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Editorial Board Member of World Journal of Gastrointestinal Oncology, William Small, MD, Professor, Department of Radiation Oncology, Northwestern Memorial Hospital, Robert H. Lurie Comprehensive Cancer Center, Chicago, IL 60611, United States

AIM AND SCOPE

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If WJGO covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, molecular mechanisms, and therapy of benign and malignant tumors of the digestive tract. The current columns of WJGO include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of gastrointestinal oncology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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Emerging molecular targets and therapy for cholangiocarcinoma

Hamzeh Kayhanian, Elizabeth C Smyth, Chiara Braconi

Hamzeh Kayhanian, Elizabeth C Smyth, Chiara Braconi, Department of Gastrointestinal Oncology, Royal Marsden Hospital, London and Surrey SM2 5PT, United Kingdom

Chiara Braconi, Institute of Cancer Research, London and Surrey SM2 5PT, United Kingdom

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Core tip: Cholangiocarcinoma (CCA) is a clinically challenging malignancy; it is rare, molecularly heterogeneous and associated with a poor prognosis. Here we review recent data on the genomic landscape of CCA and highlight the results of clinical trials using targeted agents and immunotherapy. We find a number of promising therapeutic agents in development and discuss strategies to improve diagnosis and outcomes in this patient group.

Kayhanian H, Smyth EC, Braconi C. Emerging molecular targets and therapy for cholangiocarcinoma. World J Gastrointest Oncol 2017; 9(7): 268-280 Available from: URL: http://www.wjgnet.com/1948-5204/full/v9/i7/268.htm DOI: http://dx.doi.org/10.4251/wjgo.v9.i7.268

INTRODUCTION

Cholangiocarcinoma (CCA) is a relatively infrequent malignancy arising from epithelial cells lining the biliary tree. It is associated with poor prognosis and limited
standard therapeutic options. Globally incidence varies considerably according to geographical location with significantly higher rates in South-East Asia compared to Western countries. In North-East Thailand the incidence is high at 85 per 100000[5], whilst in the United States and United Kingdom the incidence is much lower at around 1-3 cases per 100000 population[2,3]. The typical age at diagnosis of CCA is around 70 years, with slightly higher incidence in men than women[4]. Survival depends on the stage of disease at presentation, but even in patients with localised disease, five-year survival is poor at 15% and 30% for intrahepatic (ICC) and extrahepatic (ECC) CCA respectively[5]. For unclear reasons the incidence of ICC is increasing in western countries whilst rates of ECC are falling internationally[6].

CLASSIFICATION
CCA is now classified according to anatomical location into ICC, perihilar and distal subtypes; the latter two are ECC tumours. Prior to this novel classification the terms intra- and extra-hepatic CCA predominated, and we will use this nomenclature for the purposes of this review. This anatomical classification is useful as in addition to guiding surgical management, it is increasingly recognised that ICC and ECC have differing molecular profiles and may arise from differing cells of origin. There is evidence to suggest that some ICCs arise from the hepatic stem cell lineage whilst other ICCs and most ECCs arise from the biliary tree stem cell lineage[7]. Understanding the differences in tumour aetiology and biology between CCA subtypes will help guide stratification of targeted therapies.

AETIOLOGY
Parasitic infection with the liver flukes *Clonorchis sinensis* and *Opisthorchis viverrini*, which are endemic in parts of South-East Asia, are strongly associated with development of CCA. In non-Asian countries most cases occur sporadically, however conditions causing bile stasis and chronic biliary inflammation are associated with CCA development. Specific risk factors include primary sclerosing cholangitis, hepatolithiasis, choledocal cysts and Caroli’s syndrome (congenital cystic dilation of intrahepatic bile ducts). All causes of liver cirrhosis potentially predispose to CCA and studies have identified viral hepatitis and alcoholic liver disease as specific risk factors. The now banned radiocontrast, thorotrast has also been associated with CCA development.

MANAGEMENT OF CCA
Localised disease
Surgery and locoregional therapy: Less than 40% of patients present with resectable disease[8] and 5-year survival rate for patients with completely resected bile duct and GBC is in the range of 20%-50%. Loco-regional failure occurs in more than half of patients, even in absence of residual disease (R0) and provides the justification for the study of adjuvant therapy.

Previously, the role of adjuvant chemotherapy for resected patients is not clearly defined. Phase III trials in this setting had not demonstrated a survival advantage in CCA, but these studies have included a range of tumour types (including pancreatobiliary, gallbladder and ampullary carcinomas) and have lacked sufficient power to identify a survival difference specifically in CCA[9,10]. However, recently the results of the UK BILCAP study have been presented in abstract form. This large phase III randomised trial recruited patients with resected biliary cancer including 368 (plus 79 gallbladder carcinomas) cholangiocarcinoma patients and randomised between no adjuvant therapy or 6 mo of oral capecitabine. Patients treated with capecitabine had improved overall survival [53 mo vs 36 mo HR = 0.75 (95%CI: 0.58-0.97; P = 0.028)]. The results will lead to adjuvant capecitabine being adopted as a standard of care in resected biliary cancers.

Liver transplantation is not a standard treatment for CCA due historically high recurrence rates and donor shortage. More modern series, have reported more encouraging results[11]. Potential candidates, such as patients with poor hepatic reserve for extended hepatectomy or those with localised but unresectable perihilar CCA should be enrolled on to suitable clinical trials.

Locoregional therapies, including radiotherapy, photodynamic therapy, chemo/radio-embolisation and radiofrequency ablation may have a role in locally advanced disease or patients who are surgically unfit. There is a lack of comparative clinical trial evidence to support any of these modalities improving survival compared to standard of care chemotherapy[12]. However retrospective and phase II data suggest a promising rate of local control by adding radiotherapy in the management of ICC, and warrants further investigation[13,14].

Unresectable/metastatic disease
The United Kingdom phase 3 ABC-02 trial established cisplatin-gemcitabine combination therapy as standard of care for the first line treatment of advanced CCA, providing a clinically significant survival advantage compared to gemcitabine alone (median OS 11.7 mo vs 8.1 mo, HR = 0.64, 95%CI: 0.52-0.80, P < 0.001)[15]. There is currently no established second line treatment for advanced CCA and although a number of small phase II studies have shown disease activity, using single agent or doublet combinations of 5-FU, oxaliplatin, and gemcitabine, this has not been validated in a randomised trial[16]. Results from the ABC-06 (NCT01926236) phase III trial, investigating FOLFOX chemotherapy compared to supportive care in the second line setting are awaited.

Pathophysiology
Desmoplastic stroma: The tumour microenviron-
ment plays an important role in CCA pathogenesis. CCA bile ducts are typically surrounded by a dense hypovascular desmoplastic stroma, consisting of cancer associated fibroblasts (CAFs) expressing α-smooth muscle actin (SMA), activated macrophages and a fibrotic collagen rich extracellular matrix\(^\text{17}\). α-SMA positive CAFs are involved in CCA progression and tumours of patients expressing high levels of α-SMA have poorer survival\(^\text{18}\). CAFs produce a range of factors involved in autocrine and paracrine signalling, promoting oncogenic processes such as proliferation, invasion, metastasis and apoptosis evasion. Specifically, the factors produced by CAFs include periostin, tenascin-c, thrombospondin 1, Stromal cell derived factor 1 (SDF-1), Hepatocyte growth factor (HGF) and Wnt-inducible signalling protein-1v (WISP1)\(^\text{18}\). These factors interact with CCA cells to manipulate cell-signalling pathways. For instance periostin interacts with tenascin-c, HGF and SDF-1, which bind to their respective receptors, integrin, MET and CXCR4 on CCA cells, leading to activation of the PI3K/AKT signalling pathway. Cancer associated macrophages are also important in the stromal microenvironment and appear to have prognostic significance. In one study, high numbers of CD163⁺ macrophages in the stroma of resected ICC correlated with poor disease free survival\(^\text{19}\). Inflammatory macrophage infiltrates in CCA are also associated with increased WNT signalling, and abrogation of WNT signalling in preclinical models inhibits CCA growth\(^\text{20}\). In cholangiocarcinoma, sustained interleukin-6 (IL-6) signalling which promotes tumour growth via autocrine mechanisms is also associated with increasing fibrosis and dense stroma formation; it is recognised that this dense hypovascular stroma poses a challenge to cytotoxic drug delivery\(^\text{18}\). Therefore targeting stromal factors involved in cholangiocarcinogenesis or improving drug delivery through the desmoplastic stroma are attractive targets for novel therapeutics.

