

Targeted therapy in biliary tract cancer: 2009 update

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Biliary tract cancers (BTCs) include cholangiocarcinoma (intrahepatic, perihilar and extrahepatic), carcinoma of the gall bladder and ampullary carcinoma. In patients with advanced disease the prognosis is poor. There is not a consensus regarding treatment strategy. Chemotherapy has only limited efficacy. This review summarizes the new approaches for BTC patients and the rationale for targeted therapies. The prognostic factors and the molecular features of BTC are analyzed. The clinical trials evaluating the targeted agents are accurately described, especially those assessing the role of anti-EGFR and antiangiogenic drugs. The ongoing trials are also analyzed. In fact, only the results of these trials will establish which is the most effective agent or combination for this setting.

Biliary tract cancers (BTCs) are invasive carcinomas arising from the epithelial lining of the gall bladder and bile ducts. The term BTC includes both cholangiocarcinoma, which has been used to refer to cancers with origins in the intrahepatic, perihilar or distal biliary tree, carcinoma arising from the gall bladder and ampullary carcinoma. The vast majority are adenocarcinomas. Bile duct cancer, gall bladder cancer and ampullary cancer are today classified as BTCs. BTCs affect approximately 12,000 people in the USA annually [1]. Whereas the incidence of extrahepatic cholangiocarcinoma is constant and the mortality is decreasing in the USA, the mortality rates of intrahepatic cholangiocarcinoma have been increasing [2]. This group of tumors is characterized by regional lymph node metastasis, vascular invasion and distant metastases. Complete surgical resection is the only hope for a possible cure. However, only 10% of patients present with early-stage disease and are considered surgical candidates, so for the majority of BTC patients, systemic chemotherapy is the main option of their treatment plan. Chemotherapy in patients with BTC is indicated in those with unresectable advanced cancer and patients with recurrence after resection. Patients with unresectable or metastatic BTC have a poor prognosis, with a median overall survival (OS) time of 1 year [3].

Conventional chemotherapy has only limited benefit in the treatment of unresectable or metastatic BTCs.

In advanced disease, the role of radiotherapy is investigational. It is reported that radiotherapy has a better effect in improving survival time than palliative therapy. In addition, another advantage of radiotherapy is that if a stent

is indicated, the patency of the stent may be maintained and pain may be reduced by local control [4].

However, emerging target-based cancer therapies may improve therapeutic efficacy against BTC, which is typically refractory to conventional chemotherapy [5]. Among the BTCs, there are differences with respect to disease course in terms of responsiveness to chemotherapy and molecular profiles based on the site of disease (intrahepatic, distal biliary tree, gall bladder or ampulla). Thus, while these entities have traditionally been included together in clinical trials, it would be useful to separate these cancer sites in future studies [6].

Molecular characteristics & rationale for targeted therapies in BTCs

EGF receptor (EGFR), VEGF and human EGF receptor 2 (HER2) have been considered as potential therapeutic targets in cholangiocarcinoma. A recent study evaluated the EGFR, VEGF and HER2 overexpression in cholangiocarcinoma. EGFR, VEGF and HER2 overexpression were 27.4, 53.8 and 0.9% in intrahepatic cholangiocarcinoma and 19.2, 59.2 and 8.5% in extrahepatic cholangiocarcinoma, respectively. Clinicopathologically, EGFR overexpression was associated with macroscopic type ($p = 0.0120$), lymph node metastasis ($p = 0.0006$), tumor stage ($p = 0.0424$), lymphatic vessel invasion ($p = 0.0371$) and perineural invasion ($p = 0.0459$) in extrahepatic cholangiocarcinoma, and VEGF overexpression with intrahepatic metastasis ($p = 0.0224$) in intrahepatic cholangiocarcinoma. Multivariate analysis showed that EGFR expression was a significant prognostic factor ($p = 0.0006$) and also a risk factor for tumor

Keywords

- antiangiogenic therapy
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- targeted therapy

recurrence ($p = 0.0335$) in intrahepatic cholangiocarcinoma. This is the first report about the roles of EGFR/VEGF/HER2 expression in the large cohort of BTCs, and can explain the development of preclinical and clinical studies evaluating targeted agents in BTC [7].

