

## Targeted medical therapy of biliary tract cancer: Recent advances and future perspectives

Michael Höpfner, Detlef Schuppan, Hans Scherübl

Michael Höpfner, Institute of Physiology, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, 10967 Berlin, Germany

Detlef Schuppan, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, United States

Hans Scherübl, Klinik für Innere Medizin, Gastroenterologie und Gastrointestinale Onkologie, Klinikum am Urban, Vivantes Netzwerk für Gesundheit, 10967 Berlin, Germany

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**Correspondence to:** Dr. Hans Scherübl, Professor, Klinik für Innere Medizin-Gastroenterologie und Gastrointestinale Onkologie, Vivantes GmbH, 10967 Berlin,

Germany. [hans.scheruebl@vivantes.de](mailto:hans.scheruebl@vivantes.de)

Telephone: +49-30-130225201 Fax: +49-30-130225205

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### Abstract

The limited efficacy of cytotoxic therapy for advanced biliary tract and gallbladder cancers emphasizes the need for novel and more effective medical treatment options. A better understanding of the specific biological features of these neoplasms led to the development of new targeted therapies, which take the abundant expression of several growth factors and cognate tyrosine kinase receptors into account. This review will briefly summarize the status and future perspectives of antiangiogenic and growth factor receptor-based pharmacological approaches for the treatment of biliary tract and gallbladder cancers. In view of multiple novel targeted approaches, the rationale for innovative therapies, such as combinations of growth factor (receptor)-targeting agents with cytotoxic drugs or with other novel anticancer drugs will be highlighted.

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**Peer reviewer:** Ignacio Gil-Bazo, MD, PhD, Cancer Biology

and Genetics Program, Memorial-Sloan Kettering Cancer Center, 1275 York Avenue Box 241, New York 10021, United States

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### INTRODUCTION

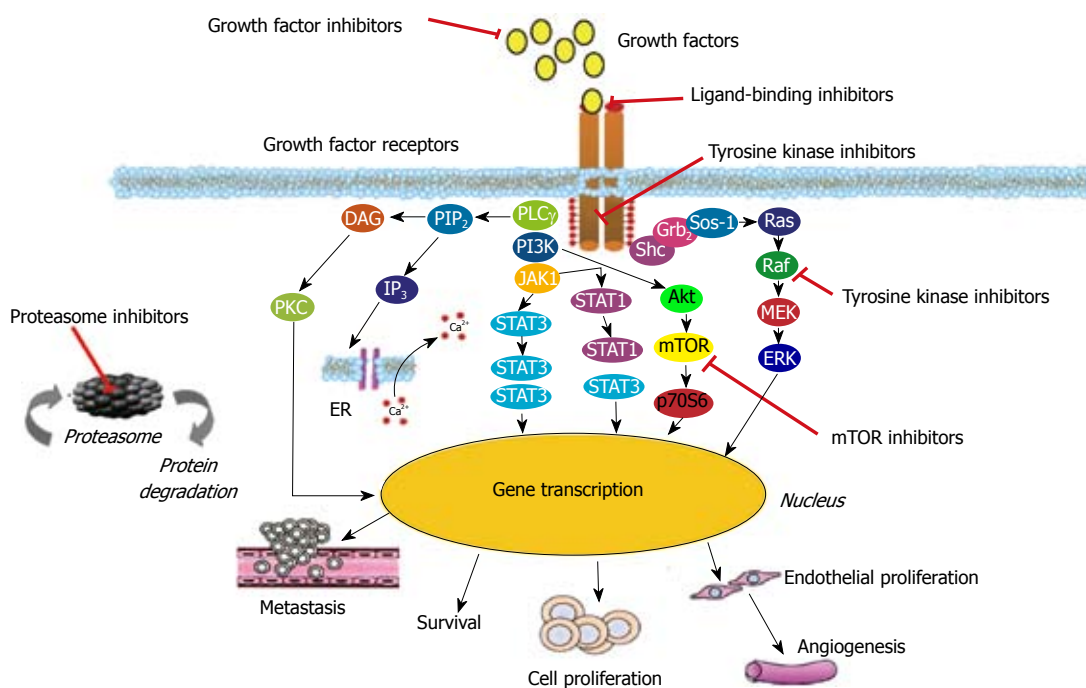
Biliary tract cancers (BTC) originate from the intra- or extrahepatic bile duct epithelium<sup>[1]</sup>. They were first described by Durand-Fardel in 1840<sup>[2,3]</sup>. The extrahepatic type (cholangiocarcinoma), primarily cancers involving the confluence of the right and left hepatic ducts, accounts for 80%-90%, and the intrahepatic type (cholangiocellular carcinoma) for the remaining 10%-20% of all biliary tract cancers. Hilar BTC as a specific sub-entity was first reported by Klatskin in 1965, hence their designation as Klatskin tumors<sup>[4]</sup>. BTC have been considered rare malignancies comprising only 3% of gastrointestinal tumors. However, interest in BTC is growing due to a rising worldwide incidence and associated mortality especially in intrahepatic BTC<sup>[5-8]</sup>.

