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Review

Biomarkers of intrahepatic cholangiocarcinoma: diagnosis and response to therapy

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Abstract

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver cancer behind hepatocellular carcinoma (HCC) and carries a dismal prognosis. Improved genetic analysis has paved the way for a better understanding of the distinct somatic genomic landscapes of ICC. The use of next generation sequencing has paved the way for more personalized medicine through identifying unique mutations which may prove to be therapeutic targets. The ability to identify biomarkers specific to ICC will assist in establishing a diagnosis, monitoring response to therapy, as well as assist in identifying novel therapies and personalized medicine. Herein, we discuss potential biomarkers for ICC and how these markers can assist in diagnosis, monitor response to therapy, and potentially identify novel interventions for the treatment of ICC.

Keywords: review; intrahepatic cholangiocarcinoma; biomarker; targeted therapy; immunotherapy

1. Introduction

Cholangiocarcinoma (CCA) is a rare malignancy that arises from the biliary tree commonly classified according to its anatomic location as intrahepatic (ICC), perihilar (PHCC), and extrahepatic (ECC) [1,2]. ICC is the second most common hepatobiliary cancer behind hepatocellular carcinoma (HCC) accounting for 5–20% of all liver malignancies and the incidence is rising [3–7]. ICCs are characterized by early nodal and vascular invasion and carry a dismal prognosis [8].

ICC is thought to occur due to chronic inflammation that can lead to an inflammatory milieu that damages DNA and induces cholangiocyte proliferation [9,10]. Another theory of ICC pathogenesis hypothesizes that hepatic progenitor cells overexpress Notch1 with oncologic transformation through a cholangiocellular pathway [11,12]. Risk factors for ICC are well established and include cirrhosis, viral hepatitis, primary sclerosing cholangitis (PSC), parasitic infections, carcinogen exposure, as well as several genetic syndromes such as Lynch syndrome, BRCA-associated protein-1 (BAP-1) tumor predisposition syndrome, cystic fibrosis, and biliary papillomatosis [13]. Additionally, ICC exists in two predominant subtypes: proliferative and inflammatory [14]. The proliferative subtype, characterized by activation of oncogenic signaling pathways, DNA amplifications, and mutations in BRAF and KRAS, tends to be more poorly differentiated than the inflammatory subtype, characterized by activation of inflammatory signaling pathways and overexpression of cytokines, and is associated with a worse prognosis [14].

Surgical resection represents the only potentially curative treatment for patients with ICC and unlike HCC or PHCC, liver transplantation is not an established treatment option [15–20]. However, approximately only one third of patients present with resectable disease and, despite surgical resection with negative margins, early disease recurrence is common [21]. Even when patients undergo surgical resection with curative intent, 5-year overall survival (OS) is only 20–35% [22]. While chemotherapy may prolong survival for select patients, drug resistance and significant toxicities, especially in patients with poor performance status, limit the success of systemic therapy [23].

Through improved genetic analysis there is a better understanding of the distinct somatic genomic landscapes of biliary tract cancers such as ICC [24]. The use of next generation sequencing has paved the way for more personalized medicine through identifying unique mutations which may prove to be therapeutic targets. The ability to identify biomarkers specific to ICC will assist in establishing a diagnosis, monitoring response to therapy, as well as assist in identifying novel therapies and personalized medicine [25]. In this review, we discuss potential biomarkers for ICC and how these markers can assist in diagnosis, monitor response to therapy, and potentially identify novel interventions for the treatment of ICC.

2. Diagnostic biomarkers of intrahepatic cholangiocarcinoma

When evaluating a liver lesion, it is important to distinguish ICC from other liver tumors such as HCC or metastatic disease. The diagnosis of ICC is primarily made with imaging in the appropriate clinical context and, if needed, subsequent biopsy [26]. Computed tomography (CT) scan and/or magnetic resonance imaging (MRI) are

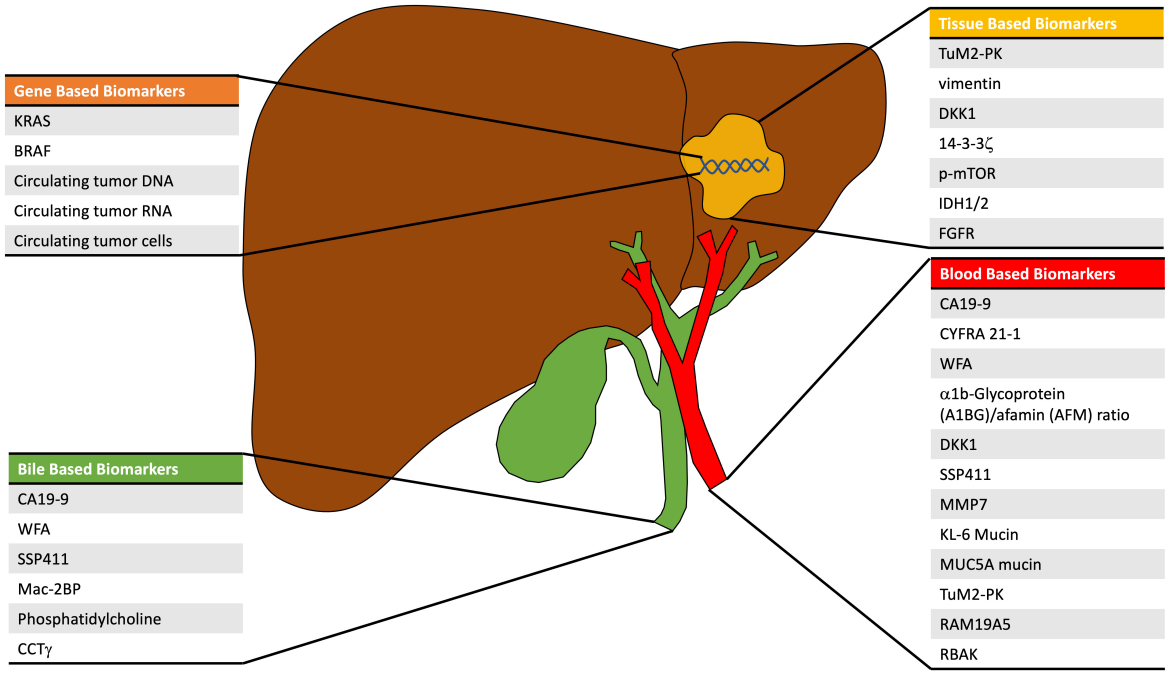

Fig. 1. Diagnostic biomarkers for intrahepatic cholangiocarcinoma.

the imaging modalities of choice for ICC. Contrast enhancement patterns on imaging may be able to distinguish ICC from HCC [27]. ICC receives its blood supply from the portal vein and thus often has a portal or delayed phase enhancement pattern while HCC receives its blood supply from the hepatic arteries and displays an arterial phase enhancement pattern on CT [28,29].