**Table 1 Mutation frequency for intrahepatic and extrahepatic cholangiocarcinoma**

| Genetic mutation | Frequency (%) in all tumours tested | Ref. |
|------------------|-----------------------------------|------|
|                  | Intrahepatic CC | Extrahepatic CC |
| IDH1/2           | 14%-36%          | 0%     | [27,59,67,99-101] |
| BAP1             | 9%-25%           | 4%-10% | [66,67,101] |
| KRAS             | 9%-24%           | 40%-47%| [62-64,96] |
| TP53             | 3%-38%           | 18%-45%| [62-64,96] |
| PBRM1            | 11%-17%          | 4%-11% | [63,97] |
| ARID1A           | 11%-36%          | 5%-16% | [62-64,95] |
| EGFR amplification | 7%               | 0%     | [101] |
| HER2             | 0%-2%            | 0%-20% | [67,101] |
| VEGF overexpression | 42%             | 31%    | [46,47] |
| PIK3CA           | 4%-6%            | 9%     | [66,101] |
| BRAF             | 4%-22%           | 6%     | [57,101-103] |
| FGFR translocation | 6%-50%          | 0%-5%  | [66] |
| MCL1 amplification | 16%-21%         | NR     | [66] |
| PTEN             | 1%-11%           | 4%     | [59,101] |
| FBXV7            | 1%-6%            | 4%-15% | [67] |
| CDK6             | 7%               | NR     | [66] |
| CDKN2A           | 7%               | 15%    | [66] |
| BRCA 1/2         | 4%               | NR     | [66] |
| SMAD4            | 1%-4%            | 11%-25%| [59,67,101] |
| mTOR             | 26%              | 40%    | [67] |

**MOLECULAR CHARACTERISATION AND POTENTIAL FOR TARGETED THERAPIES**

With recent technological advances in genomic sequencing, the mutational landscape of CCA is increasingly understood. Careful evaluation is needed to determine which genetic aberrations are true drivers of CCA. This section will review recent data on key genetic abnormalities thought to be implicated in CCA pathogenesis. There are clear differences in the prevalence of known oncogenic driver mutations between ICC and ECC, implicating distinct processes of oncogenesis for these tumour subtypes (Table 1). However it is also noteworthy that the prevalence of mutations is highly variable across studies, this heterogeneity may be reflective of regional variation, small sample size, or differences in the pathological classification of ICC and ECC prior to sequencing.

Also of interest is that liver fluke related CCA is associated with a differing pattern of genetic mutations compared to non-fluke CCA. In one of the first studies to sequence CCA, 8 liver fluke CCA were analysed, revealing novel mutations in SMAD4 (17%), MLL3 (15%), ROBO2, GNAS and RNF (9%) each and CKDN2A and PEG3 (5%) each\(^\text{21}\). In a subsequent follow up study of 208 CCA cases (108 caused by liver fluke O. viverrini), TP53 mutations were more often seen in fluke related CCA, whilst IDH1/2 and BAP1 mutations were more common in non-fluke CCA\(^\text{22}\). This highlights the impact of environmental risk factors on the pattern of somatic mutations. The prognostic value of several somatic mutations seen in CCA has been evaluated, however results are conflicting.

Interestingly IDH1/2 (Iscitrate dehydrogenase) mutations are seen almost exclusively in ICC. The IDH mutation results in reduced normal function of this enzyme and leads to increased production of 2-hydroxyglutarate (2-HG) from alpha-ketoglutarate. 2-HG is considered an oncometabolite and causes epigenetic changes, including histone and DNA methylation, which promotes tumour development\(^\text{23}\). In one study of 326 patients with resected ICC, IDH1/2 mutations were associated with improved overall survival\(^\text{24}\), however another whole exome sequencing study (\(n = 32\)) suggested worse overall survival for patients with these aberrations (3 year OS 33% mutant vs 81% wildtype, \(P = 0.003\))\(^\text{25}\), however in this study a higher proportion of patients with IDH mutations had advanced disease (50% vs 15%). Two other studies
examining the effect of IDH mutations in patients with resected and advanced ICC demonstrated no significant association with prognosis\(^26,27\). Pre-clinical data suggesting oncogenic addiction to IDH signalling can be pharmacologically abrogated resulting in control of cancer cell growth has been demonstrated in IDH mutant glioma lines\(^28\). Inhibitors of mutant IDH1 and IDH2 are currently in clinical trials (Table 2); early results for AG-120 which is an IDH1 inhibitor demonstrated tolerable toxicity with evidence of pharmacodynamic endpoint modulation with reduced circulating levels of 2-hydroxyglutarate were observed in most patients\(^29\). Of twenty CCA patients treated, one (5%) had a partial response whereas 11 (55%) had stable disease.

The genes \(BAP1\), \(ARID1A\) and \(PBRM1\), which are involved in chromatin remodelling, have been found to be frequently mutated in ICC and in one study had inactivating mutations in just under half \((n = 15/32)\) of ICC cases\(^25\). Whether these mutations can predict sensitivity to the histone deacetylase inhibitor vorinostat, which targets chromatin regulation has not yet been determined. However in a preclinical study vorinostat did show anti-cancer activity against the HuCC-T1 human CCA cell line\(^29\).

### Epidermal growth factor receptor alterations as a target in CCA

The epidermal growth factor receptor (EGFR) is abnormally activated in a number of human cancers and is therapeutically targeted using monoclonal antibodies or tyrosine kinase inhibitors\(^30\). EGFR targeted agents have demonstrated clinical efficacy in non-small cell lung cancer (NSCLC), colorectal cancer and SCC of the head and neck, where they are now established standard therapies. In NSCLC EGFR gene mutation, predicts response to EGFR inhibition\(^31\). Both activating mutations and amplifications of the \(EGFR\) gene have been observed in CCA. \(EGFR\) mutations have been observed in 10%-15% of CCA\(^32-36\), however due to small sample numbers data are conflicting regarding whether prevalence is higher in ICC or ECC. \(EGFR\) overexpression appears to be more prevalent in ICC (11%-27%) compared to ECC (5%-19%)\(^36,37\). Prognostically, EGFR expression has been found to be a negative predictor of overall survival in CCA\(^37,38\), making this an attractive target for drug intervention. There have been 3 reported phase II trials investigating anti-EGFR monoclonal antibodies (mABs) in CCA but none have demonstrated survival advantage in this patient group. In a single arm phase II trial of 30 patients with advanced biliary tract cancer (abTC) treated with first-line GEMOX-cetuximab, Gruenberger et al reported an objective response rate (CR + PR) of 63%, mOS of 15.2 mo (9.9-20.5) and 9 patients were able to undergo potentially curative resection following systemic therapy. Whilst this trial did not have a control arm and patients were unselected for EGFR expression or \(KRAS\) status, the reported response rate was considered encouraging\(^39\). Subsequently in the

### Table 2  Clinical trials of novel agents in cholangiocarcinoma

| Title                                                                 | Target                          | Phase | Estimated sample size | Expected completion date | Trial number   |
|----------------------------------------------------------------------|---------------------------------|-------|-----------------------|--------------------------|---------------|
| CX-4945 in combination with gemcitabine and cisplatin for frontline treatment of CCA | Casein kinase 2                 | I/II  | 100                   | Dec 2016                 | NCT02128282  |
| BGJ398 in patients with advanced CCA and FGFR gene fusion            | FGFR gene fusion                | II    | 55                    | Jul 2018                 | NCT02150967  |
| Dasatinib in IDH1-mutant advanced ICC                                | Epigenetic modifications        | II    | 19                    | Sep 2022                 | NCT02428855  |
| RRs-001 in second line treatment of advanced CCA prior to readministration of first line therapy | Pan-HER inhibitor               | II    | 30                    | May 2018                 | NCT0245970   |
| ASLAN001 in advanced CCA who progressed on at least 1 line of therapy | Multi-kinase inhibitor          | II    | 25                    | Dec 2017                 | NCT02609958  |
| Regorafenib as single agent in advanced CCA who failed first line    |                                 | II    | 37                    | Feb 2018                 | NCT02053376  |
| Copanlisib in combination with gemcitabine and cisplatin in advanced CCA | PI3K inhibitor                  | II    | 25                    | Dec 2018                 | NCT02631590  |
| LDK378 in ROSI/ALK overexpressed advanced CCA                        | ROSI and/or ALK                 | II    | 34                    | Jul 2018                 | NCT02374489  |
| AG120 in advanced solid tumours with IDH1 mutation                   | IDH1                           | I     | 145                   | May 2016                 | NCT02073949  |
| Study of L2801653 in advanced cancer                                | MET inhibitor                   | I     | 190                   | Nov 2017                 | NCT01285037  |
| ABC-08: Acelarin in combination with cisplatin in locally advanced/metastatic biliary tract cancers | Nucleotide analogue             | I     | 24                    | Sep 2018                 | NCT02352765  |
| Ramucirumab for advanced pre-treated biliary cancers                 | VEGFR2 antagonist               | II    | 50                    | Dec 2019                 | NCT02520141  |
| Keynote-158: Pembrolizumab in participants with advanced solid tumours | PDL-1 inhibitor                 | II    | 1100                  | Mar 2021                 | NCT02628067  |
| Immunotherapy using TILs for patients with metastatic cancer         | Adoptive T-cell therapy         | II    | 33                    | Dec 2019                 | NCT01174121  |