BTC is notoriously difficult to diagnose and is usually fatal because of its late clinical presentation and the lack of effective non-surgical treatment modalities<sup>[9]</sup>. Surgical resection or liver transplantation remain the only potentially curative therapeutic options. Unfortunately, most patients have unresectable disease at presentation and die within 12 mo. Liver failure and recurrent sepsis, secondary to biliary obstruction, also contribute to the high mortality<sup>[10]</sup>. Overall survival rate is poor, with less than 5% of BTC patients surviving to 5 years, a rate which has not changed significantly over the past 30 years<sup>[11]</sup>. Similar to BTC, there is currently no standard chemotherapy regimen for patients with advanced gallbladder cancer.

Therefore, innovative drugs are urgently needed for effective medical treatment of biliary tract and gallbladder cancers. This review will provide a perspective overview of selected agents, which are currently in development, or under consideration or testing for a more effective, targeted treatment of BTC (Table 1; Figure 1)<sup>[12-24]</sup>. Moreover, we will discuss promising approaches, which

**Table 1** Current status of clinical trials with agents that target growth factor receptors and related signaling pathways for treatment of biliary tract and gallbladder cancers

Name	Target	Mechanism	Cotreatment	Status	Clinical trials
Bevacizumab	VEGF	VEGF-neutralizing antibody	Erlotinib	Phase II	NCT00350753 <sup>[17]</sup>
			Erlotinib	Phase II	NCT00356889 <sup>[18]</sup>
			Radiation	Phase I	NCT00426829 <sup>[21]</sup>
			Floxuridine, dexamethasone	Phase II	NCT00410956 <sup>[20]</sup>
			Gemcitabine, oxaliplatin	Phase II	NCT00361231 <sup>[19]</sup>
Cediranib (AZD2171)	PAN-VEGFR, PDGFR, c-KIT	Tyrosine kinase inhibitor	AZD-0530	Phase I	NCT00475956
Cetuximab	EGFR	Monoclonal antibody	Gemcitabine, oxaliplatin	Phase II	NCT00552149 (BINGO) <sup>[22]</sup>
Erlotinib	EGFR	Tyrosine kinase inhibitor		Phase II	NCT00033462 <sup>[12]</sup>
			Gemcitabine	Phase I b	
			Oxaliplatin, gemcitabine, radiation	Phase I	NCT00266097 <sup>[16]</sup>
Lapatinib	EGFR, erbB2	Tyrosine kinase inhibitor		Phase II	NCT00107536 <sup>[14]</sup>
Sorafenib	VEGFR, PDGFR, c-Raf, B-Raf	Tyrosine kinase inhibitor	Oxaliplatin,	Phase II	NCT00238212 <sup>[15]</sup>
			Capecitabine	Phase I / II	NCT00634751 <sup>[23]</sup>
			Gemcitabine	Phase I / II	NCT00661830 (GEMSO) <sup>[24]</sup>
				Phase I	NCT00085410 <sup>[13]</sup>
Bortezomib	Proteasome	Proteasome inhibitor	Docetaxel	Phase II	



**Figure 1** Major growth factor receptor signaling pathways. TK: Tyrosine kinase; P: Phosphorylation; MEK: Mitogen-activated protein kinase; ERK: Extracellular signal-regulated kinase; PI3K: Phosphatidylinositol-3 kinase; mTOR: Mammalian target of rapamycin; JAK: Janus kinase; STAT: Signal transducer and activator of transcription.

have not yet been tested in BTC or gallbladder cancer, but warrant future evaluation.

## ANTIANGIOGENIC TREATMENT STRATEGIES

Angiogenesis plays a central role in tumor growth and progression, and its implications have been extensively investigated and described in the literature for various cancers<sup>[25,26]</sup>. In the early 1970s, Folkman J<sup>[27]</sup> was the first to develop the concept of angiogenesis-dependent tumor growth and postulated that the specific blocking

of blood flow to the tumor should be a promising strategy for cancer treatment.

Among the angiogenic factors/receptors described so far, the vascular endothelial growth factor (VEGF) and VEGF receptor family including the secreted glycoproteins VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, the placental growth factors (PlGF-1,-2), and their cognate receptors VEGFR-1 (Flt-1) and VEGFR-2 (Flk/KDR) play major roles in not only physiological but also in pathological angiogenesis. VEGF-A which binds to both VEGFR-1 and -2 is a key regulator of the development of the vascular system and is commonly

overexpressed in a variety of solid tumors<sup>[28]</sup>.

In addition, elevated levels of circulating VEGF-A are correlated with progression and metastasis of gastrointestinal cancers. A recent study confirmed that elevated VEGF expression correlated with increased metastasis of intrahepatic cholangiocarcinoma<sup>[29]</sup>. Here upregulated VEGF-C, which plays an important role in the lymph node metastasis of intrahepatic cholangiocarcinoma, was the best independent factor for a poor prognosis<sup>[30]</sup>.

In this vein VEGF protein is overexpressed in cholangiocarcinomas<sup>[31]</sup>, which is paralleled by VEGFR-1, -2 expression in the surrounding endothelial cells<sup>[32,33]</sup>. Therefore the VEGF/VEGFR system is an attractive target for the treatment of these almost chemoresistant cancers.