Several biomarkers have been identified to assist in the diagnostic work up of liver lesions (Fig. 1). Tumor markers provide useful diagnostic and prognostic information as adjunct confirmatory tests during the workup of solid tumors and in the postoperative surveillance setting to help monitor for recurrent disease; however, tumor markers should not be used as the sole means of diagnosis [30].

The diagnosis of intrahepatic cholangiocarcinoma (ICC) is primarily made with imaging and subsequent biopsy as it is important to distinguish ICC from other liver tumors such as hepatocellular carcinoma (HCC). Biomarkers have been identified in the blood, bile, and tumor tissue to assist in the diagnostic work up of liver lesions.

Carbohydrate antigen (CA) 19-9, a well-known biomarker for CCA, is a Lewis blood group antigen produced by pancreatic, biliary ductal, gastric, and colonic epithelial cells [25]. However, CA19-9 is not produced, and therefore not detectable, in 7% of the population [31,32]. An elevated CA19-9 is more commonly associated with ICC, as opposed to an elevated α-fetoprotein (AFP) that is more suggestive of HCC [33]. CA19-9 has a 72% sensitivity and 84% specificity as a diagnostic biomarker for CCA. Indeed, CA19-9 can be elevated in benign conditions such as biliary obstruction, cholangitis, and primary biliary cirrhosis.

PSC is an idiopathic, cholestatic liver disease characterized by persistent progressive biliary inflammation and fibrosis and is a risk factor for bile duct cancers [34]. In one study, a CA19-9 value of 129 U/mL demonstrated a 79% sensitivity and 98% specificity for CCA in patients with PSC [35]. In a different study, Vedeld *et al.* [36] investigated the utility of DNA methylation biomarkers in bile for early diagnosis of CCA in patients with PSC. Using droplet digital PCR (ddPCR), the authors analyzed 344 bile samples from 273 patients with sporadic and PSC-associated CCA, as well as other non-malignant liver disease for promoter methylation of CDO1, CNRIP1, SEPT9, and VIM. All four markers were associated with CCA detection among patients with PSC up to 12 months before conventional CCA diagnosis.

In addition, activating mutations in the oncogenes EGFR (ErbB1), HER2 (ErbB2), and PDGFa as well as silencing the tumor suppressor genes TP53 and CDKN2A may play a role in the pathogenesis of CCA [37]. These aberrations often correspond with the gain of chromosomal fragments 5q, 7p, 8q, 17q, and 20q and loss of 3p, 6q, 9p, and 17p [37,38]. Interestingly, patients who develop CCA in the setting of a liver fluke infection gain chromosomal fragment 21q22 and lose fragments 1p36, 9p21, 17q13, and 22q12 [39]. KRAS has been reported to be one of the most frequently mutated genes in ICC and may serve as a potential biomarker [40–42]. KRAS, as well as BRAF mutations, are present in approximately 10% of patients with ICC and approximately 30% of patients harbor mutations in the PI3K/PTEN/AKT/mTOR signaling pathway [43,44].

The biomarker serum cytokeratin 19 fragments (CYFRA 21-1) is a sensitive biomarker for gastric, breast,

and non-small cell lung cancer [45–47]. The sensitivity of CYFRA 21-1 in ICC is low as an isolated biomarker, yet can reach 92% sensitivity, 96% specificity, and 94% accuracy in combination with CA19-9, carcinoembryonic antigen (CEA), and matrix metalloproteinase-7 (MMP-7) [48]. Additionally, CYFRA 21-1 may help distinguish ICC from HCC where an elevated CYFRA 21-1 in the setting of a normal AFP suggest ICC [33,49]. Isocitrate dehydrogenase (IDH) is an enzyme involved in the Kreb cycle and exists in two isoforms IDH1 and IDH2. In patients with ICC, IDH mutations were identified in 15–30% of patients [50,51]. In addition, fibroblast growth factor receptor mutations are noted in 10–15% of patients with ICC [52].

Biomarkers may also help differentiate malignant disease from benign biliary disease. FAM19A5 and RB-associated KRAB zinc-finger protein (RBAK) were elevated in patients with CCA versus patients with benign biliary conditions [53]. Serum α1b-Glycoprotein (A1BG)/afamin (AFM) ratio greater than 1.8 differentiates patients with CCA from healthy patients with a 84.4% sensitivity and 87.5% specificity [54]. Serum and bile levels of Wisteria floribunda agglutinin (WFA) may also differentiate ICC from benign biliary diseases [55].

Bile based markers have also been used as a diagnostic aid. The fluid sample is closer to the presumed tumor tissue, a potential benefit, but sample collection requires an invasive procedure [25]. Patients with CCA have significantly lower total bile concentrations and deoxycholic acid ratios than patients with benign biliary disease due to altered bile acid transport [56]. Sperm specific protein 411 (SSP411) is elevated in the bile of CCA patients compared with patients who have benign biliary disease [57].

As cholangiocarcinoma is rather heterogeneous in terms of molecular alterations, Nakanuma *et al*. [58] proposed two histological subtypes of ICC: large bile duct type and small bile duct type. Small bile duct type is more peripherally located and mass forming [58–61]. The small duct type of ICC is typically mass forming and 10–30% have IDH1/2 mutations while 10–25% have FGFR2-fusions [50,62,63]. The large duct type typically lack IDH1/2 mutations and FGFR2-fusions, but 15–30% have KRAS mutations and 10–40% have TP53 mutations [58, 62–64].

3. Biomarker predictors of outcomes

Outcomes for patients with ICC are generally poor, and recurrence is common after resection. Additionally, a hepatectomy is a physiologically demanding procedure and patient selection is important to achieve operative success [26]. As such, biomarkers to assist in risk stratification and guide treatment decisions is an active area of investigation (Table 1). As previously discussed, CA19-9 is a useful biomarker in the diagnosis of ICC. In addition, CA19-9 may have prognostic significance for patients with ICC. Moro *et al*. [65] demonstrated that preoperative CA19-9 and CEA were prognostic of OS when a cutoff of 176.3 IU/mL for CA19-9 and 9.6 ng/mL for CEA were utilized. Other studies indicate that CA19-9 is elevated in 57% of patients with ICC, and a CA19-9 level higher than 37 U/mL was predictive of lymph node metastasis and survival [66,67]. Among patients with ICC who underwent hepatectomy, Qiu *et al*. [68] reported that a low aspartate aminotransferase (AST) to lymphocyte ratio index combined with a low CA19-9 level was associated with better OS and disease-free survival (DFS). Additionally, 237 patients with ICC who had undergone resection had arginase-1 and glypican-3 assessed via immunohistochemistry, and high arginase-1 and glypican-3 expression was associated with a poor prognosis [69].

