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Signaling Pathways as Therapeutic Targets in Biliary Tract Cancer

Jennifer Yang[1], **Matthew R. Farren**[2], **Daniel Ahn**[3], **Tanios Bekaii-Saab**[3], **Gregory B. Lesinski**[2]

[1]Molecular Cellular and Developmental Biology Graduate Program. The Ohio State University, Columbus, OH 43210

[2]The Winship Cancer Institute of Emory University, Atlanta, GA, 30322

[3]Mayo Clinic, 5777 E. Mayo Blvd, Phoenix, AZ, 85054

Abstract

Introduction: The incidence of biliary tract cancer (BTC) is increasing, and the disease is frequently diagnosed during advanced stages, leading to poor overall survival. Limited treatment options are currently available and novel therapeutic approaches are needed. A number of completed clinical trials have evaluated the role of chemotherapy for BTC, demonstrating a marginal benefit. Thus, there is increased interest in applying targeted therapies for this disease.

Areas Covered: This review article summarizes the role of chemotherapeutic regimens for the treatment of BTC, and highlights key signal transduction pathways of interest for targeted inhibition. Of particular interest are the MEK or MAP2K (mitogen-activated protein kinase kinase), phosphatidylinositol-3 kinase (PI3K) and signal transducer and activator of transcription-3 (STAT3) pathways. We discuss the available data on several promising inhibitors of these pathways, both in the pre-clinical and clinical settings.

Expert Opinion: Future treatment strategies should address targeting of MEK, PI3K and STAT3 for BTC, with a focus on combined therapeutic approaches.

Keywords

biliary tract cancer; cholangiocarcinoma; targeted therapy; STAT3; PI3K; MEK

1.0 Introduction

Biliary tract cancer (BTC) is comprised of intra- and extrahepatic cholangiocarcinoma and cancers of the gallbladder. This malignancy results from the transformation of epithelial cells that line the bile duct tree. The 5-year survival rate for this rare malignancy is dismal at only 3.2% [1]. Surgery represents an option for a subset of patients with BTC, especially those without metastases or invasion of nearby tissues. Approximately 65% of patients with

To whom correspondence should be addressed: Gregory B. Lesinski, Department of Hematology and Medical Oncology, The Winship Cancer Institute of Emory University, Building C, Room C3090, 1365 Clifton Rd. NE, Atlanta, GA 30322, USA. Tel.: (404) 778-3072; gregory.b.lesinski@emory.edu.

this malignancy are eligible to undergo surgical resection, although only 50% of those who underwent surgery achieved curative or margin-free resection (R0) [2]. Gallbladder cancers present with distant metastatic disease in approximately 85% of patients upon recurrence, whereas 60% of cholangiocarcinoma patients present primarily with a local regional pattern upon disease recurrence [3]. These data underscore the urgency for developing improved therapeutic options in this disease. Here, we review approaches for the treatment of BTC and highlight ongoing pre-clinical studies that may provide new therapeutic opportunities, with a focus on signal transduction pathways.

2.0 Combination chemotherapy treatment options in BTC

Over the last three decades, more than 50 articles reporting the results of clinical trials in BTC have been published (Table 1) [4–66]. Of these, the majority have focused on evaluation of chemotherapeutic agents. Thirty trials utilized gemcitabine (Gem)-based regimens: 7 trials included combination therapy with cisplatin (Cis), 4 with capecitabine (Cap), 9 with oxaliplatin (Ox) and 4 with S-1, oral fluoropyrimidine. In a single arm phase II study, Knox *et al* evaluated the combination of gemcitabine and capecitabine for 75 patients with BTC, of which 22 had objective responses with a median PFS and OS of 6.2 and 12.7 months, respectively [15]. Gemcitabine plus oxaliplatin (GEMOX) has also been evaluated in a phase II study of 56 patients (n = 19 GBC, n = 5 ECC, n = 3 ampulla of vater, n = 29 ICC). This trial reported a response rate of 36% in 33 patients who had not received prior treatment. These individuals demonstrated median PFS and OS of 5.7 and 15.4 months, respectively [24]. Based on the promising activity observed with the combination of gemcitabine and platinum based therapy in earlier trials, ABC-02, the largest randomized phase III trial in BTC to date, was conducted to investigate the efficacy of these agents in patients with unresectable BTC. In this study, 410 patients with locally advanced or metastatic disease, including all anatomic subgroups (cholangiocarcinoma, gallbladder and ampullary) were randomized to receive gemcitabine and cisplatin (GemCis) or gemcitabine alone, with overall survival (OS) as the primary endpoint. The combination of GemCis resulted in increased median OS (11.7 months) compared to patients treated with single agent Gem (8.1 months). GemCis also resulted in an increased median progression-free survival (PFS) of 8 months in patients receiving the combination as compared to 5 months for patients treated with Gem alone [59]. However, a more recent pooled analysis of 104 trials did not demonstrate any significant benefit of GemCis in either time to tumor progression (TTP), or median OS as compared to GemCap or GEMOX [67]. Though a phase III randomized trial would be necessary to access clinical advantages between the different gemcitabine-based regimens, GemCis has become the standard approach in treating locally advanced or metastatic BTC based on the data from the ABC-02 trial. Finally, clinical activity has been observed for advanced BTC with single agent, oral fluorpyrimidine, S-1 in the setting of a Phase II trial [12]. The combination of S-1 with gemcitabine also showed favorable activity in a randomized phase II trial versus S-1 alone with an acceptable safety profile [65]. These data have led to a randomized Phase III study of gemcitabine and S-1 that is powered to assess non-inferiority against the current standard of care consisting of gemcitabine and cisplatin [65]. Taken together, there are a number of

ongoing clinical trials utilizing chemotherapy that will provide important data upon completion (Table 2).