CCA: Cholangiocarcinoma; EGFR: Epidermal growth factor receptor; FGFR: Fibroblast growth factor receptor; IDH: Isocitrate dehydrogenase; PDI: Programmed death 1; TIL: Tumour infiltrating lymphocytes; VEGF: Vascular endothelial growth factor.
phase II BINGO trial, 150 patients were randomised to first-line gemcitabine and oxaliplatin (GEMOX) with or without the addition of cetuximab\(^{[40]}\). Median overall survival was numerically shorter in the GEMOX-cetuximab group at 11.0 mo compared to 12.4 mo in the GEMOX group indicating lack of benefit from cetuximab. Neither \textit{EGFR} overexpression (18 of 77 cases, 23\%) nor \textit{KRAS} mutation (14 of 75 cases, 19\%) was associated with patient outcome in either treatment group. More recently, Leone et al showed that the anti-\textit{EGFR} mAb panitumumab when used in combination with GEMOX chemotherapy in patients with \textit{KRAS}-WT \textit{aBTC} produced no improvement in overall survival when compared to chemotherapy alone (9.9 mo vs 10.2 mo, \(P = 0.42\))\(^{[41]}\). The small molecule \textit{EGFR} tyrosine-kinase inhibitor erlotinib has also been investigated in advanced CCA. In a randomised phase III trial of 268 patients with \textit{aBTC} (CCA, gallbladder and ampullary cancer) there was no significant difference in the primary end-point, progression free survival (PFS) in patients receiving GEMOX with or without erlotinib (5.8 mo vs 4.2 mo, HR = 0.80, 95\%CI: 0.61-1.03, \(P = 0.83\))\(^{[42]}\). However the objective response rate (CR + PR) was higher in the erlotinib group (40 vs 21 patients, \(P = 0.005\)) and in the subgroup of patients with CCA, PFS was longer in the erlotinib group (5.9 mo vs 3.0 mo, HR = 0.73, 95\%CI: 0.53-1.0, \(P = 0.049\)). \textit{EGFR} overexpression was reported in 12 of 60 patients (43\%) in the erlotinib group and of these there was 1 PR and 7 cases of SD. In summary trials investigating anti-\textit{EGFR} therapy in CCA have to date failed to demonstrate any clinically meaningful benefit over standard of care chemotherapy. The caveat to interpretation of these trials is that the inclusion of heterogenous, non-biomarker selected groups of biliary tract cancers may obscure any real survival benefit in smaller patient subsets; biomarker selected studies might be preferred for this reason.

Human epidermal growth factor 2 (\textit{HER2}) or Erb-B2 Receptor Tyrosine Kinase 2 (\textit{ERBB2}) amplification is rare in ICC, but may be present in up to 20\% of \textit{ECC}\(^{[37,43]}\) and this target has been successfully targeted in breast and gastroesophageal cancer patients. Currently only anecdotal reports\(^{[44]}\) are present in the literature regarding responses to anti-\textit{HER2} therapy in CCA, however a molecularly selected trial of trastuzumab in conjunction with GEMOX is ongoing (NCT02836847), and it remains to be seen whether this approach will be beneficial.

\section*{Angiogenesis as a target in CCA}

Vascular endothelial growth factor (\textit{VEGF}) has been targeted therapeutically in a number of malignancies, with anti-\textit{VEGF} monoclonal antibodies demonstrating efficacy in colorectal, breast and ovarian cancers, whilst TKIs targeting the \textit{VEGF} receptor are in clinical use in renal cell carcinoma and hepatocellular carcinoma\(^{[45]}\).

\textit{VEGF} expression is reported in around 30\%-40\% of \textit{CCAs} and correlates with increased lymph node metastasis and poorer survival\(^{[46,47]}\), making angiogenesis an attractive target in CCA. Bevacizumab, a humanised monoclonal antibody against \textit{VEGF}-A has been investigated in CCA. Zhu et al conducted a single arm phase II trial and treated \textit{aBTC} patients with first-line GEMOX plus bevacizumab\(^{[48]}\). They reported a median PFS of 7.0 mo, mOS of 12.7 mo and ORR of 40\%, which was considered favourable compared to historical controls. Similar results were found in a phase II trial combining gemcitabine and capecitabine with bevacizumab in patients with \textit{aBTC} where a median PFS of 8.1 mo and OS of 11.3 mo was reported\(^{[49]}\). Combining \textit{VEGF} and \textit{EGFR} inhibition has not significantly improved outcomes. Lubner et al used a combination of bevacizumab and erlotinib in a phase II trial of 49 patients with \textit{aBTC}\(^{[50]}\). This trial reported a PR in 6 (12.2\%) patients in whom the median duration of response was 8.4 mo and the reported mOS was 9.9 mo. However, overall survival in these studies (10-13 mo) did not reasonably exceed expectations for a phase II population and no control arm was available for comparison. More recently disappointing results were reported from the randomised phase II ABC-03 trial, which investigated gemcitabine-cisplatin with either cediranib or placebo as first-line treatment of patients with \textit{aBTC}\(^{[51]}\). Cediranib, a tyrosine kinase inhibitor of \textit{VEGFR}-1-3 and with additional activity against \textit{PDGF} and c-\textit{KIT}, did not improve PFS compared to the control group (PFS 8.0 mo vs 7.4 mo, HR = 0.93, 95\%CI: 0.65-1.35, \(P = 0.72\)) or overall survival (14.1 mo vs 11.9 mo, HR = 0.86, \(P = 0.44\)). Response rates were higher for cediranib treated patients (44\% vs 19\% control). An interaction between baseline \textit{PDGF}bb levels and overall survival benefit from cediranib was noted; patients with \textit{PDGF}bb concentrations above the median derived an overall survival benefit from cediranib (\(P\) value for interaction 0.002).

Sorafenib, a multi-targeted TKI of \textit{VEGFR}-2/3, \textit{PDGFR}, \textit{BRAF} and \textit{CRAF} which may also be considered an anti-angiogenic agent has been investigated in CCA with disappointing results. As single agent therapy no clinically meaningful benefit was observed in 2 phase II trials, with reported PFS of 2.3 (range 0-12) and 3 (95\%CI: 2-4) mo respectively\(^{[52,53]}\). When sorafenib was added to gemcitabine and cisplatin chemotherapy, PFS and OS of 6.5 and 14.4 mo respectively was reported which was similar to historical controls using chemotherapy alone\(^{[54]}\). Finally a phase II trial combining sorafenib with erlotinib was closed early due to failure to meet pre-determined survival criteria and reported a disappointing PFS and OS of 2 (95\%CI: 2-3) and 6 (95\%CI: 3-8) mo respectively\(^{[55]}\).

In summary, despite encouraging early trial results, therapeutic targeting of angiogenesis has not been
Table 3  Fibroblast growth factor receptor fusions according to reported frequency in cholangiocarcinoma

| FGFR fusion partner | Frequency | Ref. |
|---------------------|-----------|------|
| FGFR2-AHCYL         | 7/102 (7%) | [56] |
| FGFR2-BICC1         | 2/102 (2%) | [56] |
| FGFR2-MGEA5         | 1/6 (17%)  | [62] |
| FGFR2-PPHLN1        | 17/107 (16%) | [57] |
| FGFR2-TCACC         | 1/6 (17%)  | [62] |
| FGFR2-FGFR1         | 1/28 (4%)  | [66] |
| FGFR2-KIAA1598      | 1/28 (4%)  | [66] |

FGFR: Fibroblast growth factor receptor.

successful in CCA, although using biomarkers such as PDGFbb may improve patient selection in future.