Tsilimigras *et al*. [70] developed the LabScore scoring system based on data from 660 patients who underwent hepatectomy for ICC. The LabScore includes platelet count, CA19-9, albumin, and the neutrophil-to-lymphocyte ratio (NLR). A higher LabScore was associated with worse tumor characteristics, TNM stage of disease, and was significantly associated with 5-year OS, DFS, and disease recurrence. Furthermore, Tsilimigras *et al*. [71] created a classification tree based on the analysis of 826 patients with a history of ICC resection and divided them into 3 clusters: common, proliferative, and inflammatory according to tumor size, CA 19-9, and NLR. Although patients in the inflammatory cluster had the lowest CA19-9 levels, mid-sized tumors, and the highest NLR, these individuals had the worst median OS.

KRAS and BRAF mutations may also be associated with prognosis [43,44]. KRAS mutations are associated with perineural invasion and a worse post-operative survival in patients with ICC [72]. However, patients with KRAS mutations had a worse 5-year OS than patients with BRAF mutations (13.5 vs 23.2 months) [43]. Additionally, elevated EGFR was associated with a worse prognosis with a shorter median OS (8.5 months versus 38.5 months) [73], and reduced PTEN expression is a predictor of poor OS in patients with ICC who have undergone resection [40,74]. TP53 mutations have a prevalence of 0.7–37% in patients with ICC and are generally associated with a worse prognosis [75,76]. MET mutations occur in approximately 12–58% of ICC tumors, and MET overexpression is associated with increased invasion and poor prognosis [77,78].

DNA methylation, histone modification, and non-coding RNA-associated gene silencing may initiate and sustain epigenetic changes involved in the pathogenesis of ICC [25]. Additionally, micro-RNA expression patterns are involved in the pathogenesis of ICC and can differentiate tumor from normal tissue [79]. Alternative splicing is a critical step in post-translational modification of mRNA and can predict the prognosis and recurrence of HCC and ICC [80–83]. A cluster analysis based on differentially expressed alternative splicing (DEAS) was performed with HCC, ICC, and normal liver tissue [84]. Luo *et al*. [84] reported differences in DEAS between the samples and highlighted the

Table 1. Prognostic markers for intrahepatic cholangiocarcinoma.

Marker	Source	Prognostic indicator	Poor prognosis
A1BG/AFM ratio	Blood	OS, PRM	Yes
MMP7	Blood	OS	Yes
CYFRA21-1	Blood	LNM, ATS, IHM, VI	Yes
TuM2-PK	Blood	LNM, VI	Yes
CA19-9	Blood, Bile	OS, LNM	Yes
DKK1	Blood, Tissue	OS, ATS	Yes
MUC5AC	Blood, Tissue	OS, ATS, NI	Yes
Fibronectin	Tissue	AD, LNM	Yes
Vimentin	Tissue	OS, ATS, LNM	Yes
Gli1	Tissue	OS	Yes
Capn4	Tissue	OS, ATS, LNM	Yes
Fascin	Tissue	OS, LNM, VI, DM	Yes
IL-17	Tissue	OS	Yes
MUC1	Tissue	OS, VI	Yes
MUC16	Tissue	OS	Yes
N-cadherin	Tissue	VI	Yes
p-4EBP1	Tissue	OS	Yes
Smad4	Tissue	OS, ATS, LNM, IHM	Yes
CD151	Tissue	OS, LNM, VI, DM	Yes
S100A4	Tissue	OS	Yes
MAGE-A3/4	Tissue	OS	Yes
c-Met	Tissue	OS, LNM, DM	Yes
EGFR	Tissue	OS	Yes
Ye	Tissue	OS, NI	Yes
BRAF	Tissue	OS	Yes
TP53	Tissue	OS	Yes
Periostin	Tissue	OS	Yes
PRL-3	Tissue	OS, ATS, LNM, VI	Yes
Skp2	Tissue	OS	Yes
VEGF-C	Tissue	OS, LNM, PRM	Yes
14-3-3x	Tissue	OS, LNM	Yes
mir-200a	Tissue	ATS	Yes
mir-204	Tissue	OS, VI	Yes
mir-192	Tissue	OS, LNM	Yes
CTGF	Tissue	OS, Recurrence	No
p-AKT1	Tissue	OS	No
p-mTOR	Tissue	OS	No
PTEN	Tissue	OS	No
p27	Tissue	OS, LNM	No
P120-catenin	Tissue	OS, ATS	No
Beclin1	Tissue	OS, LNM	No
E-cadherin	Tissue	OS, ATS, LNM, NI	No
b catenin	Tissue	LNM	No
Arginase-1	Tissue	OS	Yes
Glypican-3	Tissue	OS	Yes
Core 3-synthase	Tissue	OS	No
6-sulfated N-acetyllactosamine	Tissue	OS	Yes
DUSP11	Tissue	OS, ATS	Yes
IDH	Tissue	OS	Yes

Abbreviations: OS, overall survival; PRM, positive resection margin; LNM, lymph node metastasis; ATS, advanced T stage; IHM, intrahepatic metastasis; VI, vascular invasion; NI, neural invasion; DM, distant metastasis.

prognostic significance of DEAS among the tissue samples, developing predictive models that demonstrated clinical utilization. Additionally, increased levels of the heat shock protein 70-kDa protein 1 (HSP70.1), involved in regulating the cell cycle, may be inversely correlated to OS in patients with CCA [85,86].

Tumor type M2 pyruvate kinase (TuM2-PK) can be useful to distinguish CCA from benign disease as levels are often elevated in CCA proportional to tumor burden such that high levels of TuM2-PK are seen in patients with lymph node metastasis [87–89]. In one study, the sensitivity and specificity of TuM2-PK for CCA exceeded that of CA19-9 and was able to discriminate CCA from healthy controls [25,88]. Connective tissue growth factor (CTGF) expression may be associated with longer DFS and OS, but the mechanism of how CTGF influences tumor biology remains largely unknown [90]. Wnt1-inducible signaling pathway protein 1 (WIPS1), part of the WNT pathways, is involved in regulating cell proliferation, differentiation, adhesion, migration, and survival. WISP1 expression is associated with ICC carcinogenesis, overexpressed in 49% of ICC cases, and associated with a poor prognosis [91,92]. Elevated CYFRA 21-1 was associated with poor 3-year RFS and OS [93]. MMP-7 is expressed by malignant cholangiocytes and predicts poor post-operative survival. Similarly, MMP-9 predicts lymph node metastasis [94].