3.0 Novel targeted therapies in the treatment of BTC

While up to 80% of BTC patients experience some benefit from chemotherapy, the great majority ultimately develop resistance, emphasizing the need to identify more efficacious therapies. Further, novel targeted therapies may offer greater tolerability in cancer patients. This premise is centered around the idea that malignant cells may be more dependent on the activation of the targeted pathways [68–70]. Chemotherapeutic agents typically target rapidly proliferating cells irrespective of whether they are malignant or normal. For this reason, adverse events often manifest as fatigue, diarrhea, and nausea [71], and are prevalent in the majority of patients during the course of chemotherapy. Ongoing efforts to improve BTC treatment regimens can be seen in the context of numerous ongoing clinical trials (summarized in Table 3). A growing number of trials are focused on small molecule or Ab-based targeted therapies that inhibit intracellular signal transduction pathways, kinases, survival pathways or receptors (i.e. VEGF, EGFR) [28] [46].

However, resistance to targeted therapy remains a concern, due to the cross-talk and redundancy in the maintenance of key pathways in this disease [72]. Various studies looking at known oncogenic drivers in BTC (summarized in Table 4) [73–91], as well as whole-exome sequencing of responders to single-agent selumetinib (MEK inhibitor) [92], all failed to identify any common mutational signatures, highlighting the heterogeneous nature of the disease.

Since current treatment options for BTC patients have shown limited improvement in PFS and OS, testing of new potential targeted therapies continues. Candidate pathways of interest for targeted therapy will be discussed in further detail below, with a focus on inhibitors targeting the MAPK, PI3K, and JAK/STAT pathways.

3.1 MEK Inhibitors

The Ras/Raf/MEK/ERK signaling cascade is among the most commonly dysregulated pathways in human cancers [93]. MEK, also known as MAP2K (mitogen-activated protein kinase kinase), is part of the Ras/Raf/MEK/ERK (MAPK) pathway. Depending on the stimulus, activation of this pathway can result in context-dependent effects on apoptosis or the cell cycle [94]. Aberrant activation of this pathway is frequently observed in BTC, as well as in melanoma, lung carcinoma, pancreatic, and colon cancers, among others [81, 95]. Although several studies have documented *BRAF* mutations in BTC [75, 77, 78], the existing evidence indicates a number of potential advantages to targeting MEK rather than its upstream mediators of activation, such as B-Raf. First, inhibition of MEK signaling can be accomplished without genetic testing to identify mutations leading to the aberrant activation of this pathway, as certain B-Raf inhibitors in the presence of *RAS* mutations can lead to reactivation of Raf and development of resistance necessitating such genetic screening [96]. Second, MEK1/2 have a narrow substrate specificity [95], and are only known to activate ERK1/2 [97], whereas there are 3 families of Raf proteins and ERK1/2 has numerous downstream targets [98]. Accounting for the properties of the proteins

involved, MEK represents a point of convergence for many signaling pathways, thereby making it an attractive target for mitigating the effect of pathway activation.

With the exception of E6201, most MEK inhibitors do not target ATP binding. This allows a relatively higher specificity, as ATP binding sites tend to be highly conserved [99]. Indeed, the structure of MEK1 and MEK2 allows allosteric inhibitors to bind in a hydrophobic pocket which does not overlap with the ATP-binding site [100]. A summary of the MEK inhibitors being used in both preclinical studies and in clinical trials is provided in Table 5 [95, 96, 99, 101–166]. Trametinib, a well-studied MEK inhibitor, was approved by the FDA in 2013 after a phase III trial demonstrated superior efficacy over standard chemotherapy in melanoma patients with *BRAF V600E/K* mutations. Patients who received trametinib had a median PFS of 4.8 months, versus 1.5 months for those on chemotherapy ($p < 0.001$). The OS at 6 months was 81% on trametinib compared to 67% on standard chemotherapy [134]. This MEK inhibitor is currently being evaluated in the setting of a phase I clinical trial for BTC patients ().

The MEK inhibitor MEK162 has been evaluated for safety in a phase I dose-escalation study of advanced solid tumors, and showed signs of clinical efficacy and desirable pharmacokinetics. This agent had an acceptable safety profile at 60 mg twice daily [167]. This small molecule is currently under investigation for BTC in combination with GemCis in a phase I/II clinical trial (NCT 01828034). One encouraging phase II study in metastatic BTC patients using selumetinib (another MEK inhibitor), observed clinical activity in 28 patients, with a median PFS of 3.7 months, and median OS of 9.8 months. Interestingly, the clinical activity of selumetinib in this cohort of patients was not associated with *BRAF* or *KRAS* mutations (as assessed by pre-treatment sequencing of tumor biopsies)[168]. Another notable observation from this clinical trial was the fact that administration of selumetinib led to a gain in lean muscle mass in BTC patients. These data imply that MEK inhibitors may provide some benefit to patients by limiting the cancer cachexia syndrome that accompanies BTC and other advanced malignancies. Pre-clinical studies using MEK162 in a classic model of colon-26 cancer cachexia confirmed that the muscle sparing effects of MEK inhibitors can occur in a manner independent of their action on the tumor cells [169].

