
- [55] Zhu G, Du Q, Wang X, Tang N, She F, Chen Y. TNF-alpha promotes gallbladder cancer cell growth and invasion through autocrine mechanisms. *Int J Mol Med* 2014;33:1431–40.
- [56] Gentilini A, Rombouts K, Galastri S, Caligiuri A, Mingarelli E, Mello T, et al. Role of the stromal-derived factor-1 (SDF-1)-CXCR4 axis in the interaction between hepatic stellate cells and cholangiocarcinoma. *J Hepatol* 2012;57:813–20.
- [57] Leelawat K, Leelawat S, Narong S, Hongeng S. Roles of the MEK1/2 and AKT pathways in CXCL12/CXCR4 induced cholangiocarcinoma cell invasion. *World J Gastroenterol* 2007;13:1561–8.
- [58] Yamada D, Rizvi S, Razumilava N, Bronk SF, Davila JI, Champion MD, et al. IL-33 facilitates oncogene-induced cholangiocarcinoma in mice by an interleukin-6-sensitive mechanism. *Hepatology* 2015;61:1627–42.
- [59] Wu T, Leng J, Han C, Demetris AJ. The cyclooxygenase-2 inhibitor celecoxib blocks phosphorylation of Akt and induces apoptosis in human cholangiocarcinoma cells. *Mol Cancer Ther* 2004;3:299–307.
- [60] Daoueang D, Thuwajit P, Roittrakul S, Laha T, Kaewkes S, Endo Y, et al. Secreted Opisthorchis viverrini glutathione S-transferase regulates cell proliferation through AKT and ERK pathways in cholangiocarcinoma. *Parasitol Int* 2012;61:155–61.
- [61] Wang C, Lei H, Tian Y, Shang M, Wu Y, Li Y, et al. Clonorchis sinensis granulins: identification, immunolocalization, and function in promoting the metastasis of cholangiocarcinoma and hepatocellular carcinoma. *Parasit Vectors* 2017;10:262.
- [62] Liu R, Zhao R, Zhou X, Liang X, Campbell DJ, Zhang X, et al. Conjugated bile acids promote cholangiocarcinoma cell invasive growth through activation of sphingosine 1-phosphate receptor 2. *Hepatology* 2014;60:908–18.
- [63] Utispan K, Sonongbua J, Thuwajit P, Chau-In S, Pairojkul C, Wongkham S, et al. Periostin activates integrin alpha5beta1 through a PI3K/AKTdependent pathway in invasion of cholangiocarcinoma. *Int J Oncol* 2012;41:1110–8.
- [64] Ding YB, Deng B, Huang YS, Xiao WM, Wu J, Zhang YQ, et al. A high level of integrin alpha6 expression in human intrahepatic cholangiocarcinoma cells is associated with a migratory and invasive phenotype. *Dig Dis Sci* 2013;58:1627–35.
- [65] Xiong GP, Zhang JX, Gu SP, Wu YB, Liu JF. Overexpression of ECM1 contributes to migration and invasion in cholangiocarcinoma cell. *Neoplasma* 2012;59:409–15.
- [66] Zhang Y, Liu S, Wang L, Wu Y, Hao J, Wang Z, et al. A novel PI3K/AKT signaling axis mediates Nectin-4-induced gallbladder cancer cell proliferation, metastasis and tumor growth. *Cancer Lett* 2016;375:179–89.
- [67] Heuer M, Dreger NM, Cicinnati VR, Fingas C, Juntermanns B, Paul A, et al. Tumor

- growth effects of rapamycin on human biliary tract cancer cells. *Eur J Med Res* 2012;17:20.
- [68] Weber H, Leal P, Stein S, Kunkel H, Garcia P, Bizama C, et al. Rapamycin and WYE-354 suppress human gallbladder cancer xenografts in mice. *Oncotarget* 2015;6:31877–88.
- [69] Zong H, Yin B, Zhou H, Cai D, Ma B, Xiang Y. Inhibition of mTOR pathway attenuates migration and invasion of gallbladder cancer via EMT inhibition. *Mol Biol Rep* 2014;41:4507–12.
- [70] 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.