Rationale for targeting EGFR

The main role of the EGFR in the proliferation of cancer and its overexpression in several solid tumors have provided the rationale for targeting this pathway.

The ErbB family of class I receptor tyrosine kinases consists of four distinct receptors: EGFR (ErbB1), ErbB2, ErbB3 and ErbB4. These receptors are composed of an extracellular ligand-binding domain, a transmembrane lipophilic domain and a conserved cytoplasmic tyrosine kinase domain. All these receptors, with the exception of ErbB2, bind receptor-specific ligands belonging to the EGF family of growth factors [8–11].

EGFR blockade with monoclonal antibodies and tyrosine kinase inhibitors (TKIs) is indicated in gastrointestinal tumors, including primary liver cancer [12,13].

Several reports indicate that the EGFR is frequently overexpressed in cholangiocarcinoma. Additionally, sustained EGFR activation due to defective receptor internalization has been reported for cholangiocarcinoma cells [14]. Bile acids activate EGFR signaling via a TGF- α -dependent mechanism, thus contributing to the growth characteristics of cholangiocytes and cholangiocarcinoma cells [15]. EGFR overexpression was shown to be associated with macroscopic tumor type, lymph node metastasis, tumor stage, lymphatic vessel invasion and perineural invasion in extrahepatic cholangiocarcinoma. High levels of EGFR expression and activation have been shown to increase the risk for tumor recurrence in intrahepatic cholangiocarcinoma. A recent study evaluated the overexpression of ErbB-2 in BTC, which was found in 15.7, 11.5 and 5.1% of carcinomas of the gall bladder, ampulla of Vater and extrahepatic bile duct, respectively. Overexpression of EGFR was found in 8.1% of tumors with no predominant location, and was also associated with gene amplification with high frequency (77%) [16]. Moreover, a study has shown the presence of somatic mutations of the *EGFR* gene in bile duct carcinoma. The subgroup of patients with cholangiocarcinoma or gall bladder carcinoma with somatic mutations of EGFR in the tyrosine kinase domain can elicit cell

signals sustaining survival and proliferation. These tumors might be further evaluated for their susceptibility to small-molecule inhibitor treatment [17].

The incidence of *K-ras* mutation in BTC was varied from past reports (approximately 10–60%) and from the sites of tumor [18–21]. It still remains unclear whether *K-ras* mutation is correlated with the response against EGFR inhibitors or not. The biomarker for the response to EGFR inhibitors in BTC, such as the presence of EGFR overexpression, *EGFR* gene mutation/amplification or the absence of *K-ras* mutation should be investigated in future clinical trials.

It has recently been demonstrated that TKIs targeting either EGFR or ErbB2, as well as those producing dual inhibition of EGFR and of ErbB2, are capable of effectively suppressing cellular growth and inducing significant apoptosis in human and rodent biliary cancer cell lines *in vitro*. Gefitinib, lapatinib, erlotinib and cetuximab are the molecules that show the major antitumoral activity in preclinical studies [22–24].

Particularly interesting is that the EGFR-TKI erlotinib was found to upregulate EGFR in HuCCT1 cholangiocarcinoma cells, but combined treatment with the anti-EGFR antibody cetuximab or blockade of erlotinib-induced EGFR synthesis by a small-interfering RNA abrogated this TKI effect, and resulted in an inhibition of cell proliferation and increased apoptosis in these cells [25]. Moreover, in this study cetuximab was shown to be similarly effective when administered alone or in combination with erlotinib in suppressing tumorigenic growth in HuCCT1-bearing mice, suggesting that antibody-induced downregulation of EGFR may provide an effective strategy to prevent resistance to specific EGFR-TKI inhibitors in cholangiocarcinoma and other EGFR-expressing cancer cell types [26]. Recently, preclinical evidence of synergistic antitumor activity has emerged combining EGFR inhibitors with rapamycin [27] or vandetanib, an inhibitor of VEGF receptor (VEGFR) and EGFR signaling. A recent study showed that vandetanib significantly inhibited the growth of cholangiocarcinoma cells expressing EGFR and VEGF, appearing as a promising therapeutic approach for cholangiocarcinoma. The absence of *KRAS* mutation and the presence of EGFR amplification may be a potential predictive molecular marker of sensitivity to EGFR-targeted therapy in cholangiocarcinoma [28,29].