MEK inhibition appears to be an effective therapy in a small subset of BTC patients. However, the overall effects on clinical response have been modest. That said, several ongoing clinical trials using MEK inhibitors in combination with chemotherapeutic agents in BTC may establish their role in the treatment of this disease and its utility as an agent to combat the cancer cachexia syndrome deserves further investigation (Table 3).

3.2 PI3K Inhibitors

Phosphotidylinositol-3 kinase (PI3K) is the first of the downstream proteins regulated in the PI3K/Akt pathway following activation of associated receptors. This pathway is important for numerous biological functions in malignant cells, including proliferation, senescence, and survival [170]. Disruption in the regulation of this pathway has been associated with up to one-third of all cancers [171–173], and has demonstrated a prominent role in BTC [174–178]. Constitutive activation of PI3K can occur via several mechanisms including genomic alterations in *PIK3CA* or *PTEN* [179, 180]. Due to the extensive involvement of the PI3K

pathway in different components of cell-cycle regulation and survival, there are ongoing efforts to develop and test inhibitors specific to this pathway. Table 5 lists both PI3K inhibitors in pre-clinical testing and those currently in clinical trials [96, 105, 107, 135, 139–164, 181]. Buparlisib is one orally bioavailable pan-class I PI3K inhibitor that was well tolerated in patients as a single agent. In combination with other chemotherapeutic agents, buparlisib demonstrated clinical activity in patients with advanced breast cancer [135]. Another PI3K inhibitor GDC-0941 has been tested concurrently with GDC-0973 (a MEK1/2 inhibitor). The regimen was well-tolerated, and also showed clinical responses in patients with melanoma, pancreatic cancer, NSCLC, prostate cancer, and endometrioid cancer [135]. Results from this combination are encouraging for future studies incorporating inhibitors of MEK and PI3K.

To date, inhibitors directly targeting PI3K activity have not been utilized extensively as either single agents, or as combination treatment in the context of clinical trials for BTC patients. However, one clinical trial in BTC patients was recently completed (), utilizing an Akt inhibitor (MK2206) to limit the activation of this pro-survival pathway. Results from this trial indicated this drug was tolerable, but no clinical activity was observed [11]. Thus, targeting the PI3K/Akt signaling pathway may be a potential strategy to overcome resistance if combined with other agents. Thus this pathway represents an interesting target for BTC that deserves more rigorous pre-clinical and potentially clinical evaluation.

3.3 STAT3 Inhibitors

Another pathway relevant to BTC is the JAK/STAT signaling cascade [89, 182, 183]. There are 7 Signal Transducer and Activator of Transcription (STAT) family members (STAT1–4, STAT5a, STAT5b, STAT6), all transcription factors. Though structurally similar, these proteins have distinct cellular functions. Of the STAT proteins, STAT3 and STAT5 most frequently undergo constitutive activation in malignancy [184–186]. These proteins act in an oncogenic manner and promote the expression of genes that enhance metastatic spread and survival [187–189]. STAT3 activation has been observed in numerous human cancer cell lines, including BTC, and is thought to act downstream of IL-6 or other cytokines to promote progression of the disease [190–195]. The diverse pro-tumorigenic cellular functions regulated by STAT3 signaling makes this pathway an attractive target. Agents targeting upstream JAK have also been of interest as a way of mediating STAT3 signaling, especially in myeloproliferative neoplasms given a high frequency of activating $JAK2^{V617F}$ mutations. Ruxolitinib is a JAK1/2 inhibitor, and has undergone several phase I, II, and III clinical trials and is approved in the treatment of myelofibrosis based on significant improvement in splenomegaly [196, 197]. However, in a randomized, double-blind phase II trial in patients with metastatic pancreatic cancer that failed gemcitabine therapy, ruxolitinib combined with capecitabine did not demonstrate a significant improvement in OS or PFS [198]. Furthermore, a recent phase III trial combining Jakafi (JAK1/2) with capecitabine was discontinued after interim analyses in pancreatic cancer patients (). To date, JAK/STAT inhibitors have not been evaluated in human clinical trials for BTC.

Natural products have been one driving force in the development of STAT3 inhibitors, as many synthetic products are modified from natural compounds that were identified to inhibit

STAT3 [199]. The majority of STAT3 inhibitors have been designed in an effort to prevent its phosphorylation or STAT:STAT dimerization. A variety of naturally-derived and synthetic STAT3 inhibitors that target various STAT3 interactions are summarized in Table 5 [165, 166]. One major limitation to natural product-derived compounds has been their bioavailability, which has limited their *in vivo* efficacy.

Likewise, only two STAT3 small molecule inhibitors have undergone investigation in the clinical setting. OPB-31121 is an inhibitor of STAT3 phosphorylation developed by Otsuka Pharmaceuticals Co. This molecule was evaluated in a phase I clinical trial in patients with advanced HCC [165]. Twenty-four patients were enrolled, and 26% experienced stable disease≥ 8 weeks. The second,, BBI-608, is a small molecule STAT3 inhibitor developed by Boston Biomedical, Inc. BBI-608 inhibits STAT3 and can inhibit expression of genes involved in the cancer stem cell phenotype. This approach may be of interest for BTC, given the role of cancer stem cells in this disease [200–203]. Although not investigated formally for BTC, this aspect has been the primary focus of preclinical studies using BBI-608. *In vitro* studies demonstrate this agent has activity against prostate and pancreatic cancer cell lines. BBI-608 down-regulated β-catenin and c-Myc in pancreatic cancer at the level of protein expression [204] and decreased expression of these same mRNAs in prostate cancer cell lines [205]. Further studies showed that *in vivo* treatment of mice with BBI-608 strongly inhibited the growth of PC-3 prostate cancer xenografts [205]. In pancreatic cancer xenograft models using the PaCa-2 cell line, BBI-608 likewise slowed tumor growth as compared to mice treated with gemcitabine [204]. Several ongoing phase III studies are investigating the combination of BBI-608 with chemotherapy in multiple GI malignancies, including gastric, colon and pancreatic cancer (Table 5).