## ANTIBODY-BASED ANTIANGIOGENIC THERAPY

### *Anti-VEGF treatment*

Bevacizumab is a humanized murine monoclonal anti-VEGF antibody, which when combined with standard cytostatic treatment significantly increased survival in patients with metastatic colorectal cancer as compared to standard treatment alone<sup>[34]</sup>. This positive phase III clinical trial led to approval of bevacizumab for the treatment of advanced colorectal cancer in 2005. Comparable results were obtained in a recent phase III clinical trial of bevacizumab in non-small cell lung cancer. This study was interrupted prematurely because of an obvious survival advantage in the antibody treated patients<sup>[35]</sup>.

The first clinical data on the successful treatment of cholangiocarcinoma with bevacizumab were reported in 2006, and described a patient whose metastasis from cholangiocarcinoma resolved after salvage therapy with 3 cycles of bevacizumab (5 mg/kg) combined with cisplatin (75 mg/kg) and high-dose fluorouracil and leucovorin over a period of 2 wk<sup>[36]</sup>.

Currently, several studies using bevacizumab for combination therapy of cholangiocarcinoma are ongoing. An ongoing phase II trial explores 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). In this trial, patients undergo placement of the hepatic arterial infusion (HAI) pump and a cholecystectomy and receive floxuridine and dexamethasone by HAI continuously for 2 wk. Bevacizumab is concomitantly given for 30-90 min iv at the beginning and end of each cycle. Outcome measure of the study trial is to determine the antitumor efficacy (complete and partial response, stable and progressive disease) as well as toxicity as measured by Common Toxicity Criteria (CTC), which is a standard set by the National Cancer Institute to provide standard language for reporting adverse events which occur in cancer clinical trials<sup>[37]</sup>.

Two other Phase II trials are currently determining the safety and efficacy of bevacizumab in combination with the EGFR tyrosine kinase inhibitor erlotinib in patients with metastatic or unresectable gall bladder or bile duct cancers and other advanced upper gastrointestinal carcinomas, which are refractory or intolerant to standard chemotherapy (NCT00350753<sup>[17]</sup>; NCT00033462<sup>[12]</sup>). This particular combination was chosen because dual targeting of cancer cells (EGFR) and their nutrient supply (anti-VEGF) will likely have synergistic antitumoral effects<sup>[38]</sup>. Synergy has been shown at the molecular level, since inhibition of the EGFR suppressed the release of VEGF from tumor cells *in vitro*<sup>[39,40]</sup>. Moreover, high levels of VEGF have been shown to promote resistance to anti-EGFR treatment in squamous cell carcinoma<sup>[41]</sup>. Recent clinical trials for refractory non-small-cell lung cancer and advanced renal cell carcinoma proved that this particular combination is highly effective<sup>[42-44]</sup>.

The combination of bevacizumab with radiation therapy is currently being studied in inoperable hepatocellular carcinoma and cholangiocarcinoma in a non-randomized, open label phase I trial (NCT00426829<sup>[21]</sup>). In rectal cancer patients, bevacizumab was shown to enhance tumor blood flow, to reduce tumor interstitial pressure, and to decrease mean vessel density. These physiologic changes enhanced the efficacy of radiation therapy in a neoadjuvant setting<sup>[45]</sup>. It is most probable that antiangiogenic preconditioning to normalize the tumor vasculature in primary liver tumors will equally create a “therapeutic window” whereby improved blood flow with enhanced exposure to radiosensitizing oxygen is achieved.

### *Anti-PIGF treatment*

The use of a neutralizing anti-PIGF monoclonal antibody in VEGF-inhibitor resistant tumors is an attractive alternative antiangiogenic strategy. A proof of concept study has been performed in an animal study with melanoma or pancreatic adenocarcinoma bearing mice<sup>[46]</sup>. The antibody specifically inhibits the binding of PIGF to its receptor VEGFR-1, present on tumor-associated endothelial cells and macrophages. The underlying idea of using this approach was derived from gene inactivation studies showing that endogenous PIGF is redundant in vascular development and physiological vessel maintenance, but an important contributor to the “angiogenic switch” in solid tumor growth. This led to the hypothesis that unlike VEGF inhibitors, PIGF inhibition might reduce pathological angiogenesis, without disturbing physiological blood vessel homeostasis and reduce concomitant unwanted side effects. Hence, anti-PIGF treatment could perhaps substitute for anti-VEGF therapy in the future. Moreover, as PIGF levels increase in the circulation of cancer patients receiving anti-VEGF treatment<sup>[47-49]</sup>, anti-PIGF should also counteract this potential downside of anti-VEGF therapy. Accordingly, anti-PIGF-treatment inhibits angiogenesis, lymphangiogenesis, tumor growth and motility in anti-VEGF-resistant tumor bearing

mice. Here, it blocks the so-called rescue-angiogenesis, a major problem in current antiangiogenic approaches, and showed excellent treatment tolerability. In addition, anti-PIGF treatment may permit long-term treatment of cancers in children, pregnant women, or patients at risk for thrombotic, cardiac or other complications for whom the adverse effects of other VEGF/VEGFR-inhibitors may be excessive and prohibitive.