Rationale for antiangiogenic treatment strategies

Angiogenesis is very important in tumor growth and progression [30]. Among the angiogenic factors/receptors, the VEGF and VEGF receptor (VEGFR) family, including the secreted glycoproteins VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E, the placental growth factors (PlGF-1,-2), and their related receptors VEGFR-1 (Flt-1) and VEGFR-2 (Flk/KDR), play major roles not only in physiological but also in pathological angiogenesis. VEGF-A, which binds both VEGFR-1 and -2, is a key regulator of the development of the vascular system [31].

Elevated levels of circulating VEGF-A are correlated with progression and metastasis of gastrointestinal cancers. Moreover, VEGF protein is overexpressed in cholangiocarcinomas and is associated with the VEGFR-1 and -2 expression in surrounding endothelial cells [32]. Therefore, the VEGF/VEGFR system is an interesting target for the treatment of these chemoresistant cancers.

Increased VEGF expression has been reported to be associated with a significant vascularization of human intrahepatic cholangiocarcinomas, as assessed by microvessel density. In comparison, hypovascularity of cholangiocarcinoma may be related to a downregulation of VEGF together with an upregulation of the angiogenesis inhibitor thrombospondin-1 [33,34].

Moreover, differences in protein expression exist between hilar and peripheral cholangiocarcinomas. In fact, hilar cholangiocarcinomas more often expressed MUC5AC (62 vs 22%, $p < 0.0001$), Akt2 (54 vs 27%, $p < 0.001$), CK8 (98 vs 81%, $p < 0.005$) and annexin II (92 vs 66%, $p < 0.001$). Interestingly, VEGF-A expression was more frequently encountered in peripheral cholangiocarcinoma (69 vs 25%, $p < 0.0001$) and correlated with increased vascular density. This could suggest a potential benefit for antiangiogenic therapies in peripheral cholangiocarcinomas [35].

Today, several studies with bevacizumab in metastatic cholangiocarcinoma are ongoing.

The use of an anti-PlGF monoclonal antibody in VEGF-inhibitor-resistant tumors is an interesting alternative antiangiogenic strategy. A study has been performed on mice with melanoma or pancreatic adenocarcinoma [36]. The antibody specifically inhibits the binding of PlGF to its receptor VEGFR-1, present on tumor-associated endothelial cells and macrophages. Several studies showed that endogenous

PlGF is necessary both to vessel development and maintenance, and to the 'angiogenic switch' in solid tumor growth. This led to the hypothesis that, unlike VEGF inhibitors, PlGF inhibition might reduce pathological angiogenesis, without interfering with physiological blood vessel homeostasis, reducing undesired side effects.

AZD2171 is a highly potent small molecule with pan-VEGFR-tyrosine kinase inhibiting activity. AZD2171 also inhibits VEGFR-3, PDGFR- β and c-Kit at nanomolar concentrations [37]. The antineoplastic potency of AZD2171 has been demonstrated in several tumors such as lung, hepatocellular, colorectal and prostate cancers, and in all cases the antitumor effect was associated with strong inhibition of VEGF signaling and angiogenesis [38–40].

Rationale for targeting IGF/IGF receptor pathway

Activation of the IGF-1 receptor (IGF-1R) by IGF-I and IGF-II plays a central role in tumor cell proliferation and spread, promoting cell-cycle progression, preventing apoptosis and regulating and maintaining the metastatic tumor phenotype [41–44]. Several hematological and nonhematological tumors show abnormal or overexpression of IGFs and IGF-1R, which leads to auto- and paracrine growth stimulation. This effect has been correlated with enhanced proliferation, tumor dedifferentiation, development of metastases and reduced survival. Abnormal expression of IGF-1R has also been demonstrated in BTC, so the IGF/IGFR system was shown to be centrally involved in proliferation and suppression of apoptosis of cholangiocarcinoma cells [45], making the IGF/IGFR- signaling system an attractive target for the treatment of BTCs.