In addition to the role of STAT3 and β-catenin in regulating stemness, these proteins each play important roles in regulating immune evasion in advanced malignancy. This property may also be of interest in BTC, given the immunosuppressive features of disease. As mentioned previously, STAT3 plays an important role in regulating T cell phenotypes and the expansion of immunosuppressive myeloid cells [206]. With regards to β-catenin, a recent study by Spranger *et al.* reported that activated β-catenin signaling was associated with gene expression signatures in patient melanoma tumors indicative of limited T cell infiltration [207]. This and other studies suggest that targeting STAT3 and β-catenin may augment the efficacy immunotherapy. However, it is also appreciated that Wnt/β-catenin signaling plays a key, T-cell intrinsic role in balancing the generation of $CD8^+$ memory T cells that may be instrumental in maintenance of effective anti-tumor immune responses [208, 209]. Several ongoing clinical trials are utilizing BBI-608 in combination with chemotherapy, inhibitors of VEGF or MEK, and immune checkpoint inhibitors (Table 5) and will provide valuable information.

Another clinically relevant approach to STAT3 inhibition is using AZD9150, an antisense oligonucelotide. This agent has been tested in patients with advanced lymphoma and solid tumors. With promising results observed in two-thirds of patients with diffuse large B-cell lymphoma (DLBCL), AZD9150 is currently under evaluation in several other clinical trials. For example, phase I studies of AZD9150 as a single agent are ongoing in HCC patients (), as a single agent in phase II studies in ovarian and GI cancers () and in combination with

immunotherapies such as MEDI4736, an anti-programmed death-ligand 1 (PD-L1) inhibitor and tremelimumab, an anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibody () in diffuse large B cell lymphoma. Though no results from these clinical trials are available yet, the outcome will surely have significant implications on targeting STAT3 in the setting of BTC, given the role of this pathway in its pathology.

4.0 Resistance to Targeted Inhibition

Resistance to targeted, small-molecule therapies represents an ongoing challenge for numerous malignancies including BTC. Inhibition of the MEK and PI3K signaling pathways are particularly subject to resistance based on several published studies. For example, resistance to single agent MEK inhibition is mediated via both PI3K and STAT3 signaling pathways in BTC and other cancers, providing rational approaches to combined therapy [72, 210, 211]. Further, MEK inhibition results in MYC-dependent transcriptional upregulation of ERBB3, leading to cell intrinsic drug resistance in *KRAS* mutant tumors [212]. Similarly, resistance to PI3K inhibitors can occur via multiple mechanisms. These include cell-intrinsic events such as negative feedback loops mediated via downstream mTOR signaling or MYC accumulation resulting activation [213, 214]. Alternatively, upstream receptor tyrosine kinase activation can also occur, maintaining the signaling through this pathway [215].

Further contributing to resistance to these inhibitors are the inherent crosstalk between each of these signaling pathways. This multi-directional communication occurs by virtue of common features of both the receptors and their ligands. For instance, IL-6, GM-CSF, and EGF can activate MEK, PI3K, and STAT3 signal transduction [216, 217] as these cytokines activate kinases associated with each pathway. IL-6, an inflammatory cytokine, is highly elevated in patient BTC tumors and circulating blood, and is secreted from human BTC cell lines. This and other gp130 interacting cytokines can also function in an autocrine manner to signal simultaneously via the MEK, PI3K, and STAT3 pathways (Fig. 1). Thus, inhibition of any individual pathway has the potential to promote compensatory activation of other signaling nodes, ultimately leading to drug resistance. Other proteins can behave in a manner that activates redundant signal transduction between these pathways. For example, Src can activate STAT3, Raf-1, and A-Raf, while B-Raf can be phosphorylated independently of Src [94]. Similarly, activated Ras can interact with PI3K, leading to its activation [218], while the PI3K-TOR and STAT3 signaling pathways are functionally linked [219]. Besides signaling to downstream Raf, Ras can also activate PI3K by directly binding to the catalytic subunit [220–222], thus bypassing the need for EGFR activation, and rendering EGFR inhibition ineffective.

Inhibition of multiple signaling pathways, notably those that crosstalk and can act to compensate one another, represents a rational strategy in the treatment of BTC. Several recent studies have examined the mechanisms by which cancers may bypass the inhibitory effects of these drugs. *In vitro* resistance to the MEK inhibitor AZD6244 in BTC was overcome with the addition of Akt or mTOR inhibitors (MK-2206 and AZD8055), and was most likely effective due to inhibition of the feedback mechanism on the PI3K pathway [210]. Such resistance has also been observed in cancers with *KRAS* mutations [211]. Notably, the STAT3 pathway also functions as a mechanism of resistance to MEK inhibition

in *KRAS* mutated pancreatic and colon cancers [72]. Such compensatory signaling mechanisms showcase the need for concurrent targeting of these pathways to circumvent resistance.