## ANTIANGIOGENIC THERAPY WITH SMALL MOLECULE INHIBITORS

Several agents which inhibit the tyrosine kinase activity of angiogenic growth factor receptors like the VEGFR or PDGFR, have been synthesized by combinatorial chemistry. These tyrosine kinase inhibitors are small molecules which occupy the ATP binding site of the tyrosine kinase domain of the intracellular portion of the receptor. Because of their effects on downstream signaling, these inhibitors interfere with a number of key biologic functions associated with VEGFR activation. Although drugs that are targeted to specific VEGFR kinases have shown clinical efficacy, the redundancy in the angiogenesis pathways necessitates broad spectrum inhibitors that address multiple (VEGFR) targets<sup>[50]</sup>.

### AZD2171

AZD2171 is a highly potent small molecule with pan-VEGFR-tyrosine kinase inhibiting activity (50% inhibitory concentration of < 0.002  $\mu\text{mol/L}$  for VEGFR-2 and 0.005  $\mu\text{mol/L}$  for VEGFR-1, respectively). AZD2171 also inhibits VEGFR-3, PDGFR- $\beta$  and c-Kit at nanomolar concentrations<sup>[51]</sup>. The antineoplastic potency of AZD2171 has been demonstrated in several tumors, including lung, hepatocellular, colorectal and prostate cancer and in all cases the antitumor effect was associated with strong inhibition of VEGF signaling and angiogenesis<sup>[52-54]</sup>. A phase I dose-finding study was conducted in 83 patients with advanced solid tumors<sup>[52]</sup>. The study was divided into parts A and B, with 36 patients on a dose-escalation schema, in which 3 to 8 patients received a single oral dose of AZD2171 ranging from 0.5-60 mg. After a wash-out period of 2 to 7 d, the patients continued with daily treatment at the same dose level. AZD2171 was generally well tolerated at < 45 mg/d with common side effects of fatigue, nausea, diarrhea, and vomiting. In part B, an additional 47 patients were enrolled at 20, 30 or 45 mg orally daily. All patients had liver metastases and six patients had NSCLC. The major toxicities and side-effects included hypertension, headache, diarrhea, and voice hoarseness. Three patients in the 60 mg cohort each experienced one serious adverse event (grade 4 cerebral hemorrhage, grade 4 hypoglycemia, and grade 3 hypertension). Of the 83 patients enrolled, two partial responses were observed, while stable disease was seen in 23 patients.

At present AZD2171 is being evaluated in three different studies (Horizon I - III) in patients with advanced colorectal cancer. In Horizon III, which is a

phase II/III study, AZD2171 is tested as a combination partner for FOLFOX compared to a combination of FOLFOX and bevacizumab in patients with previously untreated metastatic colorectal cancer. AZD2171 has shown encouraging signs of antitumor activity in a clinical development program, which has included over 700 patients to date. Based on the generally encouraging findings, AZD2171 is currently also being investigated in cholangiocarcinoma. A pending phase I trial (NCT00475956) explores the effects of AZD2171 in combination with AZD0530, a dual-specific inhibitor of Src and Abl. Src and Abl are protein tyrosine kinases which are overexpressed in malignancies such as chronic myeloid leukemia (CML), where AZD0530 has already been proved to be an effective anticancer agent<sup>[55]</sup>. The idea for using this particular combination for the treatment of cholangiocarcinoma may have arisen from observations that the Abl- and Src-inhibitor imatinib (Gleevec) showed apoptosis-inducing and growth-reducing effects in cholangiocarcinoma cells *in vitro*<sup>[56]</sup>. However, imatinib also inhibits other tyrosine kinases, such as c-kit and PDGFR- $\beta$ . Thus it is not clear, whether the effects of imatinib on cholangiocarcinomas are related to Src-inhibition. This is doubtful as s-src expression, which is highly correlated with the indices of early stage hepatocellular carcinoma phenotype, is not likely to be involved in the cholangiocarcinoma phenotype, as no s-src activation could be detected in cholangiocarcinoma<sup>[57]</sup>.

## STRATEGIES TARGETING THE EGFR

The central role of the epidermal growth factor receptor (EGFR) in the proliferation of tumor epithelia and its overexpression in several solid tumors have provided the rationale for targeting this key signaling network. EGFR blockade with monoclonal antibodies and tyrosine kinase inhibitors has already translated into clinical benefit in gastrointestinal tumors, including primary liver cancer<sup>[58,59]</sup>.

Over the past few years, three EGFR-specific agents have received regulatory approval: (1) The monoclonal anti-EGFR antibody cetuximab for metastatic colorectal cancer, and squamous cell carcinoma of the head and neck; (2) The tyrosine kinase inhibitor erlotinib for advanced or metastatic pancreatic cancer and NSCLC; and (3) The EGFR tyrosine kinase inhibitor gefitinib for advanced or metastatic NSCLC. However, the general FDA approval for NSCLC treatment with gefitinib was recently withdrawn after it failed to demonstrate a survival benefit either alone or with chemotherapy in three phase III trials<sup>[58,60]</sup>.