Fibroblast growth factor receptor fusions

The FGF pathway is involved in a number of cellular processes including proliferation, migration and angiogenesis. Abnormalities of this pathway have been implicated as driver events in carcinogenesis. In CCA, fibroblast growth factor receptor (FGFR) chromosomal translocations producing FGFR-fusion genes have been reported in both ICC and ECC, but are much more frequent in ICC (6%-50%) than ECC (0%-6%) (Table 3). The fusion protein is constitutively activated leading to downstream signalling though mitogen-activated protein kinase (MAPK) and PI3K/mTOR pathways.[56] Sia et al.[57] demonstrated in a cohort of 107 ICC patients that FGFR2 translocations represented the most common actionable target detected; these occurred in 16% of patients screened; this prevalence has been confirmed in other series.[58,59] One United States study has suggested that FGFR2 fusion in ICC is more common in females, and a Japanese study has implicated viral hepatitis infection in this pathway.[56,60] However, these findings require validation. FGFR2 translocation in CCA may confer a prognostic benefit. Cancer specific survival in one dataset for patients harbouring FGFR2 translocations was superior to non-translocated tumours (123 mo vs 37 mo respectively).[60] Preclinical work in cell lines and patient derived xenograft supports blockade of FGFR2 signalling in CCA as a potential effective therapy.[56,61] and early anecdotal reports of FGFR inhibitor therapy in FGFR2 translocated CCA patients have been encouraging[62]. These promising results were also reflected in an interim report from a phase II clinical trial examining the efficacy of the pan-FGFR inhibitor BGJ398 in CCA patients with an FGFR abnormality (NCT02150967)[63] in which of 36 patients eligible for assessment of response, 8 (22%) had a partial response and the disease control rate was 75%. These results compare very favourably to second line chemotherapy for CCA and serve to highlight the potential benefit of targeted therapy in appropriately selected patients.

Other potential targets in CCA

ROS1 gene rearrangements are seen in a number of cancers and result in a fusion protein with a constitutively activated kinase domain that promotes oncogenesis. In NSCLC, ROS1 rearranged tumours have shown encouraging response to the ALK/MET/ROS inhibitor Crizotinib. In CCA the prevalence of ROS1 rearrangement is reported to be around 8%-9%.[58,64] Larger series are needed to determine whether prevalence is higher in ICC or ECC, however Neia et al found that in a cohort of 65 biliary tract cancer patients FIG-ROS1 fusions were found in 4/25 ECC, 2/14 gallbladder carcinoma, 0/26 ICC.[58] In a murine allograft model of ICC the FIG-ROS fusion protein was shown to promote tumorigenesis and FIG-ROS inactivation resulted in inhibition of tumour growth.[65] Clinical trials are in progress to assess the efficacy of targeting ROS1 rearrangement in CCA (NCT02374489, NCT02034981).

The RAS/RAF/MEK/ERK MAPK pathway is a key regulator of cellular proliferation and is defective in a number of malignancies.

KRAS mutations are frequent in CCA and have a reported incidence of 9%-47%.[59,66,67] In mouse models, tissue specific activation of KRAS in the hepatic parenchyma was found to lead to development of ICC[68] and this process was accelerated by the presence of simultaneous P53[69] or PTEN loss[60]. There are currently no available drugs to directly target KRAS, however downstream proteins can be targeted, for instance using MEK inhibitors. In a phase II trial of the MEK 1/2 inhibitor selumetinib in aBCC, 12% (3/28) had objective response and 56% (14/28) prolonged stable disease (> 16 wk) resulting in a PFS of 3.7 mo and OS of 9.8 mo.[70] BRAF mutations in CCA are reported to occur at a rate of 0-22%.[71,72] In one large study, BRAF V600E mutation was detected in 3% (5/159) of ICC cases but in no (0/149) ECC cases.[71] BRAF mutation showed no correlation with prognosis in this study. Due to the infrequent occurrence of BRAF mutation in CC, molecularly targeted clinical trials are difficult to conduct, however a phase II trial of combination BRAF and MEK inhibition in rare cancers is in progress (NCT02034110).

Noncoding RNA abnormalities in CCA

MicroRNAs (miRNAs) are small non-coding RNAs that act as negative regulators of gene expression at the post transcription level. They bind to the 3’ untranslated region of target mRNAs causing inhibition of translation and mRNA degradation. miRNAs can regulate a number of cellular processes and their abnormal expression is recognised in human cancers, including CCA. The abnormal expression of miRNA in CCA has been found to impact on a number of cellular processes
involved in cell cycle progression, apoptosis and cell signalling. In one of the first reported studies on this topic Meng et al demonstrated that miR-21, miR-141, and miR-200b were highly over-expressed in malignant CCA cells, and whereas inhibition of miR-21 and miR-200b increased sensitivity to gemcitabine, inhibition of miR-141 decreased cell growth[73]. Chronic inflammation is an important risk factor for CCA. The inflammatory cytokine, IL-6 has been identified as a driver of cholangiocarcinogenesis and has been shown to alter the expression of a number of miRNAs including miRNA 7a, 370, 148a and 152[74,76]. IL-6 signalling is associated with increased expression of DNA methyltransferase (DNMT), which promotes silencing of tumour suppressor genes through DNA hypermethylation. The miRNAs 148a and 152 are believed to regulate DNMT expression, as demonstrated by decreased levels of these miRNAs in malignant cholangiocytes in in-vitro and xenograft models. Crucially in cells transfected with these miRNAs DNMT levels were shown to decrease leading to reduced cellular proliferation[74].

In another study, in a cell culture model, miRNA 29b was under-expressed in CCA cell lines compared to normal cholangiocytes, resulting in up-regulation of the anti-apoptotic protein MCL-1 and allowing tumour cells to evade apoptosis[77]. In a further study, miRNA 494 was shown to induce G1/S transition cell cycle arrest, through downregulation of cyclin dependent kinase 6. In cell-based and xenograft models of CCA, miRNA494 expression was found to be reduced and its upregulation reduced cellular proliferation[78]. miRNA 26a was shown to promote proliferation of CCA cells by lowering levels of glycogen synthase kinase 3i (GSK-3i) and preventing the degradation of β-catenin, leading to upregulation of transcription of target genes involved in carcinogenesis[79]. Other miRNAs may be related to chemoresistance; higher levels of miR-21 and miR-200b are associated with resistance to gemcitabine in cell lines whereas the converse is true for miR-29b, miR-205 and miR-221[80]. Selaru et al[81] have also demonstrated that miR-21 may be oncogenic in CCA through inhibition of programmed cell death 4 (PDCD4) and tissue inhibitor of metalloproteinases 3. As pharmacological manipulation of noncoding RNAs develops into a viable therapeutic option[82], these processes could conceivably be targeted in future to benefit CCA patients.

**Circulating miRNAs**

Pathological diagnosis of biliary tract tumours is frequently challenging due to the limited cellularity of specimens available post ERCP and also the desmoplastic stroma associated with biliary tract cancers, making the concept of a "liquid biopsy" attractive. Differential expression of several miRNAs has been demonstrated between patients with CCA and healthy controls in both tissue and blood, miR21 is known to be expressed at higher levels in biliary tract cancers, and increasing levels are also associated with more advanced clinical stage and fall following surgical resection[83]. Wang et al[84] found that miR150 was significantly elevated in the plasma of ICC patients compared to clinical controls and could be used to differentiate ICC patients from volunteers with a sensitivity of 81% and a specificity of 58%, which was enhanced when CA19.9 was used in conjunction with miRNA analysis. Other circulating miRNAs of interest include miRNA192[85] which was also linked to more advanced disease and a negative prognosis and miR106a which is downregulated in CCA compared to healthy controls and has similar prognostic value[86].

As bile secreted by the liver though the biliary ducts is more freely available to sample than tissue this also represents a potentially useful diagnostic material for CCA. In bile, miRNAs are contained in extracellular vesicles which maintain miRNA stability. Li et al designed a microvesicle based miRNA panel which was able to differentiate CCA from other causes of biliary disease or obstruction with a sensitivity of 67% and specificity of 96%[87]. The panel consisted of miR-191, miR-486-3p, miR-16, and miR-484, of which the last is the most sensitive for CCA. A Japanese study evaluating a larger panel of miRNAs in bile found ten to be upregulated in biliary tract cancer compared to benign biliary disease, and selected a combination of two (miR-9 and miR-145) as a proposed diagnostic biomarker with a specificity of 100% and high sensitivity[88]. Voigtlander et al[89] performed a study comparing miRNA expression in both serum and bile in German patients with primary sclerosing cholangitis and CCA, in addition to the serum of healthy controls. Interestingly, distinct miRNA profiles differentiated PSC and CCA in bile and in serum. In serum, lower levels of miR-1281, miR-126, miR26a, miR30b and miR-122 were found in CCA patients compared to PSC and healthy controls, whereas in bile changes in miR-412, miR-640, miR-1537 and miR-3189 predominated. Unfortunately as paired samples from each patient were not available a predictive panel containing blood and bile biomarkers was unable to be generated, however in future such an approach could be of significant utility. Although the use of circulating miRNAs is of significant interest, these studies are relatively small and require validation before becoming clinically applicable. Furthermore consideration of the geographic region of origin of each of the above studies (impacting on CCA aetiology and biology) must be considered before generalising these findings.