5.0 Conclusion

Applying targeted therapeutic approaches to the treatment of BTC represents an area that remains largely unexplored. Given the complicated pathology and heterogeneity of BTC, inhibiting a single oncogenic signaling pathway is unlikely to elicit durable complete responses. Combined targeting of multiple signaling pathways may be an important strategy to improve efficacy and limit resistance. The small molecule inhibitors discussed also have the potential to be tested in combination with other modalities, including radiotherapy and immunotherapy. Given that MEK, PI3K, and JAK/STAT pathways may be involved in the radio-resistance or immune suppression observed in cancer patients, concurrent inhibition of these pathways may impact efficacy.

Expert Opinion

BTC is a refractory tumor that has poor outcomes. Chemotherapeutic regimens have been evaluated extensively in clinical trials and have provided only incremental benefit. Recent data has uncovered a role for many key pro-survival and inflammatory pathways that are candidates for targeting therapeutically. These findings hold strong potential for identification of new treatment approaches that may produce durable clinical activity in this aggressive malignancy. It is likely that targeting constitutively active signal transduction pathways could benefit patients with advanced biliary cancer, when they are administered together with chemotherapeutic approaches, or when applied in combination based on supportive pre-clinical data. Among the most notable of these pathways which can be subject to pharmacologic inhibition are the MEK, PI3K and STAT3 pathways. These pathways are particularly interesting based on their role in the malignant phenotype, the tumor microenvironment and immune dysregulation that occurs in BTC patients. However, additional pre-clinical data will be necessary to achieve the goal of translating combination therapy approaches into human clinical trials. In the coming years, it will be critical to adapt our understanding of cross-talk between these and other pathways to identify the most promising therapeutic combinations to move forward. Given the recent renaissance in immunotherapy, it is also desirable to identify small molecules that can be administered with the goal of potentiating the efficacy of these immune stimulatory modalities. This is especially important in light of a recent report indicating that adoptive immunotherapy can produce clinical activity in BTC [223].

One of many challenges to date in advancing therapy for BTC has been a lack of *in vivo* pre-clinical models that approximate human disease. The fact that most BTC patients diagnosed already possess metastatic disease also leaves little time to attempt multiple lines of treatment and learn in the clinical setting. Further, it is likely that because these tumors have heterogeneous etiology, genetic profiles, and anatomic location, development of relevant pre-clinical models will remain a challenge. These factors necessitate the need for a

collaborative, coordinated effort within the field to collect and study patient material whenever possible to gain the most information about this disease.

It is conceivable that small molecule inhibitors targeting MEK, PI3K and STAT3 will have utility as second line therapy in patients who receive limited benefit from chemotherapy and radiotherapy. This particularly relevant as the activation of these pathways could be altered in response to many of these conventional therapy approaches. However, a key endeavor will be to more carefully define how the order of treatment alters activation of these pathways, and importantly, the mechanisms of resistance to these modalities. It will also be critical to gain further information about resistance of patient tumors on an individual level, which could allow for prioritizing among the many pathways that could be targeted. As new treatment combinations targeting these pathways enter clinical trials, we must take several factors into consideration when planning both patient assessment and laboratory correlative studies. These include characterizing the safety profile, optimizing dose, schedule and potential for synergy or antagonism on tumor, immune and stromal cell compartments.

Several opportunities are available to fine-tune the use of these and other drugs to improve clinical outcomes for BTC patients in the future. First, we need to continue to improve our ability to classify patients in a more personalized manner via unique mutation profiles in the tumor. This may identify distinct subsets of patients with greater likelihood to respond to these targeted agents. In the coming years, coordinated efforts to conduct both targeted and unsupervised sequencing may identify key genetic features that could be used as prognostic or predictive markers in the clinical setting.

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* Denotes references of importance

** Denotes references of considerable importance

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Article Highlight Box

- Poor survival and limited treatment options for biliary tract cancer (BTC) demonstrate the need for novel therapeutic approaches.
- A summary of the various chemotherapeutic regiments of both published and ongoing clinical trials in BTC has been presented.
- Key signal transduction pathways are of interest for targeted therapy in BTC, with particular focus and rationale for targeting mitogen-activated protein kinase kinase (MEK), phosphatidylinositol-3 kinase (PI3K), and signal transducer and activator of transcription-3 (STAT3) pathways.
- Several small molecule inhibitors designed to inhibit MEK, PI3K, and STAT3 are available for potential application to BTC.