The mucin family of glycoproteins may help in the diagnosis and prognosis of CCA. For example, KL-6 mucin may help differentiate ICC from HCC [95]. Additionally, MUC4 and MUC5AC may distinguish benign from malignant biliary disease [96–98]. MUC1, MUC4 and MUC16 can predict poor post-operative outcomes, while MUC2 positive tumors have a more favorable prognosis [99–103]. Interestingly, MUC5AC is associated with liver fluke-associated ICC [104].

Core 3 synthase plays an important role in the digestive system, and cells expressing core 3 synthase show lower migratory and invasive rates, as well as lower metastatic activity. Indeed, in CCA, the expression of core 3 synthase, identified by the antibody G8-144, was associated with lower mortality rates [105]. On the other hand, expression of 6-sulfated *N*-acetyllactosamine on the extended core-1 *O*-glycans, identified by the antibody MECA-79, was associated with an unfavorable prognosis [105]. Additionally, dual-specificity phosphatase 11 (DUSP11) was evaluated in eight pairs of ICC, PHCC, and distal CCA, and their corresponding adjacent tissue by qPCR. In all types of CCA, DUSP11 was elevated compared with the adjacent tissue. In ICC, high DUSP11 was associated with an advanced T stage and poor prognosis, which was not the case for ECC or PHCC [106].

DDK1 expression in tumor tissue from ICC is associated with elevated MMP-9 and vascular endothelial growth factor-C (VEGF-C) expression which, in turn, is associated with tumor invasion and a high incidence of lymph node metastasis [107]. Additionally, IDH mutations were more common in tumors with poor histology and are associated with worse survival after resection [50].

4. Biomarkers to guide therapy

Systemic chemotherapy options for ICC are limited as drug resistance and drug-related toxicities are common [23]. Gemicatine with cisplatin is the standard systemic therapy for advanced cholangiocarcinoma [108]. However, through better genetic analysis and understanding of distinct biomarkers, targeted therapies have been developed in an attempt to improve treatment response and survival [24,25]. As previously discussed, IDH mutations may be present in 15–30% of patients and are a poor diagnostic marker [50,51]. IDH inhibitors have been utilized in the treatment of ICC with limited success. The ClarIDHy study was a phase III randomized controlled trial (RCT) involving 185 patients with IDH1-mutated cholangiocarcinoma. The patients were assigned to the IDH1 inhibitor, ivosidenib, or placebo. Patients who received ivosidenib had significantly longer PFS compared to placebo (2.7 versus 1.4 months, respectively) [109].

Lapatinib, an inhibitor of EGFR and HER-2, as well as trastuzumab, a HER2 inhibitor, have some demonstrated efficacy in CCA [110]. Similarly, among patients with advanced biliary cancer, including ICC, erlotinib, an EGFR inhibitor, with or without bevacizumab, a VEGF inhibitor, has clinical efficacy in ICC [111,112]. However, the addition of erlotinib to GEMOX did not improve OS or PFS [113].

Fibroblast growth factor receptor (FGFR) alterations are present in 10–15% of patients with ICC [52]. Pemigatinib, an inhibitor of FGFR, has been reported to have a 35% objective response rate in ICC patients harboring an FGFR mutations [114]. However, 42% of patients died from disease progression and 45% of patients had serious adverse events [114]. Nevertheless, pemigatinib is currently under investigation in a phase 3 RCT that compares the efficacy and safety of pemigatinib versus gemcitabine and cisplatin among patients with advanced or metastatic cholangiocarcinoma with an FGFR2 fusion or rearrangement (NCT03656536) [115]. Similarly, futibatinib, a different inhibitor of FGFR, was investigated in patients with ICC and FGFR2 alterations who had disease progression after first-line therapy [116]. Futibatinib had a 34% objective response rate and 76% disease control rate at $\geq$6 months follow-up, but serious adverse events occurred in 73% of patients [117]. Futibatinib is currently being compared with gemcitabine-cisplatin in a phase 3 study of patients with advanced cholangiocarcinoma and an *FGFR2* fusion or rearrangement (NCT04093362) [115].

A study by Chen *et al*. [118] performed targeted next generation sequencing in 98 patients with advanced biliary tract cancer treated with camrelizumab plus gemcitabine and oxaliplatin. The authors noted that KRAS and TP53 mutations were much more frequent in advanced-stage biliary tract cancers than in early-stage disease. KRAS-TP53 co-mutations were favored in advanced CCA, with a favorable response to immunotherapy and single KRAS mutations predicted poor prognosis and immunotherapy outcomes for CCA. Tsilimigras *et al*. [119] reported on the role of tumor burden as a predictor of outcomes in 1101 patients with ICC who received surgical treatment. Patients were divided into groups of low, medium, and high tumor burden. The 5-year OS was incrementally worse as the tumor burden increased. In subgroup analysis, patients with high tumor burden that received adjuvant chemotherapy had significantly better outcomes than individuals who did not.

5. Immune signature and ICC

Immune based therapies have changed the landscape of cancer care from directly targeting the tumor itself to manipulation and activation of the immune system to eradicate tumor cells. However, a minority of patients respond to immunotherapies and advances are needed in immune based biomarkers to predict response to therapy and guide treatment decisions. Immune checkpoint inhibitors (ICIs) are currently approved for patients with solid gastrointestinal malignancies that have mismatch repair deficiency that includes ICC [120,121]. Unfortunately, mismatch repair deficiencies are reported in only 1–10% of patients with ICC [122]. Mismatch repair deficiencies were detected more frequently among patients with liver-fluke associated tumors [58]. Pembrolizumab, an anti-PD-1 ICI, is currently approved for the treatment of solid tumors with mismatch repair deficiencies [120]. Nivolumab, another anti-PD1 ICI, and pembrolizumab have demonstrated acceptable response rates in early phase clinical trials in patients with biliary tract cancers [123–126]. The LEAP-005 study is currently investigating lenvatinib, a kinase inhibitor, in combination with pembrolizumab in patients with advanced solid tumors. Interim analysis has demonstrated an overall response rate of only 10% with a disease response rate of 21% and duration of response of 5.3 months [127].