**Immunotherapy**

**Immune checkpoint inhibitors:** Immune checkpoints, which provide co-stimulatory and co-inhibitory signals to T-cells are exploited by a number of cancers to evade the host immune system and checkpoint inhibition has been used therapeutically, most notably in melanoma and non-small cell lung cancer amongst other malignancies. There may also be a role for
checkpoint inhibition in CCA. Ye et al\(^{95}\) studied the expression of the co- inhibitory immune checkpoint, Programmed Death Ligand 1 (PD-L1) in 31 surgically resected ICC samples from Asian patients and found PD-L1 expression to be upregulated in tumour tissue compared to adjacent tissue. Tumours with high levels of PD-L1 expression were associated with poor differentiation, higher TNM stage and higher levels of apoptotic CD8\(^+\) tumour infiltrating lymphocytes (TIL). Poorer survival has also been demonstrated in Western patients with ICC with positive tumour PD-L1 expression\(^ {91,92}\). Sabbatino et al\(^ {92}\) also found that downregulation of HLA class I antigen expression by tumour cells was associated with poorer clinical outcome. These data indicate PD-L1 upregulation and HLA class I antigen downregulation may be mechanisms of immune escape in CCA and could be potential biomarkers of response to anti-PD1/PDL1 immunotherapy. Chemotherapy may also have a role in modulating the immune system via inducing immunogenic cell death and upregulating expression of tumour associated antigens. Koido et al\(^ {91}\) found treatment of ICC cells with gemcitabine resulted in upregulation of the tumour antigen WT1, calreticulin (a protein that provides a pro-phagocytic signal) and PD-L1. Thus there may be a rationale for combining standard chemotherapy drugs with immune checkpoint inhibitors.

Trials investigating immune checkpoint inhibitors in CCA are in progress but early signals of efficacy have recently been reported. Keynote-028 is a multicohort phase I/II trial of pembrolizumab in PD-L1 positive pretreated advanced solid tumours\(^ {94}\). Early data from the biliary tract cohort of this trial reported an objective response rate of 17% (n = 4/17, all partial responses) and a further 17% (n = 4/17) achieved stable disease. Responses appeared to be durable with all responding patients remaining on treatment at 40-42 wk. Le et al\(^ {95}\) also reported data from a phase II trial of 17 patients with mismatch repair deficient non-colorectal gastrointestinal cancers treated with the PD-1 inhibitor pembrolizumab. Of the 3 patients with CCA there was one complete response, one partial response and one stable disease, with durable and ongoing responses at median follow up of 5.3 mo. More mature data with larger sample sizes are eagerly awaited but mismatch repair deficiency appears to be a promising predictive biomarker for checkpoint inhibition (although of relatively rare prevalence). The Keynote-158 phase II trial is recruiting 1100 patient with advanced solid tumours to be treated with pembrolizumab and will include a cohort of patients with biliary tract cancer (NCT02628067).

**Mutation specific adoptive T-Cell therapy:** The use of T-cells with specificity to cancer antigens is an emerging field and efficacy has been demonstrated in metastatic melanoma\(^ {96}\) and B cell leukaemia\(^ {97}\). T-cell based therapy for epithelial malignancies, such as CCA is under investigation.

Tran et al\(^ {98}\) treated a female patient with metastatic CCA who had progressed on multiple lines of chemotherapy, with autologous T-1 tumour infiltrating lymphocytes (TILs) specific to a mutated antigen expressed by the patient’s cancer. In this novel approach, TILs from the patient’s lung metastases were retrieved and whole exome sequencing performed on tumour tissue to identify somatic mutations present. Further testing revealed that CD4\(^+\) T-1 TILs recognised mutated erb2 interacting protein (ERBB21P) in the tumour tissue. These mutation specific TILs were clonally expanded and the patient underwent lymphodepletive chemotherapy, before receiving 42.4 billion TILs (25% ERBB2IP-mutation reactive T cells). There was impressive reduction in size of metastatic lesions and prolonged stable disease for more than 1 year. When the disease progressed after 13 mo the patient was retreated and again achieved disease response. Whilst this demonstrated an important proof of concept for T-cell based therapy in CCA, reproducibility in further patients is needed. Furthermore the highly personalised nature of this approach has high cost implications.

**CONCLUSION**

CCA is a molecularly heterogeneous malignancy with currently limited treatment options beyond first line systemic chemotherapy. Genomic profiling studies have highlighted differing patterns of mutation signatures between ICC and ECC, helping to stratify patients for targeted therapies. FGFR fusions and IDH mutations appear to be frequently mutated in ICC and hold promise as therapeutic targets. Immunotherapy has considerable potential but requires a validated biomarker to guide selection of patients for this approach. Circulating miRNAs are of interest in improving early diagnosis and detecting disease relapse. However given the relative rarity of this cancer and the molecular heterogeneity, multi-centre collaboration is essential in order to design adequately powered clinical trials of targeted agents.

**REFERENCES**

1. Vatanasapt V, Uttaravichien T, Maariang EO, Pairojkul C, Chart-banchachai W, Haswell-Elkins M. Cholangiocarcinoma in north-east Thailand. Lancet 1990; 335: 116-117 [PMID: 1967406]
2. Public Health England. National Cancer Intelligence Network Rare and less common cancers. Incidence and Mortality in England, 2010 to 2013. 2015. Available from: URL: http://www.ncin.org.uk/publications/reports/
3. Altekruse SF, Petrick JL, Rolin AI, Cuccinelli JE, Zoa Z, Tatalovich Z, McGlynn KA. Geographic variation of intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and hepatocellular carcinoma in the United States. PLoS One 2015; 10: e0120574 [PMID: 25837669 DOI: 10.1371/journal.pone.0120574]
4. Blechacz B, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. Hepatology 2008; 48:
Kayhanian H et al. Molecular targets in cholangiocarcinoma

308-321 [PMID: 18536057 DOI: 10.1002/hep.22310]

American Cancer Society. Survival statistics for bile duct cancers. Available from: URL: http://www.cancer.org/cancer/bileductcancer/detailedguide/bile-duct-cancer-survival-by-stage-2014

Khaw SF, Hobbs HC, Davidson NC, Taylor-Robinson SD. Cholangiocarcinoma. Lancet 2005; 366: 1303-1314 [PMID: 16214602 DOI: 10.1016/s0140-6736(05)67530-7]

Cardinale V, Carpino G, Reid L, Gaudio E, Alvaro D. Multiple cells of origin in cholangiocarcinoma underlie biological, epidemiological and clinical heterogeneity. World J Gastrointest Oncol 2012; 4: 94-102 [PMID: 22645632 DOI: 10.4251/wjgi.v4.i3.94]

Jarnagin WR, Feng Y, DeMatteo RP, Gonen M, Burke EC, Boddisiewicz BS J, Youssef BA M, Klimstra D, Blumgart LH. Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. Ann Surg 2001; 234: 507-517, discussion 517-519 [PMID: 11537044 DOI: 10.1097/00000658-200110000-00 010]

Takada T, Amano H, Yasuda H, Nimura Y, Matsushiro T, Kato H, Nakagawa T, Nakayama T. Is postoperative adjuvant chemotherapy useful for gallbladder carcinoma? A phase III multicenter prospective randomized controlled trial in patients with resected pancreaticoduodenal carcinoma. Cancer 2002; 95: 1685-1695 [PMID: 12365016 DOI: 10.1002/encr.10835]

Neoptolemos JP, Moore MJ, Cox TF, Valle JW, Palmer DH, McDonald AC, Carter R, Tebbutt NC, Dervenis C, Smith D, Czito BG. Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma. J Hepatol 2002; 37: 95-109 [PMID: 11835931 DOI: 10.1016/s0168-8278(01)02452-2]