Several reports indicate that the EGFR is frequently (over-)expressed in cholangiocarcinoma. Additionally, sustained EGFR activation due to defective receptor internalization has been reported for cholangiocarcinoma cells<sup>[61]</sup>. Of note, bile acids activate EGFR-signaling *via* a TGF- $\alpha$ -dependent mechanism, thereby contributing to the growth characteristics of cholangiocytes and cholangiocarcinoma cells<sup>[62]</sup>. Clinicopathologically,



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 increased the risk for tumor recurrence in intrahepatic cholangiocarcinoma<sup>[29]</sup>. EGFR-inhibitors inhibited cholangiocarcinoma cell growth *in vitro* and *in vivo*<sup>[33,61,63]</sup>.

These encouraging preliminary findings on the general suitability of anti-EGFR-based approaches for the treatment of cholangiocarcinoma spawned several clinical trials. In a cohort composed of 24 chemotherapy-refractory patients and 18 chemotherapy-naïve patients administered oral erlotinib (150 mg/d) as monotherapy, the progression free survival at 6 mo was determined<sup>[64,65]</sup>. Seventeen percent of the patients achieved this primary end point, while disease control was obtained in 50% of patients with a median duration of 5.1 mo. Seven percent of the patients showed a partial response of 4 to 14 mo duration. The results suggest an astonishing therapeutic benefit for EGFR blockade with erlotinib in patients with advanced biliary cancer, however, this has to be confirmed in future larger controlled trials and in trials which use erlotinib in combination with other targeted agents.

An ongoing multicenter phase II trial in patients with advanced BTC (BINGO; NCT00552149) evaluates the efficacy of the EGFR-antibody cetuximab (Huether *et al.*, 2006), combined with gemcitabine-oxaliplatin chemotherapy (GEMOX). Patients will be randomized 1:1 to receive GEMOX (1000 mg/m<sup>2</sup> gemcitabine; 100 mg/m<sup>2</sup> oxaliplatin) alone or GEMOX + cetuximab (500 mg/m<sup>2</sup>) every other week. The BINGO trial also comprises ancillary basic research and functional imaging studies, in order to identify markers that predict treatment efficacy of bile duct cancer. The primary outcome measure of the study is progression-free survival at 4 mo. Secondary outcome measures are the feasibility and toxicity of the treatments, and an evaluation of the degree and duration of objective tumor response or tumor control in a time frame of one year.

A third study used cetuximab in combination with GEMOX in a small number of nine GEMOX resistant patients with advanced, metastatic and unresectable intrahepatic cholangiocarcinoma<sup>[66]</sup>. Patients received cetuximab 400 mg/m<sup>2</sup> on day 1, then 250 mg/m<sup>2</sup> weekly, combined with gemcitabine 1000 mg/m<sup>2</sup> on day 1 and oxaliplatin 85 mg/m<sup>2</sup> on day 2, every 3 wk. Results of the study were encouraging. Cetuximab was well tolerated and provided good palliative effects in advanced cholangiocarcinoma. Moreover, adding cetuximab bypassed tumor resistance to GEMOX<sup>[67]</sup>.

Taken together, anti-EGFR-based therapies for treating BTC appear to have their greatest potential when given in combination either with conventional cytostatics or with other targeted agents. The rationale for using combination therapies is the existence of multilevel receptor cross-stimulation or of redundant signaling pathways which lead to neoplasia. Blocking only one of

these pathways allows others to act as salvage or escape mechanisms for cancer cells. Preclinical evidence of synergistic antitumor activity achievable by combining targeted agents that block multiple signaling pathways has recently emerged<sup>[68-70]</sup>. The multi-target approach can be accomplished by using either combinations of selective agents or single agents, which address various targets<sup>[71]</sup>.

## IGF/IGFR-BASED STRATEGIES

Activation of the insulin-like growth factor (IGF) receptor 1 (IGF-1R) by IGF- I and IGF- II plays a pivotal role in tumor cell proliferation and spread, by promoting cell cycle progression, preventing apoptosis, and by regulating and maintaining the metastatic tumor phenotype<sup>[70,72-75]</sup>. A wide variety of tumors show abnormal or enhanced expression of IGFs and IGF-1R, which leads to auto- and paracrine growth stimulation, and which has been correlated with enhanced proliferation, tumor de-differentiation, disease stage, development of metastases and reduced patient survival. Enhanced expression of IGF-1R has also been demonstrated in BTC, and the IGF/IGFR system was shown to be centrally involved in proliferation and suppression of apoptosis of cholangiocarcinoma cells<sup>[76]</sup>, making the IGF/IGFR-signaling system an attractive target for the treatment of BTC. Thus IGF-1R blocking antibodies, IGF-1R antisense oligonucleotides, or IGF-1R siRNA have all been shown to effectively interfere with IGF-1R mediated signaling *in vitro* and with tumor growth and spread *in vivo*<sup>[76-80]</sup>.