- [71] Jung KS, Lee J, Park SH, Park JO, Park YS, Lim HY, et al. Pilot study of sirolimus in patients with PIK3CA mutant/amplified refractory solid cancer. *Mol Clin Oncol* 2017;7:27–31.
- [72] Heits N, Heinze T, Bernsmeier A, Kerber J, Hauser C, Becker T, et al. Influence of mTOR-inhibitors and mycophenolic acid on human cholangiocellular carcinoma and cancer associated fibroblasts. *BMC Cancer* 2016;16:322.
- [73] Moolthiya P, Tohtong R, Keerachamroen S, Leelawat K. Role of mTOR inhibitor in cholangiocarcinoma cell progression. *Oncol Lett* 2014;7:854–60.
- [74] Verzoni E, Pusceddu S, Buzzoni R, Garanzini E, Damato A, Biondani P, et al. Safety profile and treatment response of everolimus in different solid tumors: an observational study. *Future Oncol* 2014;10:1611–7.
- [75] Costello BA, Borad MJ, Qi Y, Kim GP, Northfelt DW, Erlichman C, et al. Phase I trial of everolimus, gemcitabine and cisplatin in patients with solid tumors. *Invest New Drugs* 2014;32:710–6.
- [76] Buzzoni R, Pusceddu S, Bajetta E, De Braud F, Platania M, Iannacone C, et al. Activity and safety of RAD001 (everolimus) in patients affected by biliary tract cancer progressing after prior chemotherapy: a phase II ITMO study. *Ann Oncol: official J Eur Soc Med Oncol* 2014;25:1597–603.
- [77] Lau DK, Tay RY, Yeung YH, Chionh F, Mooi J, Murone C, et al. Phase II study of everolimus (RAD001) monotherapy as first-line treatment in advanced biliary tract cancer with biomarker exploration: the RADiChol Study. *Br J Cancer* 2018;118:966–71.
- [78] Kim ST, Lee J, Park SH, Park JO, Park YS, Kang WK, et al. Prospective phase II trial of everolimus in PIK3CA amplification/mutation and/or PTEN loss patients with advanced solid tumors refractory to standard therapy. *BMC Cancer* 2017;17:211.
- [79] LoRusso PM. Inhibition of the PI3K/AKT/mTOR pathway in solid tumors. *J Clin Oncol* 2016;34:3803–15.
- [80] Yoon H, Min JK, Lee JW, Kim DG, Hong HJ. Acquisition of chemoresistance in intrahepatic cholangiocarcinoma cells by activation of AKT and extracellular signal-regulated kinase (ERK)1/2. *Biochem Biophys Res Commun* 2011;405:333–7.
- [81] Jin L, Jin MH, Nam AR, Park JE, Bang JH, Oh DY, et al. Anti-tumor effects of NVP-BKM120 alone or in combination with MEK162 in biliary tract cancer. *Cancer Lett* 2017;411:162–70.
- [82] Guest RV, Boulter L, Dwyer BJ, Kendall TJ, Man TY, Minnis-Lyons SE, et al. Notch3 drives development and progression of cholangiocarcinoma. *Proc Natl Acad Sci USA* 2016;113:12250–5.
- [83] McRee AJ, Sanoff HK, Carlson C, Ivanova A, O'Neil BH. A phase I trial of mFOLFOX6 combined with the oral PI3K inhibitor BKM120 in patients with advanced refractory solid tumors. *Invest New Drugs* 2015;33:1225–31.
- [84] Bendell JC, Rodon J, Burris HA, de Jonge M, Verweij J, Birlle 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;30:282–90.
- [85] Hyman DM, Snyder AE, Carvajal RD, Gerecitano JF, Voss MH, Ho AL, et al. Parallel phase Ib studies of two schedules of buparlisib (BKM120) plus carboplatin and paclitaxel (q21 days or q28 days) for patients with advanced solid tumors. *Cancer Chemother Pharmacol* 2015;75:747–55.
- [86] Kim RD, Alberts SR, Pena C, Genvresse I, Ajavon-Hartmann A, Xia C, et al. Phase I dose-escalation study of copanlisib in combination with gemcitabine or cisplatin plus gemcitabine in patients with advanced cancer. *Br J Cancer* 2018;118:462–70.