PD-L1 expression may be present in up to 70% of ICC tumors and be associated with worse survival [122,128]. PD-L1 expression correlates with response to ICIs in patients with non-small cell lung cancer, gastric cancer, and urothelial cancer, but efficacy data is limited among patients with ICC [122,129–131]. There has been increased interest in the possible utility of DNA damage repair (DDR) gene mutations as a predictive biomarker to immunotherapy response. DDR gene mutations, such as in poly (ADP-ribose) polymerase 1 and 2 (PARP) or breast related cancer antigens (BRCA), prevent the ability of cells to repair DNA damage effectively repair DNA damage resulting in genomic instability [122,132]. DDR deficiency may lead to antitumor immunity by activating the innate immune response [133]. Additionally, other immune or inflammatory markers such as C-reactive protein (CRP) are associated with tumor recurrence after resection. For example, a CRP level $<$1.0 mg/dL was a favorable prognostic factor among patients with biliary tract cancers receiving chemotherapy [134]. Furthermore, interleukin-6 (IL-6) is proportional to pre-operative and post-operative tumor burden in patients with CCA [135]. Therefore, IL-6 may be useful as a potential diagnostic marker but low specificity limits its utility in this manner [136,137]. Transforming growth factor (TGF)-β plays a role in cancer development as it is essential for cellular proliferation and differentiation. The expression of TGF-β is an indicator of early tumor recurrence [138]. Similarly, SMAD4, a protein involved in TGF-β signaling, is downregulated in approximately 55% of patients with ICC and associated with increased lymph node metastasis and poor tumor differentiation [139,140].

Recent data have noted four immune subsets of ICC characterized based on the composition of the tumor microenvironment. The immune desert phenotype is the most common, comprising 48% of cases, and is characterized by weak expression of immune markers. Meanwhile, the immunogenic pattern is characterized by a high amount of innate and adaptive immune cells and strong activation of inflammatory and immune checkpoint pathways. The myeloid rich subset is characterized by moderate to strong expression of myeloid signatures and a low lymphocytic signature. The last subtype has mesenchymal features with strong expression of activated fibroblast and is most associated with a poor prognosis [141].

The use of adoptive cell transfer may benefit patients with metastatic cholangiocarcinoma. Tran *et al*. [142] utilized whole-exome-sequencing to demonstrate that tumor infiltrating lymphocytes (TIL) from a patient with metastatic cholangiocarcinoma contained CD4+ T cells that recognized a mutation in erbb2 interacting protein (ERBB2IP) and the transfer of TIL decreased metastatic tumor burden. In addition, upon disease progression, the patient was again treated with TIL and experienced disease regression. This case report demonstrated the utility of sequencing to target unique mutations to provide a highly personalized therapy for patients with advanced disease. Evolving immune therapies may show promising results in the treatment of ICC (Table 2).

6. Future directions

Biomarkers assist in the diagnosis of multiple malignancies and emerging data support their use to guide patient selection for both surgery and systemic therapy. An important pathway to improve survival for patients with ICC is to identify biomarkers that appropriately select patients who might benefit from either neoadjuvant or adjuvant systemic therapy, as well as identify patients with tumors at high risk

Table 2. Immune based therapies in the treatment of intrahepatic cholangiocarcinoma.

NCT Number	Title	Population	Interventions	Characteristics	Enrollment	Location
NCT03633773	Safety and Efficacy Evaluation of MUC-1 CAR T in the Treatment of ICC	ICC	MUC-1 CAR T cell immunotherapy	Phase I/II	9	China
NCT04238637	Immunotherapy Combined With Y-90 SIRT Therapy in Advanced Stage Intrahepatic BTC	ICC	Durvalumab Tremelimumab	Phase II	50	Germany
NCT04989218 ICC	Durvalumab and Tremelimumab With Platinum-based Chemotherapy in ICC	CCA	Durvalumab Tremelimumab Gemcitabine Cisplatin	Phase I/II	20	USA
NCT03820310	Clinical Trial of Autologous Tcm Immunotherapy in ICC	ICC- After resection	autologous Tcm cellular immunotherapy	Phase II	20	China
NCT04413734	Combination of Anti-PD-1 Antibody and Chemotherapy for Unresectable ICC	ICC	Triprilumab Gemcitabine Cisplatin	Phase II	120	China
NCT04834674	DEB-TACE Combined With Apatinib and PD-1 for the Treatment of ICC	ICC	DEB-TACE apatinib carrelizumab	Phase II	20	China
NCT03898895 CORRECT	Combination of Radiotherapy With Anti-PD-1 Antibody for unresectable ICC	ICC	Radiotherapy Camrelizumab Gemcitabine Cisplatin	Phase II	184	China
NCT04708067	Hypofractionated Radiation Therapy and Bintrafusp Alfa for the Treatment of Advanced ICC	ICC	Bintrafusp Alfa Hypofractionated Radiation Therapy	Phase I	15	USA
NCT03201458	Atezolizumab With or Without Cobimetinib in Treating Patients With Metastatic BTC That Cannot Be Removed by Surgery or GBC	CCA, GBC	Atezolizumab Cobimetinib	Phase II	76	USA
NCT04301778	Durvalumab in Combination With a CSF-1R Inhibitor (SNDX-6532) Following Chemo or Radio- Embolization for Patients With ICC	ICC	Durvalumab SNDX-6352	Phase II	30	USA
NCT04068194	Testing the Combination of New Anti-cancer Drug Peposertib With Avelumab and Radiation Therapy for Advanced/Metastatic Solid Tumors and Hepatobiliary Malignancies	CCA, GBC	Avelumab Hypofractionated Radiation Therapy Peposertib	Phase I/II	39	USA
NCT02520141	Ramucirumab in Treating Patients With Advanced or Metastatic, Previously Treated BTC That Cannot Be Removed by Surgery	CCA, GBC	Ramucirumab	Phase II	61	USA

Table 2. Continued.

NCT Number	Title	Population	Interventions	Characteristics	Enrollment	Location
NCT04941287	Testing A New Combination of Anti-cancer Immune Therapies, Atezolizumab and CDX-1127 (Varlilumab) With or Without the Addition of A Third Anti-cancer Drug, Cobimetinib, for Advanced-Stage BTC	CCA, GBC	Atezolizumab Cobimetinib Varlilumab	Phase II	64	USA
NCT02834013	Nivolumab and Ipilimumab in Treating Patients With Rare Tumors	Many	Ipilimumab Nivolumab	Phase II	818	USA
NCT04466891 HERIZON-BTC-01	A Study of ZW25 (Zanidatamab) in Subjects With Advanced or Metastatic HER2-Amplified BTC	HER2-amplified CCA and GBC	Zanidatamab	Phase II	100	USA
NCT03929666	A Safety and Efficacy Study of ZW25 (Zanidatamab) Plus Combination Chemotherapy in HER2-expressing Gastrointestinal Cancers	HER2-expressing Gastrointestinal Cancers, Including Gastroesophageal Adenocarcinoma, BTC and Colorectal Cancer	Zanidatamab Capecitabine Cisplatin Fluorouracil Leucovorin Oxaliplatin Bevacizumab Gemcitabine	Phase II	362	USA

Abbreviations: CAR, chimeric antigen receptor; ICC, intrahepatic cholangiocarcinoma; CCA, cholangiocarcinoma; GBC, gallbladder cancer; BTC, biliary tract cancer; SIRT, selective internal radiation therapy; DEB-TACE, drug eluding bead-transarterial chemoembolization.