Rea DJ, Heimbach JK, Rosen CB, Haddock MG, Alberts SR, Park JW, Kim BH, Woo SM, Kim TH, Koh YH, Lee SY, Choo SP, Tan IB, Duda D, Nastase A, Myint WS, Wong BH, Gan A, Rajasegaran V, Ng CC, Nagarajan S, Jasakul Z, Zhang S, Vohra P, Wu Y, Huang D, Smitthawporn W, Yongvanit P, Wongkham S, Khuntikeo N, Bhudhisawadi V, Rozen SG, Tan P, Teh BT. Exome sequencing of liver fluke-associated cholangiocarcinoma. Nat Genet 2012; 44: 690-693 [PMID: 22562150 DOI: 10.1038/ng.2273]

Chann-On W, Naisrmsig ML, Ong CK, Lim WK, Dima S, Park JW, Pairojkul C, Lim KH, McPherson JR, Cutcute I, Heng HL, Ooi L, Chung A, Chow P, Cheow PC, Lee SY, Choo SP, Tan IB, Duda D, Nastase A, Myint WS, Wong BH, Gan A, Rajasegaran V, Ng CC, Nagarajan S, Jasakul Z, Zhang S, Vohra P, Wu Y, Huang D, Smitthawporn W, Yongvanit P, Wongkham S, Khuntikeo N, Bhudhisawadi V, Popescu I, Rozen SG, Tan P, Teh BT. Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. Nat Genet 2013; 45: 1474-1478 [PMID: 24185513 DOI: 10.1038/ng.2806]

Grassian AR, Pagliarini R, Chiang DY. Mutations of isocitrate dehydrogenase 1 and 2 in intrahepatic cholangiocarcinoma. Curr Opin Gastroenterol 2014; 30: 295-302 [PMID: 24569570 DOI: 10.1097/MOG.0000000000000050]

Wang P, Dong Q, Zhang C, Kuan PF, Liu Y, Jeeck WR, Andersen JB, Jiang W, Savich GL, Tan TX, Auman JT, Hoskins JM, Misher AD, Moser CD, Yourstone SM, Kim JW, Cibuulsks K, Getz G, Hunt HV, Thorgerisson SS, Roberts LR, Ye D, Guan KL, Xiong Y, Qin LX, Chiang DY. Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. Oncogene 2013; 32: 3091-3100 [PMID: 22824796 DOI: 10.1038/onc.2012.315]

Jiao Y, Pavlik TM, Anders RA, Sellar FM, Stepped MM, Lucas DJ, Niknafs N, Guthrie VB, Maitra A, Argani P, Offerhaus GJ, Roa JC, Roberts LR, Gores GJ, Popescu I, Alexandrescu ST, Dima S, Fassan M, Simbolo M, Mafficini A, Capelli P, Lawlor RT, Ruzzenente A, Guglielmii A, Tortora G, de Braud F, Scarpa A, Jarnagin W, Klimstra D, Karchin R, Velculescu VE, Hruban RH, Vogelstein B, Kinzler KW, Weinstein JN, Broomer AJ, Sugarbaker DJ. Exome sequencing of liver fluke-associated cholangiocarcinoma. Gastroenterology 2014; 146: 1685-1695 [PMID: 24815513 DOI: 10.1053/j.gastro.2013.12.029]

Weigt J, Malfertheiner P. Cisplatin plus gemcitabine versus gemcitabine alone for patients with advanced-stage unresectable intrahepatic cholangiocarcinoma. Radiat Oncol 2013; 8: 292 [PMID: 24359879 DOI: 10.1186/1748-717X-8-292]

Rohle D, Popovic-Muller J, Palaskas N, Turcan S, Grommes C, Campos C, Tsoi J, Clark O, Oldrini B, Komisopoulou E, Kunii R, Pedraza A, Schaln S, Silverman L, Miller A, Wang F, Yang H, Chen Y, Kernytsky A, Rosenblum MK, Liu W, Biller SA, Su SM, Brennan CW, Chan TA, Graeber TG, Ye KE, Mellinofg IK. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. Science 2013; 340: 626-630 [PMID: 23558169 DOI: 10.1126/science.1236602]
29 Buirrs H, Mellinghoff I, Maher E, Wen P, Beermann M, Toata M, Faris J, Azad N, Cloughesy T, Gore L, Trent J, Hoff D Von, Goldwasser M, Fan B, Agresta S. Abstract PL04-05: The first reported results of AG-I-120, a first-in-class, potent inhibitor of the ID1H mutant protein, in a Phase I study of patients with advanced ID1H-mutant solid tumors, including gliomas. Mol Cancer Ther 2015; 14: PL04-05 [DOI: 10.1158/1535-7163]

30 Kwak TW, Kim DH, Jeong YI, Kang DH. Antitumor activity of vorinostat-incorporated nanoparticles against human cholangiocarcinoma cells. J Nanobiotechnology 2015; 13: 60 [PMID: 26410576 DOI: 10.1186/s12951-015-0122-4]

Yeawle C, Baradad I, Vhore I, Patil S, Misra A. Epidermal growth factor receptor targeting in cancer: a review of trends and strategies. Biomaterials 2013; 34: 8690-8707 [PMID: 23953842 DOI: 10.1016/j.biomaterials.2013.07.100]

32 Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350: 2129-2139 [PMID: 15118073 DOI: 10.1056/NEJMoa040938]

34 Leone F, Cavalloni G, Pignochino Y, Sarotto I, Ferraris R, Picciobello W, Venesio T, Capussotti L, Risio M, Aglietta M. Somatic mutations of epidermal growth factor receptor gene in bile duct and gallbladder carcinoma. Clin Cancer Res 2006; 12: 1680-1685 [PMID: 16551849 DOI: 10.1158/1078-0432.CCR-05-1692]

35 Gwak GY, Yoon JH, Shin CM, Ahn YJ, Chung JK, Kim YK, Kim TY, Lee HS. Detection of response-predicting mutations in the kinase domain of the epidermal growth factor receptor gene in cholangiocarcinomas. J Cancer Res Clin Oncol 2005; 131: 649-652 [PMID: 16032426 DOI: 10.1007/s00432-005-0016-1]

36 Chang YT, Chang MC, Huang KW, Tung CC, Hsu C, Wong KY, Dobashi Y, Suzuki S, Fujii H, Takeda Y, Ooi A. Clinicopathological and prognostic significances of EGFR, KRAS and BRAF mutations in biliary tract carcinomas in Taiwan. J Gastroenterol Hepatol 2014; 29: 1119-1125 [PMID: 24372748 DOI: 10.1111/j.1440-1746.2014.07128.x]

38 Nakazawa K, Dobashi Y, Suzuki S, Fujita H, Takeda Y, Ooi A. Amplification and overexpression of c-erbB-2, epidermal growth factor receptor, and c-met in biliary tract cancers. J Pathol 2005; 206: 356-365 [PMID: 15892172 DOI: 10.1002/path.1799]

39 Yoshikawa D, Ojima H, Iwaski M, Hiraoka N, Kosuge T, Kasi S, Hirohashi S, Shibata T. Clinicopathological and genetic characterization of cholangiocarcinomas. J Pathol 2006; 202: 1051-1063.e15 [PMID: 16978559 DOI: 10.1002/path.1925]

41 Leone F, Marino D, Cereda S, Filippi R, Belli C, Spadi R, Nasti G, Montano M, Amatu A, Aprile G, Cagnazzo C, Fasola G, Siena S, Ciuffreda L, Reni M, Aglietta M. Panitumumab in combination with gemcitabine and oxaliplatin does not prolong survival in wild-type KRAS advanced biliary tract cancer: A randomized phase 2 trial (Venti-BIL study). Cancer 2016; 122: 574-581 [PMID: 26540314 DOI: 10.1002/cncr.29778]

42 Lee J, Park SH, Han HM, Kim JS, Choi HJ, Lee MA, Jang JS, Jeong HC, Kang JI, Lee HW, Shin DB, Kang HJ, Sun JM, Park JO, Park YS, Kang WK, Lim HY. Cetuximab and oxaliplatin with or without erlotinib in advanced biliary-tract cancer: a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2012; 13: 181-188 [PMID: 22192731 DOI: 10.1016/S1470-2045(11)70301-3]

43 Kim HJ, Yoo TW, Park DI, Park JH, Cho YK, Sohn CI, Jeon WK, Kim BI, Kim MK, Chae SW, Sohn JH. Gene amplification and protein overexpression of HER-2/neu in human extrapancreatic cholangiocarcinoma as detected by chromogenic in situ hybridization and immunohistochemistry: its prognostic implication in node-positive patients. Ann Oncol 2007; 18: 892-897 [PMID: 17322545 DOI: 10.1093/annonc/mdn006]