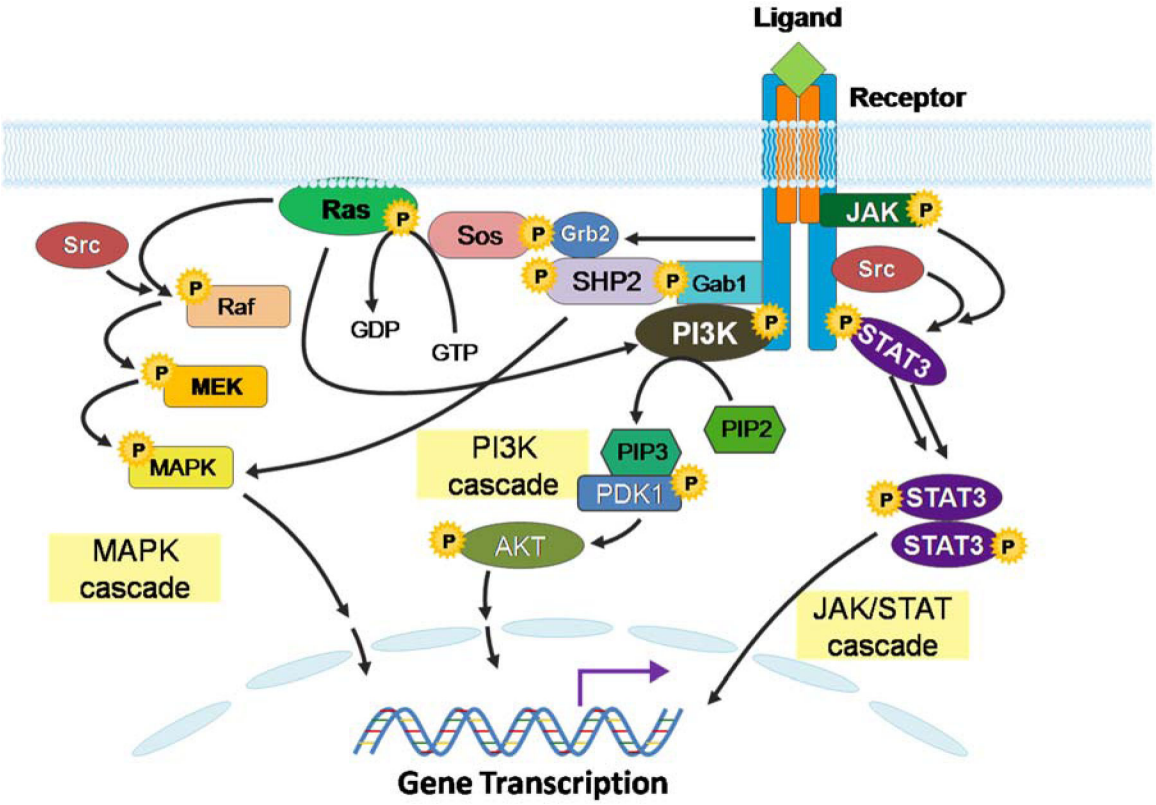

Figure 1.

Network of gp130-interacting signaling pathways. This figure illustrates the network of integral signaling pathways that are aberrantly activated in a variety of cancers, as well as in BTC. Mitogen-activated protein kinases (MAPK), phosphoinositide 3-kinase (PI3K), and Janus kinase/signal transducer and activator of transcription (Jak/STAT) signaling cascades cross talk and contribute to tumor growth, proliferation and treatment resistance.

Table 1.

Published clinical trials on BTC.

Single agent trials	Reference
Capecitabine	[4]
Docetaxel	[5, 6]
Erlotinib	[7]
Gemcitabine	[8, 9]
Irinotecan	[10]
MK2206	[11]
S-l	[12]
Sunitinib	[13]
Gemcitabine (Gem) combination based trials	**Reference**
Gem + capecitabine	[14–18]
Gem + cetuximab	[19]
Gem + cisplatin	[20–22]
Gem + docetaxel	[23]
Gem + oxaliplatin	[24–27]
Gem + oxaliplatin + bevacizumab	[28]
Gem + oxaliplatin + cetuximab	[29]
Gem + cisplatin + S-l	[30, 31]
Fluorouracil (5-FU) combination based trials	**Reference**
5-FU + cisplatin	[32]
5-FU + IFNα−2b	[33]
5-FU + leucovorin	[34–36]
5-FU + carboplatin + leucovorin	[37]
5-FU + cisplatin + epirubicin	[38]
5-FU + cisplatin + leucovorin	[39]
5-FU + doxorubicin + mitomycin C	[40]
5-FU + epirubicin + leucovorin + methotrexate	[41]
FOLFIRI + bevacizumab	[42]
Gemcitabine + 5-FU combination based trials	**Reference**
Gem + 5-FU + leucovorin	[43]
Gem + 5-FU + cisplatin + epirubicin	[44]
Gem + oxaliplatin + 5-FU	[45]
Other combination trials	**Reference**
Bevacizumab + erlotinib	[46]
Capecitabine + cisplatin	[47, 48]
Capecitabine + oxaliplatin	[49]

Single agent trials	Reference
Capecitabine + cisplatin + epirubicin	[50]
S-l + oxaliplatin	[51]
S-l + valproic acid	[52]
2-arm Comparison trials	**Reference**
5-FU vs. 5-FU + doxorubicin + mitomycin C	[53]
Gem vs. Gem + 5-FU + leucovorin	[54]
Mitomycin C + Gem vs. mitomycin C + capecitabine	[55]
5-FU + etoposide + leucovorin vs. 5-FU + cisplatin + epirubicin,	[56]
Gem vs. Gem + cisplatin	[57–59]
Gem + oxaliplatin vs. Gem + oxaliplatin + erlotinib	[60, 61]
Gem + oxaliplatin vs. Gem + oxaliplatin + cetuximab	[62]
Gem vs. Gem + sorafenib	[63]
5-FU vs. capecitabine vs. 5-FU + irinotecan vs. 5-FU + oxaliplatin vs. 5-FU + cisplatin vs. sunitinib	[64]
S-l vs. S-l + Gem	[65]
Photodynamic therapy (PDT) vs. PDT + S-l	[66]

5-FU - fluorouracil, FOLFIRI - irinotecan with fluorouracil and folinic acid, Gem - gemcitabine

Table 2.

Completed clinical trials in BTC.