Table 3. Ongoing clinical trials for patients with cholangiocarcinoma.

NCT Number	Title	Population	Interventions	Characteristics	Enrollment	Location
NCT02807181 SIRCCA	SIRT Followed by CIS-GEM Chemotherapy Versus CIS- GEM Chemotherapy Alone as 1st Line Treatment of Patients With Unresectable ICC	ICC	Gemcitabine SIRT + cisplatin-gemcitabine	Phase II/III	89	International
NCT04961970	HAIC With FOLFOX Versus Systemic Chemotherapy With GP for Unresectable ICC	ICC	FOLFOX, Gemcitabine, cisplatin	Phase II/III	188	China
NCT04077983	HAIC Versus Systemic Chemotherapy for Unresectable ICC	ICC	Irinotecan, oxaliplatin, fluorouracil, and leucovorin gemcitabine and oxaliplatin	Phase III	188	China
NCT04077983	Nab-Paclitaxel Combined With Gemcitabine Adjuvant Chemotherapy After Radical Resection of ICC	ICC	nab-paclitaxel and gemcitabine	Phase II	40	N/A
NCT04891289	Gemcitabine and Oxaliplatin Chemotherapy With or Without a Floxuridine and Dexamethasone Pump in People With CCA That Cannot Be Removed With Surgery	ICC	Gemcitabine, oxaliplatin, dexamethasone, FUDR	Phase II	164	USA
NCT04527679	Cisplatin and Gemcitabine Chemotherapy and Lenvatinib for Patients With Unresectable ICC	ICC	Cisplatin and Gemcitabine combined Lenvatinib	Phase II	40	China
NCT01862315	Hepatic Arterial Infusion With FUDR and Dexamethasone Combined With Systemic Gemcitabine and Oxaliplatin in Patients With Unresectable ICC	CCA	FUDR, dexamethasone, Gemcitabine, Oxaliplatin	Phase II	55	USA
NCT04251715	mFOLFIRINOX Followed by Hepatic Arterial Infusion of Floxuridine and Dexamethasone With Systemic mFOLFIRI for Unresectable Liver-dominant ICC	ICC	Floxuridine, Ironotecan, Oxaliplatin, Leucovorin, Dexamethasone	Phase II	30	USA
NCT03364530 GEMOXIA-02	Hepatic Arterial Infusion of Gemcitabine-oxaliplatin for Second- line Therapy in Non-metastatic Unresectable ICC	CCA	Gemcitabine, Oxaliplatin	Phase II	40	France
NCT01648023 DELTIC	Drug-Eluting Bead, Irinotecan Therapy for Unresectable ICC w/Concomitant Gemcitabine and Cisplatin or Carboplatin	ICC	Gem-Cis or Gem- Carbo ONCOZENE Bead with Gem-Cis or Gem-Carbo	Phase II	49	USA
NCT03086993	Percutaneous Hepatic Perfusion vs. Cisplatin/Gemcitabine in Patients With ICC	ICC	Cisplatin and Gemcitabine Melphalan/HDS	Phase II/III	295	USA

Table 3. Continued.

NCT Number	Title	Population	Interventions	Characteristics	Enrollment	Location
NCT04546828	A Single-arm Study of Gemcitabine, Cisplatin, and Nab-Paclitaxel as Neoadjuvant Therapy for Resectable Oncologically High-Risk ICC in Korea	ICC	Gemcitabine, Cisplatin, and Nab-Paclitaxel	Phase II	34	N/A
NCT01938729	Hepatic Arterial Infusion With Floxuridine and Dexamethasone in Combination With Gemcitabine as Adjuvant Treatment After Resection of ICC	CCA	Liver resection and placement of HAIC, Floxuridine, dexamethasone, gemcitabine	Phase I	8	USA
NCT03579771	Gemcitabine, Cisplatin, and Nab- Paclitaxel Before Surgery in Patients With High-Risk Liver Bile Duct Cancer	ICC	Cisplatin, Gemcitabine, Nab-paclitaxel	Phase II	31	USA
NCT02392637	Gemcitabine Hydrochloride, Cisplatin, and Nab-Paclitaxel in Treating Patients With Advanced or Metastatic Biliary Cancers	CCA, GBC	Gemcitabine, cisplatin, Nab-paclitaxel	Phase II	62	USA
NCT03768414	Gemcitabine Hydrochloride and Cisplatin With or Without Nab- Paclitaxel in Treating Patients With Newly Diagnosed Advanced Biliary Tract Cancers	CCA	Gemcitabine, cisplatin, Nab-paclitaxel	Phase III	452	USA
NCT01247337	Intra-hepatic Chemotherapy in Patient With Non-resectable Liver Metastases From Cholangiocarcinoma	CCA	Oxaliplatin, capecitabine, gemcitabine, cetuximab	Phase II	56	Denmark
NCT01825603	ADH-1, Gemcitabine Hydrochloride and Cisplatin in Treating Patients With Locally Advanced or Metastatic Pancreatic or Biliary Tract Cancer That Cannot Be Removed by Surgery	CCA, GBC, Pancreatic cancer, Ampullary cancer	Gemcitabine, cisplatin, ADH-1	Phase I	17	USA
NCT04068194	Testing the Combination of New Anti-cancer Drug Peposertib With Avelumab and Radiation Therapy for Advanced/Metastatic Solid Tumors and Hepatobiliary Malignancies	CCA, GBC, malignant solid neoplasm	Avelumab, peposertib	Phase I/II	39	USA
NCT02162914 REACHIN	Regorafenib Versus Placebo to Treat Cholangiocarcinoma	CCA	Regorafenib	Phase II	66	Belgium

Abbreviations: ICC, intrahepatic cholangiocarcinoma; CCA, cholangiocarcinoma; GBC, gallbladder cancer; HAIC, hepatic artery infusion chemotherapy; FUDR, Floxuridine.

for recurrence after resection. While systemic therapy provides a modest benefit to some patients with ICC, other patients may be unlikely to enjoy therapeutic benefit while experiencing toxicity. As such, the appropriate selection of patients may help avoid morbidity and minimize unnecessary exposure to toxic therapies for patients unlikely to derive clinical benefit. Currently, gemcitabine in combination with cisplatin remains the standard systemic treatment for advanced ICC with a median OS of 11.7 months versus 8.1 months among patients receiving gemcitabine alone [108]. Additional studies of systemic and locoregional therapies for ICC are underway (Table 3).