We and others validated the selective IGF-1R tyrosine kinase inhibitor NVP-AEW541 as a promising novel agent for the therapy of several cancers<sup>[81-85]</sup>. Moreover, we showed that a combination of IGF-1R inhibitors together with the multi-kinase inhibitor sorafenib, offer additive antitumoral efficacy for cholangiocarcinoma *in vitro*<sup>[86]</sup>. The antineoplastic properties of NVP-AEW541 and related compounds such as NVP-ADW742 have been demonstrated in preclinical studies on Ewing's sarcoma-bearing mice, fibrosarcoma, breast cancer and musculoskeletal sarcoma<sup>[81-83]</sup>.

Specific IGFR-antibodies potently suppressed prostate and breast cancer cell growth *in vitro*<sup>[87]</sup>. The clinically most advanced anti-IGFR antibody is CP-751871, which is currently being tested in three phase II trials for advanced breast cancer, NSCLC and prostate cancer ([www.clinical-trials.gov](http://www.clinical-trials.gov)). Importantly, the preliminary clinical data indicate that IGFR-inhibition is well-tolerated<sup>[88-90]</sup>. Safety is important, since IGFR-based inhibition has long been regarded as a high-risk intervention, because of the high homology of the IGF-1R receptor with the related insulin-receptor, and the fear that IGF-1R tyrosine kinase inhibitors may lead to insulin resistance and overt diabetes<sup>[91]</sup>. However, the current *in vivo* data do not support this assumption, resulting in a growing interest in anti-IGFR-based therapies<sup>[92]</sup>.

Crosstalk between the signaling of the IGF/IGFR

system and other growth factor receptors will likely attenuate the antineoplastic effect of monotherapeutic approaches, necessitating combinations of IGF/IGFR-targeting therapies with other therapies to enhance efficacy<sup>[93,94]</sup>. This can be achieved by dual-targeting the IGF-1R and the EGFR, since the EGFR is activated by the IGF/IGFR-system leading to mitogenic EGFR-tyrosine kinase activity without ligand stimulation of the EGFR<sup>[95]</sup>. In this line IGFR- combined with EGFR-inhibition can over-additively enhance the antineoplastic effect of the respective monotherapies in gastrointestinal cancers<sup>[96-98]</sup>.

## DUAL-TARGETING SMALL MOLECULE INHIBITORS

The use of dual-targeting small molecule inhibitors, simultaneously blocking less related kinases such as VEGFR and EGFR tyrosine kinases, may also be promising for the future treatment of BTC. These agents inhibit both tumor cell proliferation/survival by blocking mitogenic EGFR signaling of the tumor cells and angiogenesis by inhibiting endothelial VEGFRs. Recent *in vivo* studies of non-cholangiocarcinoma models (colon, prostate, NSCLC) demonstrated that the dual-targeting tyrosine kinase inhibitor NVP-AEE788 displayed significant antineoplastic efficacy<sup>[99-101]</sup>. NVP-AEE788 was recently also shown to be a potent inhibitor of cholangiocarcinoma cell growth<sup>[33]</sup>, further emphasizing the possible suitability of EGFR/VEGFR-dual targeting agents for the treatment of cholangiocarcinoma.

ZD6474 (Zactima) is another EGFR/VEGFR tyrosine kinase inhibitor with potent antineoplastic properties in phase II/III trials on NSCLC and thyroid cancer. In these trials response rates of 30% in patients with locally advanced medullary thyroid cancer<sup>[102]</sup> as well as significant prolongation in the progression-free survival of NSCLC patients<sup>[103,104]</sup> were observed.

Clinical studies on BTC using these dual target kinase inhibitors have not yet been conducted. Nevertheless, the idea of simultaneously inhibiting these two growth factor receptor systems is currently under clinical investigation using a combination of EGFR-inhibiting erlotinib together with VEGF-neutralizing bevacizumab (see before). Indeed, the University of Colorado together with Astra Zeneca only recently started a phase I trial (NCT00551096) to determine the highest dose of Zactima that can be safely given as a single agent or in combination with gemcitabine and capecitabine in advanced solid tumors. This study is explicitly planned with an expanded cohort of patients with biliary cancers (BTC and gallbladder cancer), who will be treated at the highest determined dose in further studies.

## OTHER STRATEGIES

### Targeting the AKT/mTOR pathway

The activated PI3K/AKT/mTOR pathway has emerged as a novel contributor to BTC development<sup>[105]</sup>. PI3K associates with the intracellular domain of several

growth factor receptors. Upon receptor activation, PI3K triggers the generation of phosphatidylinositol 3,4,5-trisphosphate (PIP3), which provokes the subsequent activation of AKT, a serine/threonine kinase that activates multiple cellular target proteins, such as the mammalian target of rapamycin (mTOR) subfamily. mTOR is a serine-threonine kinase that downregulates apoptosis, and *via* stimulation of cell cycle progression enhances proliferation and cell growth. Specifically, mTOR is involved in the activation of mRNA-translation into proteins, which are necessary for cell cycle progression from G1 to S-phase, including the E4-binding protein (E4-BP1), and p70<sup>S6</sup> kinase<sup>[106]</sup>. In nontransformed cells the PI3K/AKT/mTOR pathway is controlled by the phosphatase and tensin homolog deleted on chromosome ten (PTEN), a tumor suppressor which inhibits this pathway by reversing PI3K and subsequent AKT activation. Mutation or silencing of the PTEN gene leads to activation of the mTOR pathway and promotion of carcinogenesis.