Type of Trial	Patient Characteristics	Treatments	Outcome	Reference/ ClinicalTrials.gov Identifier
Phase I	Patients at risk of CCA recurrence	Gem + cisplatin + sirolimus (rapamycin)	Completed, results not yet posted	
Phase I/II	CCA	Gem + cisplatin + floxuridine	Completed, results not yet posted	
Phase II	Advanced and/or inoperable CCA	Gem + capecitabine	Completed, results not yet posted	
Phase II	Metastatic or unresectable biliary cancers	Erlotinib + bevacizumab	Median survival: 9.9 months; TTP: 4.4 months	
Phase II	CCA	Gem + capecitabine + bevacizumab	Completed, results not yet posted	
Phase I/II	CCA	Gem + cisplatin	Completed, results not yet posted	
Phase II	Advanced or metastatic BTC	Imatinib + 5-FU + leucovorin	Completed, results not yet posted	
Phase II	BTC	Gem + oxaliplatin vs. panitumumab + gem + oxaliplatin	Completed, results not yet posted	

5-FU - fluorouracil, CCA - cholangiocarcinoma, TTP - time-to-progression

Table 3.

Ongoing clinical trials in BTC.

Type of Trial	Treatments	ClinicalTrials.gov Identifier
Phase 0	HAI of FURD + dexamethasone vs. HAI of FURD + dexamethasone + gem vs. HAI of FURD + dexamethasone + gem + oxaliplatin	
Phase I	Acelarin + Cisplatin	
Phase I	DKN-01 + gem + cisplatin	
Phase I	HAI of FURD + dexamethasone + gem	
Phase I	Pazopanib + GSK1120212	
Phase I/II	Amphinex + gem vs. gem + cisplatin	
Phase I/II	ARQ 087	
Phase I/II	CX-4945 + gem + cisplatin	
Phase I/II	Cytokine induced killer cells	
Phase I/II	Gem + cisplatin + MEK162	
Phase II	Abraxane + gem + cisplatin	
Phase II	ASLAN001	
Phase II	*BGJ398 (pan FGFR kinase inhibitor)*	
Phase II	Cabozantinib	
Phase II	Ceritinib (LDK378)	
Phase II	FOLFIRINOX	
Phase II	Gem + cisplatin + selumetinib	
Phase II	Gem + oxaliplatin + capecitabine vs. gem + oxaliplatin + capecitabine + panitumumab	
Phase II	Gem + oxaliplatin + capecitabine + panitumumab vs. gem + oxaliplatin + catecitabine + bevacizumab	
Phase II	HAI of FURD + dexamethasone + gem + oxaliplatin	
Phase II	Low-dose radiation + gem + cisplatin	
Phase II	Nab-paclitaxel + gem	
Phase II	Pembrolizumab	
Phase II	Ramucirumab	
Phase II	Refametinib	
Phase II	Regorafenib	
Phase II	RRx-001 + gem + cisplatin	
Phase II	Sunitinib	
Phase Ha	Trametinib	
Phase II/III	Radiofrequency ablation + cytokine-induced killer cells (CIK) transfusion	
Phase III	Gem + cisplatin	
Phase III	Gem + oxaliplatin vs. capecitabine	
Phase III	Oxaliplatin	

5-FU - fluorouracil, FOLFIRI - irinotecan with fluorouracil (5FU) and folinic acid, FOLFIRINOX - FOLFIRI plus oxaliplatin, FURD - floxuridine, Gem - gemcitabine, HAI - hepatic arterial infusion.

Table 4.

Genetic alterations and abnormal protein expression found in BTC.

Gene	Percentage of patients	Function/Alterations	Reference
Proliferation			
KRAS	24.6% of BTC	Mutation at codon 12 or 13, constitutively active protein	[73]
	54% of BTC		[74]
	45% of BTC		[75]
	33% of BTC		[76]
	7.4% ICC		[77]
	5% of ICC and 23% of ECC		[78]
BRAF	22% of BTC	Mutations at various codons	[75]
	7.4 of ICC	V600E substitution, constitutively	[77]
	3% of ICC	active protein	[78]
EGFR	13.6% of BTC	Mutations at various codons	[79]
	13–15% of BTC		[80]
	26.5% of BTC		[81]
RASSF1A	69% of BTC	Promoter hypermethylation, decreased transcriptional activity	[82]
	68.75% of ECC		[83]
	27% of BTC		[84]
PIK3CA	8.2% of BTC	Gain of function mutation	[81]
	9% of ICC and 0% of ECC		[85]
Cell-cycle regulation			
SMAD4	45.2% of ICC	Loss of protein expression	[86]
CDKN2A (p16)	35.7% of ICC	Loss of protein expression	[86]
	16.1%, 57.1%, 20% of BTC	Hypermethylation, mutation, LOH	[87]
TP53	5% of ICC and 14% of ECC	G245S and R175H substitution, loss of protein function	[78]
	37% of ICC	Loss of protein function	[88]
Chronic inflammation			
SOCS3	27% of ICC	Promoter hypermethylation	[89]
Metabolism			
IDH1/2	23% of ICC and 0% of ECC	Mutation decrease protein function	[78]
	10% of ICC		[90]
	28% of ICC and 7% of ECC		[91]

BTC - biliary tract cancer, CDKN2A - cyclin-dependent kinase inhibitor 2A, ECC - extrahepaticcholangiocarcinoma, EGFR - epidermal growth factor receptor, ICC - intrahepatic cholangiocarcinoma, IDH1/2 - isocitrate dehydrogenase, LOH - loss of heterozygosity, PIK3CA – phosphatidylinositol−4,5-bisphosphate 3-kinase, RASSF1A - Ras association domain family 1 isoform A, SMAD4- mothers against decapentaplegic homolog 4, SOCS3 - suppressor of cytokine signaling 3,TP53- tumor suppressor protein p53

Table 5.