Rationale for targeting Akt/mammalian target of rapamycin pathway

The activated phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway has an emerging role in BTC development [46]. PI3K links the intracellular domain of several growth factor receptors. Once the receptor has been activated, PI3K causes the activation of AKT, a serine/threonine kinase that starts multiple cellular target proteins, such as the mTOR subfamily. mTOR downregulates apoptosis, and via stimulation of cell-cycle progression enhances proliferation and cell growth [47]. In normal cells the PI3K/AKT/mTOR pathway is checked by the phosphatase and tensin homolog deleted on chromosome ten (PTEN), a tumor suppressor that inhibits this pathway by

reversing PI3K and subsequent AKT activation. Alterations or silencings of the *PTEN* gene may activate the mTOR pathway and promote tumor development and growth.

VQD-002 is a small-molecule inhibitor of AKT signaling. Similarly, AKT inhibition by VQD-002 promotes the suppression of cell growth and induces the apoptosis in human cancer cells and in tumor xenograft mouse models, with high selectivity for those tumors with aberrant Akt [48].

The natural antibiotic rapamycin is a powerful inhibitor of mTOR [49]. Recently, three analogs of rapamycin with superior pharmacokinetic and biological properties have emerged: temsirolimus (CCI-779), everolimus (RAD001) and AP23573. In preclinical studies, the antimigratory, antiproliferative and anti-invasive potency of rapamycin in cholangiocarcinoma cells has been recently illustrated [50]. For BTC patients, activated mTOR was also demonstrated to be a negative prognostic factor [51].

Rationale for targeting Ras/Raf/mitogen-activated protein kinase pathway

BTCs show a common spectrum of mutations in cancer-related genes, including frequent activation of *K-ras* (mutated in 45% of BTC) and loss of function of the *P16*, *P53* and *SMAD4* tumor suppressor genes [52,53]. Activating missense *B-raf* mutations were also identified in 22% of a series of cholangiocarcinomas. Significantly, these mutations were mutually exclusive of *K-ras* alterations, which occurred in 45% of tumors within this series, with a consequent activation of the Raf/mitogen-activated protein kinase (MAPK) signaling pathway within many of these tumors [54].

Sorafenib is an oral multikinase inhibitor, which targets kinases of wild-type B-Raf, mutant B-Raf and C-Raf, and receptor tyrosine kinases involved in angiogenesis, including VEGFR-2 and -3, and PDGFR [55].

Sorafenib alone or in combination with conventional cytostatics (doxorubicin, 5-fluorouracil and gemcitabine) or IGF-1R inhibition induces a potent growth suppression of cholangiocarcinoma cells *in vitro* [56]. Antitumor efficacy was even superior when sorafenib was combined with the histone deacetylase inhibitor MS-275 [57,58].

Rationale for targeting the proteasome

Another interesting target for cancer treatment is the inhibition of the 26S proteasome, a large protease present both in the nucleus and the

cytoplasm of eukaryotic cells. The proteasome is involved in programmed cellular death functions, refereed by the ubiquitin system. The so-called ubiquitin–proteasome pathway (UPP) is the major nonlysosomal proteolytic system in eukaryotic cells, and elicits degradation of proteins involved in cell-cycle progression, apoptosis and angiogenesis. UPP also degrades mutant, damaged and misfolded proteins [59]. As these pathways are critical for cell survival and proliferation, inhibition of the proteasome has emerged as an interesting target for cancer therapy.

Bortezomib is a proteasome inhibitor that blocks multiubiquitinated protein degradation by reversibly and competitively inhibiting the active site threonine residue of the 26S proteasome [60]. Antineoplastic activity of bortezomib has already been shown in several *in vitro* and *in vivo* studies [61,62]. Recent studies demonstrated that bortezomib induces a strong apoptosis and inhibits the growth of cholangiocarcinoma cells [63].