### AKT-inhibition

The tricyclic nucleoside VQD-002 (tricitabine phosphate monohydrate, TCN-P, Vioquest Pharmaceuticals) is a small molecule inhibitor of AKT signaling. Identified by the Moffitt Cancer Center through screening the NCI diversity set, VQD-002 was shown to be highly selective for Akt without affecting the activation of other related kinases, such as PI3K, PKC, phosphoinositide-dependent kinase-1, serum and glucocorticoid-inducible kinase, PKA, STAT-3 or ERK1/2. Accordingly, AKT-inhibition by VQD-002 resulted in suppression of cell growth and induction of apoptosis in human cancer cells and in tumor xenograft mouse models, with high selectivity for those tumors with aberrant Akt<sup>[107]</sup>. An ongoing phase I / II a trial (NCT00363454) on metastatic solid tumors overexpressing AKT, such as pancreatic, breast, ovarian and colorectal cancer is promising, as preliminary results indicate that VQD-002 was well tolerated and prolonged the stable disease period of patients (<http://www.vioquestpharm.com>). VQD-002 is already earmarked for combination with the EGFR antagonist erlotinib, since preclinical studies showed that coadministration of VQD-002 can help to overcome resistance to EGFR-antibody therapy in breast cancer patients with PTEN-deficiency<sup>[108]</sup>.

### mTOR-inhibition

The natural antibiotic rapamycin (sirolimus) is a potent inhibitor of mTOR<sup>[109]</sup>. Recently, three analogues of rapamycin with superior pharmacokinetic and biological properties have emerged. The cell cycle inhibitor-779 (CCI-779, temsirolimus) is a soluble ester analogue. RAD001 [40-O-(2-hydroxyethyl)-rapamycin, everolimus] is a derivative of rapamycin with high oral bioavailability, and AP23573 is a non-pro-drug analogue of rapamycin. These agents have been successfully tested for their antineoplastic potency and/or tolerability in various malignancies in early clinical trials (e.g. CCI-779 in renal, breast and lung cancers),

or are currently being studied in open clinical trials for the treatment of colorectal, endometrial, and brain tumors (RAD001, everolimus)<sup>[110-112]</sup>. AP23573 has been successfully tested in a phase II trial in sarcomas<sup>[113]</sup>, and two phase I studies in patients with refractory or advanced solid tumors showed partial responses and disease stabilization in individual patients<sup>[114]</sup>. In preclinical investigations, the antiproliferative, antimigratory and anti-invasive potency of rapamycin in cholangiocarcinoma cells has recently been described<sup>[115]</sup>. Activated mTOR was also demonstrated to be a negative prognostic factor for patients with BTC, and patients with activated mTOR are likely to benefit from targeted therapy with mTOR inhibitors in the future<sup>[116]</sup>. However, so far no trials exploring mTOR-inhibitors for BTC have been initiated.

### **Targeting the Ras/Raf/MARK pathway**

The proliferative Ras/Raf/MEK/ERK pathway is one of the key signaling cascades that underlies the development and maintenance of cancers. This pathway transduces extracellular signals from the various growth factor receptor tyrosine kinases (e.g. EGFR, IGFR, VEGFR and PDGFR) to the nucleus with a series of specific phosphorylation events, resulting in the expression of proteins for cell cycle progression, apoptosis resistance, extracellular matrix remodeling, cellular motility, angiogenesis or drug resistance<sup>[117]</sup>. Dysregulation of this crucial pathway occurs due to oncogenic transformation of Ras and Raf isoforms, or to overexpression and/or overactivation (*via* phosphorylation) of the Ras and Raf genes<sup>[118,119]</sup>. Activating B-Raf mutations are relatively common in cholangiocarcinomas and disruption of the Raf/MEK/ERK (MAPK) kinase pathway, either by B-Raf or Ras mutations, is detected in more than 60% of all BTC, which is therefore one of the most frequent defects in cholangiocellular carcinogenesis<sup>[120]</sup>.

### **Sorafenib**

The bi-aryl urea derivative sorafenib (Nexavar<sup>TM</sup>) is an oral multi-kinase inhibitor, which targets kinases of wild-type B-Raf, mutant V559E B-Raf and C-Raf, and importantly receptor tyrosine kinases involved in angiogenesis, including VEGFR-2, and -3, and PDGFR<sup>[121]</sup>. Sorafenib has been approved by the FDA for the treatment of advanced renal cell carcinoma and of inoperable hepatocellular cancer.

The effect of sorafenib on several molecular targets in addition to the Raf isoforms makes it difficult to determine which of its targets contributes most to its antitumor activity in a given tumor type. For instance, a recent HCC trial suggested that inhibition of the Raf/MEK/ERK pathway was central to sorafenib's mode of antitumor action<sup>[122]</sup>, whereas in other cancers, such as renal cell carcinoma or NSCLC, the antineoplastic activity was attributed mainly to its antiangiogenic activity<sup>[121,123]</sup>.