44 Law LY. Dramatic response to trastuzumab and paclitaxel in a patient with human epidermal growth factor receptor 2-positive metastatic cholangiocarcinoma. J Clin Oncol 2012; 30: e271-e273 [PMID: 22851567 DOI: 10.1200/JCO.2012.42.3661]

45 Vasudev NS, Reynolds AR. Anti-angiogenic therapy for cancer: current progress, unresolved questions and future directions. Angiogenesis 2014; 17: 471-494 [PMID: 24842243 DOI: 10.1007/s10456-014-9420-y]

46 Hida Y, Morita T, Fujita M, Miyasaka Y, Horita S, Fujioka Y, Nagashima K, Katoh H. Vascular endothelial growth factor expression is an independent negative predictor in extrapancreatic biliary tract cancer carcinomas. Anticancer Res 1999; 19: 2257-2260 [PMID: 10472340]

47 Park BK, Paik YH, Park JY, Park KH, Bang S, Park SW, Chung JB, Park YN, Song SY. The clinicopathologic significance of the expression of vascular endothelial growth factor-C in intrapancreatic cholangiocarcinoma. Am J Clin Oncol 2006; 29: 138-142 [PMID: 16601431 DOI: 10.1097/01.coc.0000204402.29830.08]

48 Zhu AX, Meyerhardt JA, Blaszkowsky LS, Kambadakone AR, Muzikansky A, Zheng H, Clark JW, Abrams TA, Chan JA, Enzinger PC, Bhagrava P, Kwak EL, Allen JN, Jain SR, Stuart K, Horgan K, Sheehan S, Fuchs CS, Ryan DP, Sahani DV. Efficacy and safety of cetuximab, oxaliplatin, and bevacizumab in advanced biliary-tract cancers and correlation of changes in 18-fluorodeoxyglucose PET with clinical outcome: a phase 2 study. Lancet Oncol 2010; 11: 48-54 [PMID: 19932054 DOI: 10.1016/S1470-2045(09)70333-X]

49 Iyer RV, Groman A, Ma WW, Malhotra U, Iancu D, Grande C, Bekaii-Saab TS. Cetuximab (C) and bevacizumab (BV) in patients with advanced biliary cancers (ABC): final results of a multicenter phase II study. J Clin Oncol 2015; 33: 4078 [DOI: 10.1200/jco.2015.33.15_suppl.4078]

50 Lubner SJ, Mahoney MR, Kolesar JL, Loconte NK, Kim GP, Pitot HC, Philip PA, Picus J, Yong WP, Horvath L, Van Hazel G, Erlichman C, Holden KD. Report of a multicenter phase II trial testing a combination of biweekly bevacizumab and daily erlotinib in patients with unresectable biliary cancer: a phase II Consortium study. J Clin Oncol 2010; 28: 3491-3497 [PMID: 20530271 DOI: 10.1200/JCO.2010.28.0475]

51 Valle JW, Wasan H, Lopes A, Backen AC, Palmer DH, Morris K, Duggan M, Cunningham D, Anthony DA, Corrie P, Madhusudan S, Maraveyas A, Ross PJ, Waters JS, Steward WP, Rees C, Beare S, Dive C, Bridgewater JA. Cediranib or placebo in combination with cisplatin and gemcitabine chemotherapy for patients with advanced biliary tract cancer (ABC-03): a randomised phase 2 trial. Lancet Oncol 2015; 16: 979-987 [PMID: 26179201 DOI: 10.1016/S1470-2045(15)00139-4]

52 Bengala C, Bertolini F, Malavasi N, Boni C, Atini E, Dealis C, Zironi S, Depenni R, Fontana A, Del Giovane C, Luppi G, Conte P. Sorafenib in patients with advanced biliary tract carcinoma: a phase II trial. Br J Cancer 2010; 102: 68-72 [PMID: 19935794 DOI: 10.1038/sj.bjc.6605485]

53 El-Khoueiry AB, Rankin CJ, Ben-Josef E, Lenz HJ, Gold PJ, Hamilton RD, Govindarajan R, Eng C, Blanke CD. SWOG 0514:
a phase II study of sorafenib in patients with unresectable or metastatic gallbladder carcinoma and cholangiocarcinoma. *Invest New Drugs* 2012; 30: 1646-1651 [PMID: 21748296 DOI: 10.1007/s10637-011-9710-0]

54 Lee JK, Capano M, O'Reilly EM, Ma J, Chou JF, Shia J, Katz SS, Gansukh B, Reidy-Lagunes D, Segal NH, Yu KH, Chung KY, Salz LB, Abou-Alfa GK. A phase II study of gemcitabine and cisplatin plus sorafenib in patients with advanced biliary adenocarcinomas. *Br J Cancer* 2013; 109: 915-919 [PMID: 23900219 DOI: 10.1038/bjc.2013.432]

55 El-Khoueiry AB, Rankin C, Siegel AB, Iqbal S, Gong IY, Kayhanian H. Novel approaches to the treatment of altered cholangiocarcinoma (CCA) who failed or are intolerant to sorafenib. *Cancer* 2012; 118: 2307-2314 [PMID: 22349318 DOI: 10.1002/cncr.27074]

56 Arai Y, Totoki Y, Hosoda F, Shirato T, Hama N, Nakamura H, Ojima H, Furuta K, Shimada K, Okusaka T, Kosuge T, Shibata T. Fibroblast growth factor receptor 2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma. *Hepatology* 2014; 59: 1427-1434 [PMID: 24122810 DOI: 10.1002/hep.26890]

57 Stu D, Losic B, Moeini A, Cabelllos L, Hao K, Revill K, Bonal D, Miltiadous O, Zhang Z, Hoshiya Y, Corenlla H, Castillo-Martin M, Pinayi R, Kasai Y, Raoayie S, Thung SF, Fuster J, Schwartz ME, Wuxman S, Cordon-Cardo C, Schadt E, Mazzaferrro V, Llovet JM. Massive parallel sequencing uncovering actionable FGFR2-PPHLN1 fusion and ARAF mutations in cholangiocarcinoma. *Nat Commun* 2015; 6: 6087 [PMID: 25686663 DOI: 10.1038/ncomms7087]

58 Peraldo Neia C, Cavalloni G, Balsamo A, Venesio T, Napoli C, Sassi F, Martin V, Frattini M, Leone F. Screening for the FG-ROS1 fusion in biliary tract carcinomas by nested PCR. *Genes Chromosomes Cancer* 2014; 53: 1033-1040 [PMID: 25231053 DOI: 10.1002/gcc.22212]

59 Zhou S, Li J, Zhou H, Frech C, Jiang X, Chu JS, Zhao X, Li Y, Li Q, Wang H, Hu J, Kong G, Wu M, Ding C, Chen N, Hu H. Mutational landscape of intrahepatic cholangiocarcinoma. *Nat Commun* 2014; 5: 5396 [PMID: 25526346 DOI: 10.1038/ncomms6696]

60 Graham RP, Barr Frichler EG, Pestova E, Schulz J, Sitalo IA, Vasmatzis G, Murphy SJ, McWilliams RR, Hart SN, Halling KC, Roberts LR, Gores GJ, Couch FJ, Zheng L, Borad MJ, Kipp BR. Fibroblast growth factor receptor 2 translocations in patients with advanced gallbladder carcinoma. *Hum Pathol* 2014; 45: 1630-1638 [PMID: 24837065 DOI: 10.1016/j.humpath.2013.04.014]

61 Wang Y, Ding X, Wang S, Moser CD, Shalhe H, Mohamed EA, Chaiteerakij R, Allotey LK, Chen G, Miyabe K, McNulty MS, Meng F, Jiang X, Chu JS, Zhao X, Li Y, Zhou H, Frech C, Wang S, Moser CD, Wehbe-Janek H, Smith H, Patel T. Application of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Mod Pathol* 2014; 27: 1386-1391 [PMID: 25190260 DOI: 10.1016/j.modpathol.2013.09.003]

62 Bekaii-Saab T, Phelps MA, Li X, Saij M, Gold LF, Kahl JS, O’Neil BH, Balsam S, Balint C, Liersmann R, Vasko VV, Bloomston M, Marsh W, Doyle TA, Ellison G, Grever M, Ringel MD, Villalona-Calero MA. Multi-institutional phase II study of selumetinib in patients with metastatic biliary cancers. *J Clin Oncol* 2011; 29: 2357-2363 [PMID: 21519026 DOI: 10.1200/JCO.2010.33.9473]