Clinical trials of targeted agents in BTC

Anti-EGFR therapy: clinical trials

Based on the preclinical evidence, many clinical trials are ongoing to evaluate anti-EGFR therapy in BTC patients. A trial of 42 patients with BTC treated with oral erlotinib (150 mg/day) as monotherapy demonstrated a 6-month progression-free survival (PFS) of 17%, and 7% of the patients showed a partial response (PR). Of these patients, 57% had received first-line chemotherapy. In this study, *EGFR* mutation status was not tested, so it is unknown if there is a correlation between response and *EGFR* mutation status [64]. This result suggests a benefit of erlotinib in patients with advanced BTC, even if only larger controlled trials and trials evaluating erlotinib in combination with other targeted agents will confirm these data. The role of EGFR inhibitors in BTC was further evaluated with EGFR gene amplification studies, and case reports on the efficacy of cetuximab in combination with either gemcitabine or gemcitabine and oxaliplatin [65]. A small study evaluated cetuximab in combination with gemcitabine and oxaliplatin (GEMOX) in nine GEMOX-resistant patients with advanced, metastatic and unresectable intrahepatic cholangiocarcinoma. Cetuximab was well tolerated, the median time to progression (TTP) was 4 months and the median OS was 7 months. Therefore, the addition of cetuximab seemed to reverse the resistance to GEMOX [66,67]. A multicenter, randomized Phase II trial in patients with advanced BTC (BINGO trial)

is evaluating the efficacy of GEMOX alone or in combination with biweekly cetuximab in first-line. The primary end point is PFS at 4 months. Secondary end points are response rate (RR), PFS, OS, toxicity, early response assessment by positron emission tomography (PET) and blood/tumor EGFR signaling pathway member analyses. From October 2007 to October 2008, 101 patients were enrolled. At the interim analysis, the 4-month PFS rate was 44% versus 61% in the arm with cetuximab, so the addition of cetuximab to GEMOX showed promising activity. Moreover, the treatment was well tolerated. This trial is still ongoing. The EGFR pathway analyses will show if there is a correlation between EGFR overexpression and response to cetuximab therapy [68]. Recently, a single-center Phase II study evaluated the correlation between K-ras status and response in 30 patients with advanced or metastatic cholangiocarcinoma or gall bladder cancer treated with cetuximab plus GEMOX. The RR was 63.3%, including three patients with a complete response (CR). A total of five patients (16.7%) achieved stable disease (SD), and only six patients (20%) progressed under chemotherapy. *K-ras* mutation was detected in three patients (12%). All three patients did not progress under chemotherapy. Neither PFS nor OS were affected by K-ras status. The median PFS of all 30 patients was 8.3 months, and median OS was 12.7 months. Therefore, the authors concluded that there is no correlation between responses and K-ras status [69]. The role of lapatinib in patients with BTC and hepatocarcinoma has been evaluated in a Phase II trial. In 17 patients with BTC, no responses were

observed and five patients had SD. Therefore, the authors concluded that lapatinib is not active in BTC [70].

These trials demonstrated that anti-EGFR therapies seems to be effective in BTCs, especially if combined with chemotherapy or with other biological agents. In fact, by targeting only one pathway, escape mechanisms of cancer cells prevent the inhibition of tumor growth. TABLE 1 shows studies evaluating EGFR inhibitors in BTC patients.

Antiangiogenic therapy: clinical trials

In 2006 the first evidence regarding the activity of bevacizumab in cholangiocarcinoma was published. In fact a case report described a patient with liver metastasis from cholangiocarcinoma that was treated with bevacizumab (5 mg/kg) combined with cisplatin (75 mg/kg), fluorouracil and leucovorin. A rapid resolution of the metastases was described [71]. Some interesting studies of antiangiogenic therapy in patients with BTC have been conducted (TABLE 2). A multicenter Phase II trial tested the combination of bevacizumab with gemcitabine and oxaliplatin in BTC patients. A total of 24 patients were included, and seven had PRs and six had SD. A PET analysis after two cycles of treatment was also promising. Among the 23 patients analyzed, 17 had PRs, five had SD and one had progressive disease (PD) [72]. At ASCO 2009, Zhu *et al.* presented a Phase II study evaluating 35 patients with BTC receiving bevacizumab in combination with gemcitabine and oxaliplatin. A total of 13 patients (45%) had PR and ten patients (34%) had SD. With a median follow up of 9.9 months, the

Table 1. Clinical studies of EGFR inhibitors in biliary tract cancers.