Novel methods for biomarker detection are also currently under investigation such as liquid biopsies. Liquid biopsies involve the detection of markers in patient fluid samples (e.g., blood, urine, or bile) that can be used to evaluate disease biology. Furthermore, liquid biopsies allow for serial detection of these markers that can provide information on changes in tumor biology [143]. Circulating tumor DNA (ctDNA) or RNA, circulating tumor cells (CTC), and tumor-derived exosomes, cytokines, and proteins are all biomarkers of interest. ctDNA may be used to assess response to systemic therapy. Ettrich *et al*. [144] investigated tumor tissue and corresponding ctDNA samples collected from patients with CCA prior to and during chemotherapy. Of note, blood and tissue were concordant in 92% of ICCs, and variant allele frequency in ctDNA correlated with tumor load and progression-free survival (PFS). Yang *et al*. [145] studied the use of CTCs in 88 patients with CCA and reported that 15 patients were positive for CTC; CTCs were associated with the extent of disease, more aggressive tumors, and predicted survival. Additionally, Han *et al*. [146] investigated the use of circulating microRNA as a bile-derived biomarker in cholangiocarcinoma and noted that microRNA represented the oncogenic characteristics of CCA tissue.

Identification of extracellular vesicles (EV) may play a role as an emerging biomarker for CCA. EVs are endocytic-oriented membrane vesicles released by tumor cells and are vital to regulate cellular microenvironments by transporting biologic material [147]. EVs may be involved in tumor-induced inflammation and chemoresistance [148,149]. Xu *et al*. [150] identified CCA-associated circRNA, circ-CCAC1, upregulated in bile EVs and tissues. circ-CCAC1 may serve as a biomarker or therapeutic target for cholangiocarcinoma. Likewise, the role of circRNA in ICC is limited, but trials are ongoing [151]. Expression of circSMARC5 was decreased in ICC tissue and negatively correlated with advanced stage [152]. Similarly, circACTN4 promotes tumor cell growth by regulating Wnt signaling pathways and thus promotes tumor cell growth in ICC [153]. Therapeutic strategies could be developed to reduce the pro-oncogenic activity of circRNA. For example, target site blockers (TSBs) could target the miRNA response elements carried by circRNAs [151,154].

7. Conclusions

Patients with ICC have a poor prognosis despite multimodality therapy including resection with curative intent and systemic therapy. Furthermore, these therapies come with morbidity and toxicity for many patients. Emerging biomarkers may provide diagnostic utility and assist with treatment decisions for patients with ICC including appropriate patient selection for surgical intervention and individualized perioperative systemic therapy regimens. Furthermore, innovative investigative techniques, such as next generation sequencing, are expected to expand our knowledge of tumor biology and the underlying genetic and epigenetic drivers of disease. As a result, novel biomarkers will play an increasingly significant role in the management of patients with ICC. Future studies are required to evaluate novel biomarkers, as well as further define how to apply biomarkers in the clinical setting.

Author contributions

ZJB, DBH and TMP—Study Design; ZJB, DBH and TMP—Preparing the Manuscript; ZJB, DBH and TMP—critical review and revision of the manuscript.

Ethics approval and consent to participate

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Conflict of interest

The authors declare no conflict of interest.

References

[1] Spolverato G, Bagante F, Ethun CG, Poultsides G, Tran T, Idrees K, *et al*. Defining the Chance of Statistical Cure among Patients with Extrahepatic Biliary Tract Cancer. World Journal of Surgery. 2017; 41: 224–231.

[2] Rizvi S, Khan SA, Hallemeier CL, Kelley RK, Gores GJ. Cholangiocarcinoma — evolving concepts and therapeutic strategies. Nature Reviews Clinical Oncology. 2018; 15: 95–111.

[3] Beal EW, Tumin D, Moris D, Zhang X, Chakedis J, Dilhoff M, *et al*. Cohort contributions to trends in the incidence and mortality of intrahepatic cholangiocarcinoma. HepatoBiliary Surgery and Nutrition. 2018; 7: 270–276.

[4] Wu L, Tsilimigras DI, Paredes AZ, Mehta R, Hyer JM, Merath K, *et al*. Trends in the Incidence, Treatment and Outcomes of Patients with Intrahepatic Cholangiocarcinoma in the USA: Facility Type is Associated with Margin Status, Use of Lymphadenectomy and Overall Survival. World Journal of Surgery. 2019; 43: 1777–1787.

[5] Brown KM, Parmar AD, Geller DA. Intrahepatic cholangiocarcinoma. Surgical Oncology Clinics of North America. 2014; 23: 231–246.