63 Goepbert P, Frauenshuh L, Renner M, Roessler S, Stenzinger A, Klauschen F, Warth A, Vogel MN, Mehrabi A, Hafezi M, Boelmer K, von Deimling A, Schirmacher P, Weichert W, Capper D. BRAF V600E-specific immunohistochemistry reveals low mutation rates in biliary tract cancer and restriction to intrahepatic cholangiocarcinoma. *Cancer Res* 2012; 72: 1557-1567 [PMID: 22266220 DOI: 10.1158/0008-5472.CAN-11-3596]

64 Marsh V, Davies EJ, Williams GT, Clarke AR. PTEN loss and KRAS activation cooperate in murine biliary tract malignancies. *J Pathol* 2013; 230: 165-173 [PMID: 23483557 DOI: 10.1002/path.4189]

65 Goldenberg D, Rosenbaum E, Argani P, Wistuba II, Sidransky D, Thuluvath PJ, Hidalgo M, Califano J, Maitra A. The V599E BRAF mutation is uncommon in biliary tract cancers. *Cancer* 2008; 112: 1386-1391 [PMID: 18355590 DOI: 10.1002/cncr.23598]

66 Meng F, Henson R, Lang M, Webhe H, Marwaha S, Wendell JT, Jiang J, Schmittgen TD, Patel T. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology* 2006; 130: 2113-2129 [PMID: 16762633 DOI: 10.1053/j.gastro.2006.02.057]

67 Braconi C, Huang N, Patel T. MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interferon-6 in human malignant cholangiocytes. *Hepatology* 2010; 51: 881-890 [PMID: 20146264 DOI: 10.1002/hep.23381]

68 Meng F, Webhe-Janek H, Henson R, Smith H, Patel T. Epigenetic regulation of microRNA-370 by interferon-6 in malignant human cholangiocytes. *Oncogene* 2008; 27: 378-386 [PMID: 17621267 DOI: 10.1038/sj.onc.1210648]

69 Meng F, Henson R, Webhe-Janek H, Smith H, Ueno Y, Patel T. The MicroRNA let-7a modulates interferon-6-dependent STAT-3 survival signaling in malignant human cholangiocytes. *J Biol Chem* 2007; 282: 8256-8264 [PMID: 17220301 DOI: 10.1074/jbc.C605601200]
M60771200

77 Mott JL, Kobayashi S, Bronk SF, Gores GJ. mir-29 regulates Mel-1 protein expression and apoptosis. Oncogene 2007; 26: 6133-6140 [PMID: 17445774 DOI: 10.1038/sj.onc.1210436]

78 Olaor AV, Giaour G, Yamanauka S, Luvsanjav D, An F, Popescu L, Alexiadescu S, Allen S, Pawlik TM, Tomberson M, Georgiades C, Roberts LR, Gores GJ, Ferguson-Smith A, Meimda M, Calin GA, Mezey E, Sellar F. MicroRNA down-regulated in human cholangiocarcinoma control cell cycle through multiple targets involved in the G1/S checkpoint. Hepatology 2011; 54: 2009-2018 [PMID: 2189359 DOI: 10.1002/hep.24591]

79 Zhang J, Chan, C, Wu T. MicroRNA-26a promotes cholangiocarcinoma growth by activating β-catenin. Gastroenterology 2012; 143: 246-256.e8 [PMID: 22848410 DOI: 10.1016/j.gastro.2012.03.045]

80 Okamoto K, Miyoshi K, Murawaki Y. miR-29b, miR-205 and miR-221 enhance chemosensitivity to gemcitabine in HuH28 human cholangiocarcinoma cells. PLoS One 2013; 8: e77623 [PMID: 24147037 DOI: 10.1371/journal.pone.0077623]

81 Sellar FM, Olaor AV, Kan, T, David, C, Shen, C, Mory, Y, Jiang, J, Paun, B, Jin, Z, Agarwal R, Hamilton JP, Abraham J, Georgiades C, Alvarez H, Vivekanandan P, Wu Y, Maitra A, Tomberson M, Thuluvath PJ, Gores GJ, LaRusso NF, Hruban R, Meltzer SJ. MicroRNA-21 is overexpressed in human cholangiocarcinoma and regulates programmed cell death 4 and tissue inhibitor of metalloproteinase 3. Hepatology 2009; 49: 1591-1601 [PMID: 19296468 DOI: 10.1002/hep.22838]

82 Li Z, Rana TM. Therapeutic targeting of miRNAs: current status and future challenges. Nat Rev Drug Discov 2014; 13: 622-638 [PMID: 24954372 DOI: 10.1038/nrd4294]

83 Kishimoto T, Eguchi H, Nagano H, Kobayashi S, Akita H, Hama N, Wada H, Kawamoto K, Tomokuni A, Tomimaru Y, Umesaki H, Doki Y, Mori M. Plasma miR-21 is a novel diagnostic biomarker for biliary tract cancer. Cancer Sci 2013; 104: 1626-1631 [PMID: 24118467 DOI: 10.1111/cas.12300]

84 Wang S, Yin J, Li T, Yuan L, Wang D, He J, Du X, Lu J. Upregulated circulating miR-150 is associated with the risk of intrahepatic cholangiocarcinoma. Oncol Rep 2015; 33: 819-825 [PMID: 25482382 DOI: 10.3892/ort.2014.3641]

85 Silakri R, Loilome W, Yongvanit P, Chusorn P, Techasen A, Vina, J, Veerakamee V, Bhan, U, Robert, G, Almeida M, Hong K, Doki Y, Mori M. Plasma miR-21 is a novel diagnostic biomarker for cholangiocarcinoma. Oncol Rep 2015; 33: 627-632 [PMID: 25011539 DOI: 10.1038/onc.2014.359]

86 Cheng Q, Feng F, Zhu L, Zheng Y, Luo X, Liu C, Yi B, Jiang X. Circulating mir-106a is a Novel Prognostic and Lymph Node Metastasis Indicator for Cholangiocarcinoma. Sci Rep 2015; 5: 16103 [PMID: 26534789 DOI: 10.1038/srep16103]

87 Li L, Masica D, Ishida M, Tomuleasa C, Umegaki S, Kalloo AN, Georgiades C, Singh VK, Khashab M, Amato A, Li Z, Okolo P, Lennon AM, Saxena P, Geschwind JF, Schlachter T, Park JH, Yang JC, Sherry R, Hughes MS, Royal R, Vaccarella A, Fisher GA, DiCenzo TS, Sterling J, Restifo NP, Thomasian A, Downey SG, Smith FO, Kupper J, Morton K, Laurenco C, White DE, Rosenberg SA. Adoptive cell therapy for patients with metastatic melanoma: evolution of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol 2008; 26: 5233-5239 [PMID: 18809613 DOI: 10.1200/JCO.2008.16.5449]

88 Park JH, Riviere I, Wang X, Bernal Y, Purdon T, Halton E, Curran KD, Saperas CS, Sadelain M, Brentjens RJ. Efficacy and safety of CD19-targeted 19-28z CAR modified T cells in adult patients with relapsed or refractory B-ALL. 2015 ASCO Annual Meeting, 2015 [PMID: 25174625 DOI: 10.1002/jhbp.155]

89 Andersen JB. Molecular pathology of intrahepatic cholangiocarcinoma. J Hepatobiliary Pancreat Sci 2015; 22: 101-113 [PMID: 25174625 DOI: 10.1002/jhbp.155]

90 Borger DR, Tanabe KK, Fan KC, Lopez HU, Fanit VR, Strelay KS, Schenken DP, Hezel AF, Anckarvik M, Liebman HJ, Swain J, D'Angio G, Ryan RP, Deshpande V, Dias-Santagata D, Ellisien LW, Wang AZ, Hafner AJ. Frequent mutation of isocitrate dehydrogenase (IDH1) and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. Oncologist 2012; 14: 27-79 [PMID: 22180306 DOI: 10.1634/theoncologist.2011-0386]
Kayanian H et al. Molecular targets in cholangiocarcinoma

102 Ruzzene A, Fassan M, Conci S, Simbolo M, Lawlor RT, Pedrazzani C, Capelli P, D’Onofrio M, Iacono C, Scarpa A, Guglielmi A. Cholangiocarcinoma Heterogeneity Revealed by Multigene Mutational Profiling: Clinical and Prognostic Relevance in Surgically Resected Patients. Ann Surg Oncol 2016; 23: 1699-1707 [PMID: 26717940 DOI: 10.1245/s10434-015-5046-6]

103 Tannapfel A, Sommerer F, Benicke M, Katalinic A, Uhlmann D, Witzigmann H, Hauss J, Wittekind C. Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. Gut 2003; 52: 706-712 [PMID: 12692057 DOI: 10.1136/gut.52.5.706]

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