Author (year)	Regimen	Phase	No. patients	Line	Results	Ref.
Philip <i>et al.</i> (2006)	Erlotinib	II	42	I/II	RR: 7%; PFS (6 months): 17%	[64]
Paule <i>et al.</i> (2007)	Cetuximab + GEMOX	II	9	II (PD after GEMOX)	Median TTP: 4 months Median OS: 7 months	[67]
Malka <i>et al.</i> (2009)	Cetuximab + GEMOX vs GEMOX	II	101	I	PFS (4 months): 61% vs 44%	[68]
Gruenberger <i>et al.</i> (2009)	Cetuximab + GEMOX	II	30	I	RR: 63%, median PFS: 8.3 months Median OS: 12.7 months No correlation between K-ras and response	[69]
Ramanathan <i>et al.</i> (2006)	Lapatinib	II	17	I/II	RR: 0%	[70]

BTC: Biliary tract cancer; EGFR: EGF receptor; GEMOX: Gemcitabine–oxaliplatin; OS: Overall survival; PD: Progressive disease; PFS: Progression-free survival; RR: Response rate.

Table 2. Clinical studies of VEGF inhibitors in biliary tract cancers.

Author (year)	Regimen	Phase	No. patients	Results	Ref.
Clark <i>et al.</i> (2007)	Bevacizumab + GEMOX	II	24	PR: 7; SD: 6	[72]
Zhu <i>et al.</i> (2009)	Bevacizumab + GEMOX	II	35	PR: 45%; SD: 34%; PFS: 7 months; OS: 13.2 months	[73]
El-Khoueiry <i>et al.</i> (2007)	Sorafenib	II	31	PR: 6%; SD: 29%; PFS: 2 months; OS: 6 months	[74]

BTC: Biliary tract cancer; GEMOX: Gemcitabine–oxaliplatin; OS: Overall survival; PFS: Progression-free survival; PR: Partial response; SD: Stable disease.

median OS was 13.2 months and the median PFS was 7 months. The PET analysis showed significant differences in standardized uptake value changes between the groups with PR/SD and PD ($p = 0.006$) [73]. To date, several studies evaluating bevacizumab in BTC are ongoing. An ongoing Phase II trial is evaluating the combination of systemic bevacizumab with floxuridine and dexamethasone given as a hepatic arterial infusion in patients with unresectable hepatocellular carcinoma or intrahepatic cholangiocarcinoma (NCT00410956). The end points of this study are to determine the anti-tumor efficacy (CR and PR, SD and PD) and the safety profile. Two other Phase II trials are currently ongoing to assess the safety and efficacy of bevacizumab in combination with erlotinib in patients with metastatic or unresectable gall bladder cancer or BTCs and other advanced upper gastrointestinal carcinomas, which are refractory or intolerant to standard chemotherapy (NCT00350753; NCT00033462). A nonrandomized, open-label Phase I trial (NCT0042689) is evaluating the combination of bevacizumab with radiotherapy in inoperable hepatocarcinoma and cholangiocarcinoma.

AZD2171, a small molecule with pan-VEGFR-tyrosine kinase inhibiting activity, is currently also being investigated in patients with cholangiocarcinoma. In fact an ongoing Phase I trial is assessing the effects of the combination between AZD2171 and AZD0530, a dual-specific inhibitor of Src and Abl in patients with BTC (NCT00475956).

An ongoing Phase II trial is evaluating sorafenib monotherapy in patients with unresectable or metastatic gall bladder cancer or BTC (NCT00238212). An interim analysis showed that sorafenib was well tolerated in the 31 patients enrolled. A total of 20 (66.7%) patients had grade 3–4 toxicities, including hand–foot syndrome, thrombosis/embolism, elevated liver transaminases and abdominal pain. Two patients (6%) had PR and nine patients (29%) had SD. At the time of the report, 27 patients had progressed on therapy, so the median PFS was 2 months, with a median OS of 6 months [74]. These promising results suggest the importance of targeting angiogenesis in BTC, even if it should be considered that the PFS of 2 months and the OS of 6 months do not seem good when compared with results of erlotinib or other agents. Novel therapeutic strategies that will combine antiangiogenic drugs with multikinase inhibition, chemotherapy or unrelated pathway inhibitors are required to better understand the role of bevacizumab or sorafenib in BTC.