- [87] Wilson JM, Kunnimalaiyaan S, Kunnimalaiyaan M, Gamblin TC. Inhibition of the AKT pathway in cholangiocarcinoma by MK2206 reduces cellular viability via induction of apoptosis. *Cancer Cell Int* 2015;15:13.
- [88] Ahn DH, Li J, Wei L, Doyle A, Marshall JL, Schaaf LJ, et al. Results of an abbreviated phase-II study with the Akt Inhibitor MK-2206 in patients with advanced biliary cancer. *Sci Rep* 2015;5:12122.
- [89] Ewald F, Norz D, Grottko A, Hofmann BT, Nashan B, Jucker M. Dual Inhibition of PI3K-AKT-mTOR- and RAF-MEK-ERK-signaling is synergistic in cholangiocarcinoma and reverses acquired resistance to MEK-inhibitors. *Invest New Drugs* 2014;32:1144–54.
- [90] Sakamoto Y, Yamagishi S, Tanizawa Y, Tajimi M, Okusaka T, Ojima H. PI3K-mTOR pathway identified as a potential therapeutic target in biliary tract cancer using a newly established patient-derived cell panel assay. *Jpn J Clin Oncol* 2018;48:396–9.
- [91] Li Q, Mou LJ, Tao L, Chen W, Sun XT, Xia XF, et al. Inhibition of mTOR suppresses human gallbladder carcinoma cell proliferation and enhances the cytotoxicity of 5-fluorouracil by downregulating MDR1 expression. *Eur Rev Med Pharmacol Sci* 2016;20:1699–706.
- [92] Hollebecque A, Houede N, Cohen EE, Massard C, Italiano A, Westwood P, et al. A phase Ib trial of LY2584702 tosylate, a p70 S6 inhibitor, in combination with erlotinib or everolimus in patients with solid tumours. *Eur J Cancer* 2014;50:876–84.
- [93] Azaro A, Rodon J, Calles A, Brana I, Hidalgo M, Lopez-Casas PP, et al. A first-in-human phase I trial of LY2780301, a dual p70 S6 kinase and Akt Inhibitor, in patients with advanced or metastatic cancer. *Invest New Drugs* 2015;33:710–9.
- [94] Angevin E, Cassier PA, Italiano A, Goncalves A, Gazzah A, Terret C, et al. Safety, tolerability and antitumour activity of LY2780301 (p70S6K/AKT inhibitor) in combination with gemcitabine in molecularly selected patients with advanced or metastatic cancer: a phase IB dose escalation study. *Eur J Cancer* 2017;83:194–202.
- [95] Zhang P, Guo Z, Wu Y, Hu R, Du J, He X, et al. Histone deacetylase inhibitors inhibit the proliferation of gallbladder carcinoma cells by suppressing AKT/mTOR signaling. *PLoS ONE* 2015;10:e0136193.
- [96] Weber H, Valbuena JR, Barbhuiya MA, Stein S, Kunkel H, Garcia P, et al. Small molecule inhibitor screening identified HSP90 inhibitor 17-AAG as potential therapeutic agent for gallbladder cancer. *Oncotarget* 2017;8:26169–84.
- [97] Chen MH, Chiang KC, Cheng CT, Huang SC, Chen YY, Chen TW, et al. Antitumor activity of the combination of an HSP90 inhibitor and a PI3K/mTOR dual inhibitor against cholangiocarcinoma. *Oncotarget* 2014;5:2372–89.
- [98] Ling S, Feng T, Ke Q, Fan N, Li L, Li Z, et al. Metformin inhibits proliferation and enhances chemosensitivity of intrahepatic cholangiocarcinoma cell lines. *Oncol Rep* 2014;31:2611–8.
- [99] Bi T, Zhu A, Yang X, Qiao H, Tang J, Liu Y, et al. Metformin synergistically enhances antitumor activity of cisplatin in gallbladder cancer via the PI3K/AKT/ERK pathway. *Cytotechnology* 2018;70:439–48.
- [100] Chaiterakij R, Yang JD, Harmsen WS, Slettedahl SW, Mettler TA, Fredericksen ZS, et al. Risk factors for intrahepatic cholangiocarcinoma: association between metformin use and reduced cancer risk. *Hepatology* 2013;57:648–55.