Inhibitors of MEK/PI3K/STAT3

Pathway	Name	Target	Clinical trial	Cancers	References
MAPK	PD098059	MEK1/2	Preclinical	Advanced hematological and advanced solid cancers	[101–104]
	U0126	MEK1/2	Preclinical	Advanced hematological and advanced solid cancers	[103–105, 107, 108]
	AZD8330	MEK1/2	Phase I	Advanced solid tumors	[109]
	E6201	MEK1	Phase I	Melanoma	[99, 110, 111]
	PD-0325901	MEK1/2	Phase I	Melanoma, NSCLC	[110, 112, 113]
	Pimasertib (AST03026)	MEK1/2	Phase I	Colorectal, multiple myeloma	[115]
	RO4987655	MEK1	Phase I	Melanoma	[116]
	RO5126766	Raf/MEKl/2	Phase I	Melanoma	[117]
	TAK733	MEK1/2	Phase I	Melanoma, NSCLC, colorectal, breast	[114, 118]
	MEK162	MEK1/2	Phase I/II	*NRAS* mutant melanoma, NSCLC, pancreatic, BTC	[119]
	Selumetinib (AZD6244)	MEK1	Phase I, II	Melanoma HCC, pancreatic, colon, lung, breast, NSCLC	[120–133]
	Refametinib (RDEA119)	MEK1/2	Phase II	HCC, melanoma, colorectal	[95]
	WX-554	MEK1/2	Phase II	Advanced solid tumors	[95]
	Trametinib (GSK1120212)	MEK1/2	Phase III	Melanoma, colorectal	[134]
	Cobimetinib (GDC-0973)	MEK1	Phase I, II, III	Advanced solid tumors, melanoma	[135–138]
PI3K	LY294002	PI3K and other related kinases	Preclinical	Fibrosarcoma	[105, 107, 139–141]
	PI-103	PI3K, mTORC1/2, DNA-PK	Preclinical	Glioma prostate, colon, NSCLC	[142–146]
	PWT-458	PI3K	Preclinical	NSCLC, glioblastoma, renal	[147, 148]
	Wortmannin	PI3K, mTOR, DNA-PK, MAPK	Preclinical	Advanced hematological and advanced solid cancers	[135, 142, 149]
	ZSTK474	PI3Ks	Preclinical	NSCLC, melanoma, ovarian, prostate	[150, 151]
	BAY 80–6946	PI3K(p110α, p)	Phase I	Lymphoma, esophageal, pancreatic	[135]
	GDC-0032	PI3K(pl00α,-δ,-γ)	Phase I	Breast, NSCLC	[135]
	GSK-2126458	PI3K, mTOR	Phase I	Renal cell, bladder	[152]
	IPI-145	PI3K(p110 δ, γ)	Phase I	Leukemia, lymphoma	[135]
	BEZ-235	PI3K, mTOR	Phase I, II	Breast, glioma, melanoma, pancreatic	[135, 153–156]
	BGT-226	PI3K, mTOR	Phase I/II	Solid tumors, breast	[157]
	BKM120 (Buparlisib)	PI3K	Phase I/II	Breast, glioblastoma, NSCLC	[135,158]
	BYL-719	PBK(p110 α)	Phase I/II	Breast, cervical, ovarian, head and neck	[135]

Pathway	Name	Target	Clinical trial	Cancers	References
	GDC-0941	PI3K(p110α),Flt3	Phase I/II	Lymphoma, NSCLC, breast, melanoma, pancreatic endometrial	[135, 159–161]
	GDC-0980	PI3K, mTOR	Phase I/II	Prostate	[135]
	PX-866	PI3K	Phase I/II	Glioblastoma, breast, colon, prostate, NSCLC, pancreatic, ovarian	[135, 162, 163]
	XL-147	PI3K	Phase I/II	NSCLC, solid tumors, glioblastoma	[96, 135]
	XL-765	PI3K, mTOR	Phase I/II	Glioma, NSCLC	[164]
	PF-04691502	PI3K, mTOR	Phase II	Endometrial	[135]
	PF-05212384	PI3K(p110 α, γ), mTOR	Phase II	Solid tumors, colon	[135]
	CAL-101 (Idelalisib)	PI3K(p110δ)	Phase III	Leukemias, lymphomas, myeloma	[135]
STAT3	OPB-31121	STAT3	Phase I	Advanced HCC	[165]
	AZD9150	STAT3	Phase I/II	Lymphoma, HCC, ovarian, GI	, , , , [166]
	BBI6018 (napabucasin)	STAT3	Phase I/II/III	Hematologic malignancies, colorectal, GI, pancreatic, HCC, glioblastoma, NSCLC, mesothelioma,	, , , , , , , , , , , , , , , ,

BTC - biliary tract cancer, DNA-PK - DNA-dependent protein kinase, Flt3 - Fms-related tyrosine kinase 3, GI - gastrointestinal, HCC - hepatocellular carcinoma, MEK - extracellular signal-regulated kinase (ERK) kinase, mTOR - mammalian target of rapamycin, NSCLC – non-small cell lung cancer,PI3K - phosphoinositide 3-kinase, STAT3 - signal transducer and activator of transcription 3