New evidence: clinical trials of imatinib & bortezomib

At ASCO 2009, a multicenter Phase II trial investigating the disease control rate (DCR) of the combination between fluorouracil and imatinib in patients with unresectable or metastatic BTC or gall bladder cancer was presented. Since May 2007, 41 patients were enrolled. Treatment-related grade 3/4 toxicities included diarrhea,

Table 3. Clinical studies of other targeted therapies in biliary tract cancers.

Author (year)	Regimen	Phase	No. patients	Results	Ref.
Sprenger <i>et al.</i> (2009)	Imatinib + 5-FU/LV	II	41	DCR: 58%	[75]
Costello <i>et al.</i> (2009)	Bortezomib	II	20	PFS: 48 days; OS: 284 days (accrual halted for futility)	[77]

5-FU/LV: 5-fluorouracil/leucovorin; BTC: Biliary tract cancer; DCR: Disease control rate; OS: Overall survival; PFS: Progression-free survival.

edema, neutropenia, nausea and transient transaminase elevation. The DCR of 26 patients available for response assessment at time of analysis was 58%, with one CR, one PR and 13 SD of at least four cycles. A total of 11 patients showed PD. Three patients had SD after 12 cycles and continue on treatment [75].

The proteasome inhibitor bortezomib has also been studied in patients with BTC. A Phase I trial evaluated the safety profile of the combination of bortezomib with docetaxel in patients with advanced solid tumors, including cholangiocarcinoma. Bortezomib showed good tolerability [76]. At ASCO 2009, a Phase II trial exploring bortezomib in patients with recurrent or metastatic adenocarcinoma of the bile duct or gall bladder (NCI #6135) was presented with a total of 20 patients enrolled. There was only one patient with an unconfirmed PR, nine patients with SD and ten patients with PD. Accrual was halted for futility. Median PFS was 48 days and median OS was 284 days. The authors concluded that bortezomib is not an active agent in the treatment of metastatic gall bladder or BTC [77]. A Phase II trial exploring bortezomib as first-line systemic therapy of patients with unresectable or metastatic adenocarcinoma of the bile duct or gall bladder is currently ongoing (NCT00085410). TABLE 3 shows the results of the trials evaluating imatinib and bortezomib in advanced BTCs.

Future perspective

Conventional chemotherapeutic drugs have achieved only modest results in patients

with BTC. Therefore, innovative therapeutic approaches are needed to obtain significant results in this setting of patients. The first standard of care has been proposed at ASCO 2009. In fact, the combination of cisplatin and gemcitabine has been shown to be more effective than gemcitabine alone in a multicenter randomized Phase III trial [78], and new Phase III trials evaluating the combination of chemotherapy and biological therapy are needed. Preliminary results have been obtained with targeted therapies that specifically inhibit growth factor receptors, blocking the downstream signaling. Specifically, strategies targeting the EGFR and antiangiogenic drugs are the most intriguing approaches. To date, these drugs as monotherapy or combined with conventional cytotoxic drugs have obtained interesting results. The future will be the combination of targeted drugs inhibiting different pathways. The results of the ongoing trials are keenly awaited to definitely identify the role of targeted agents in BTC.

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Executive summary

Preclinical rationale for targeted therapy in biliary tract cancers

- EGR receptor (EGFR) is often overexpressed in biliary tract cancers (BTCs), and is an independent prognostic factor.
- Preclinical studies have shown that anti-EGFR antibodies caused an inhibition of cell proliferation, increasing apoptosis.
- VEGF is overexpressed in cholangiocarcinomas with differences between intra- and extra-hepatic cholangiocarcinoma.
- Other potential targets are the Akt/mammalian target of rapamycin pathway, the Ras/Raf/mitogen-activated protein kinase pathway and the proteasome, with intriguing preclinical evidence.

Clinical trials of targeted therapy in biliary tract cancers

- Anti-EGFR therapies seem to be effective in BTCs, especially if combined with chemotherapy or with other biological agents.
- Phase II trials have evaluated the activity of bevacizumab and sorafenib in BTCs with promising results, but Phase III trials of antiangiogenic agents in combination with chemotherapy have to be conducted to confirm these results.
- Randomized clinical trials evaluating bortezomib and imatinib are needed to establish their role in BTC.

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