Sorafenib alone or in combination with conventional cytostatics (5-fluorouracil, gemcitabine, doxorubicin) or IGF-1R inhibition induces a potent growth suppression

of cholangiocarcinoma cells *in vitro*<sup>[86]</sup>. Antitumor efficacy was even higher when sorafenib was combined with the histone deacetylase inhibitor MS-275<sup>[124,125]</sup>. These encouraging findings have resulted in an ongoing phase II trial which evaluates sorafenib monotherapy in patients with unresectable or metastatic gallbladder cancer or BTC (NCT00238212). In an intermediate evaluation of this study, sorafenib was well tolerated, but as a single agent it did not lead to a clinically significant response rate in these patients, while its impact on survival was comparable to commonly used chemotherapy regimens. These promising results of sorafenib monotherapy will likely facilitate novel therapeutic strategies which will combine multikinase inhibition with conventional cytostatic therapy or with unrelated pathway inhibitors, such as histone deacetylase or proteasome inhibitors (see below) for enhanced and well tolerated medical treatment of advanced BTC<sup>[126]</sup>.

### **Targeting the proteasome**

Another interesting therapeutic approach for innovative cancer treatment is the inhibition of the 26S proteasome, which is a large protease that is present in both the nucleus and the cytoplasm of eukaryotic cells. The proteasome functions as an identifier and proteolytic graveyard for proteins branded for destruction by the ubiquitin system. The so-called ubiquitin-proteasome pathway (UPP) is the major non-lysosomal proteolytic system in eukaryotic cells and triggers degradation of proteins involved in cell cycle progression, apoptosis, nuclear factor kappa B (NF- $\kappa$ B) activation, and angiogenesis. UPP also degrades mutant, damaged, and misfolded proteins<sup>[127]</sup>. Since these signaling pathways are critical for cell survival and proliferation, especially in cancer cells, inhibition of the proteasome has emerged as an attractive target for cancer therapy.

### **Bortezomib**

Bortezomib (Velcade<sup>TM</sup>) is a proteasome inhibitor, which blocks multi-ubiquitinated protein degradation by reversibly and competitively inhibiting the active site threonine residue of the 26S proteasome<sup>[128]</sup>. Antineoplastic activity of bortezomib has already been shown in several *in vitro* and *in vivo* studies<sup>[129,130]</sup>. Only recently we and others showed the potent apoptosis inducing and growth inhibiting features of bortezomib in cholangiocarcinoma cells<sup>[125,131]</sup>. Bortezomib is the first proteasome inhibitor to be approved for cancer therapy and based on the results of a phase II trial<sup>[127]</sup> has recently been approved by the FDA for the treatment of mantle cell lymphoma<sup>[132,133]</sup>. Other cancers, including neuroendocrine tumors, RCC, NSCLC, or metastatic sarcomas have also been evaluated in recent phase II clinical trials. In some of these studies a significant antineoplastic effect with bortezomib monotherapy was observed, while in other studies no or only marginal responses were found<sup>[134-136]</sup>. However, in the latter cases further investigation on the role of bortezomib in combination with other antitumoral drugs was recommended, since proteasome

inhibition will likely sensitize cancer cells to other therapeutic agents. Combinations with encouraging results have been reported in two studies of lung cancer and lymphoma<sup>[157,158]</sup>. In another phase I trial, bortezomib was tested in combination with the cytotoxic agent docetaxel in advanced solid tumors, including cholangiocarcinoma, where it showed generally good tolerability<sup>[13]</sup>. A phase II trial exploring bortezomib as first-line systemic therapy of patients with unresectable or metastatic adenocarcinoma of the bile duct or gallbladder is currently ongoing (NCT00085410). A comparable study in HCC was recently reported to have resulted in disease stabilization in some patients, with generally good tolerability. Here it was again suggested that the focus should next be on combinations of bortezomib with HCC-relevant cytostatics such as doxorubicin<sup>[159]</sup>. In the *in vitro* studies on cholangiocellular carcinoma cells we found that bortezomib shows over-additive antitumoral effects when combined with multikinase inhibitors like sorafenib or histone deacetylase inhibitors, such as MS-275<sup>[125]</sup>.

## CONCLUSION

Targeted-therapies, which specifically inhibit growth factor receptors and their related signaling pathways, are promising approaches for the innovative medical treatment of biliary tract and gallbladder cancers. In particular, antiangiogenic strategies as well as combination treatments with cytostatics have proved particularly efficient, as they leave fewer mechanisms of escape for the tumor cells. Combinations of these targeted drugs are especially intriguing, and in the future multi-kinase inhibitors such as sorafenib will be combined with other growth factor receptor inhibitors, proteasome inhibitors, histone deacetylase inhibitors, farnesyltransferase inhibitors or cytostatics to effectively control advanced biliary tract or gallbladder cancers. The advantage of such novel combination therapies is their higher tumor cell specificity and higher efficacy, combined with acceptable toxicity and side effects. These novel combination treatments will widen the therapeutic spectrum for biliary tract and gallbladder cancers; the results of (ongoing) clinical studies are eagerly awaited.

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