[6] Florio AA, Ferlay J, Znaor A, Ruggieri D, Alvarez CS, Laversanne M, *et al*. Global trends in intrahepatic and extrahepatic cholangiocarcinoma incidence from 1993 to 2012. Cancer. 2020; 126: 2666–2678.
[7] Van Dyke AL, Shiels MS, Jones GS, Pfeiffer RM, Petrick JL, Beebe-Dimmer JL, *et al*. Biliary tract cancer incidence and trends in the United States by demographic group, 1999–2013. Cancer. 2019; 125: 1489–1498.
[8] Blechacz B. Cholangiocarcinoma: Current Knowledge and New Developments. Gut and Liver. 2017; 11: 13–26.
[9] Sia D, Tovar V, Moeini A, Llovet JM. Intrahepatic cholangiocarcinoma: pathogenesis and rationale for molecular therapies. Oncogene. 2013; 32: 4861–4870.
[10] Andersen JB. Molecular pathogenesis of intrahepatic cholangiocarcinoma. Journal of Hepato-Biliary-Pancreatic Sciences. 2015; 22: 101–113.
[11] Komuta M, Govaere O, Vandecaveye V, Akiba J, Van Steenbergen W, Verslype C, *et al*. Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes. Hepatology. 2012; 55: 1876–1888.
[12] Roskams T. Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. Oncogene. 2006; 25: 3818–3822.
[13] Khan SA, Tavolari S, Brandi G. Cholangiocarcinoma: Epidemiology and risk factors. Liver International. 2019; 39: 19–31.
[14] Sia D, Hoshida Y, Villanueva A, Roayaie S, Ferrer J, Tabak B, *et al*. Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. Gastroenterology. 2013; 144: 829–840.
[15] Doherty B, Nambudiri VE, Palmer WC. Update on the Diagnosis and Treatment of Cholangiocarcinoma. Current Gastroenterology Reports. 2017; 19: 2.
[16] Cillo U, Fondevila C, Donadon M, Gringeri E, Mocchegiani F, Schlitt HJ, *et al*. Surgery for cholangiocarcinoma. Liver International. 2019; 39: 143–155.
[17] Lauterio A, De Carlis R, Centonze L, Buscemi V, Incarbone N, Vella I, *et al.* Current Surgical Management of Peri-Hilar and Intra-Hepatic Cholangiocarcinoma. Cancers. 2021; 13: 3657.
[18] Beal EW, Cloyd JM, Pawlik TM. Surgical Treatment of Intrahepatic Cholangiocarcinoma: Current and Emerging Principles. Journal of Clinical Medicine. 2020; 10: 104.
[19] Benson AB, D'Angelica MI, Abbott DE, Abrams TA, Alberts SR, Saenz DA, *et al*. NCCN Guidelines Insights: Hepatobiliary Cancers, Version 1.2017. Journal of the National Comprehensive Cancer Network. 2017; 15: 563–573.
[20] Rea DJ, Heimbach JK, Rosen CB, Haddock MG, Alberts SR, Kremers WK, *et al*. Liver transplantation with neoadjuvant chemoradiation is more effective than resection for hilar cholangiocarcinoma. Annals of Surgery. 2005; 242: 451–461.
[21] Hu L, Zhang X, Weiss M, Popescu I, Marques HP, Aldrighetti L, *et al*. Redefining Conditional Overall and Disease-Free Survival after Curative Resection for Intrahepatic Cholangiocarcinoma: a Multi-institutional, International Study of 1221 patients. Journal of Gastrointestinal Surgery. 2020; 24: 2756–2765.
[22] Zhang X, Beal EW, Bagante F, Chakedis J, Weiss M, Popescu I, *et al*. Early versus late recurrence of intrahepatic cholangiocarcinoma after resection with curative intent. The British Journal of Surgery. 2018; 105: 848–856.
[23] Valle JW, Furuse J, Jitlal M, Beare S, Mizuno N, Wasan H, *et al*. Cisplatin and gemcitabine for advanced biliary tract cancer: a meta-analysis of two randomised trials. Annals of Oncology. 2014; 25: 391–398.
[24] Sicklick JK, Fanta PT, Shimabukuro K, Kurzrock R. Genomics of gallbladder cancer: the case for biomarker-driven clinical trial design. Cancer Metastasis Reviews. 2016; 35: 263–275.
[25] Rahnemai-Azar AA, Weisbrod A, Dillhoff M, Schmidt C, Pawlik TM. Intrahepatic cholangiocarcinoma: Molecular markers for diagnosis and prognosis. Surgical Oncology. 2017; 26: 125–137.
[26] Hewitt DB, Brown ZJ, Pawlik TM. Surgical management of intrahepatic cholangiocarcinoma. Expert Review of Anticancer Therapy. 2021; 22: 27–38.
[27] Fábrega-Foster K, Ghasabeh MA, Pawlik TM, Kamel IR. Multimodality imaging of intrahepatic cholangiocarcinoma. Hepatobiliary Surgery and Nutrition. 2017; 6: 67–78.
[28] Valls C, Gumà A, Puig I, Sanchez A, Andía E, Serrano T, *et al*. Intrahepatic peripheral cholangiocarcinoma: CT evaluation. Abdominal Imaging. 2000; 25: 490–496.
[29] Seo N, Kim DY, Choi J. Cross-Sectional Imaging of Intrahepatic Cholangiocarcinoma: Development, Growth, Spread, and Prognosis. AJR. American Journal of Roentgenology. 2017; 209: W64–W75.
[30] El-Diwany R, Pawlik TM, Ejaz A. Intrahepatic Cholangiocarcinoma. Surgical Oncology Clinics of North America. 2019; 28: 587–599.
[31] Nehls O, Gregor M, Klump B. Serum and Bile Markers for Cholangiocarcinoma. Seminars in Liver Disease. 2004; 24: 139–154.
[32] Viterbo D, Gausman V, Gonda T. Diagnostic and therapeutic biomarkers in pancreaticobiliary malignancy. World Journal of Gastrointestinal Endoscopy. 2016; 8: 128–142.
[33] Tao L, Cai L, He X, Liu W, Qu Q. Comparison of serum tumor markers for intrahepatic cholangiocarcinoma and hepatocellular carcinoma. The American Surgeon. 2010; 76: 1210–1213.
[34] Lazaridis KN, LaRusso NF. Primary Sclerosing Cholangitis. New England Journal of Medicine. 2016; 375: 1161–1170.
[35] Levy C, Lymp J, Angulo P, Gores GJ, Larusso N, Lindor KD. The value of serum CA 19-9 in predicting cholangiocarcinomas in patients with primary sclerosing cholangitis. Digestive Diseases and Sciences. 2005; 50: 1734–1740.
[36] Vedeld HM, Grimsrud MM, Andresen K, Pharo HD, Seth E, Karlsen TH, *et al*. Early and accurate detection of cholangiocarcinoma in patients with primary sclerosing cholangitis by methylation markers in bile. Hepatology. 2021; 75: 59–73.
[37] McKay SC, Unger K, Pericleous S, Stamp G, Thomas G, Hutchins RR, *et al*. Array comparative genomic hybridization identifies novel potential therapeutic targets in cholangiocarcinoma. HPB. 2011; 13: 309–319.
[38] Homayounfar K, Gunawan B, Cameron S, Haller F, Baumhoer D, Uecker S, *et al*. Pattern of chromosomal aberrations in primary liver cancers identified by comparative genomic hybridization. Human Pathology. 2009; 40: 834–842.
[39] Dachrut S, Banthaisong S, Sripa M, Paeyao A, Ho C, Lee SA, *et al.* DNA copy-number loss on 1p36.1 harboring RUNX3 with promoter hypermethylation and associated loss of RUNX3 expression in liver fluke-associated intrahepatic cholangiocarcinoma. Asian Pacific Journal of Cancer Prevention. 2009; 10: 575–582.
[40] Xu RF, Sun JP, Zhang SR, Zhu GS, Li LB, Liao YL, *et al*. KRAS and PIK3CA but not BRAF genes are frequently mutated in Chinese cholangiocarcinoma patients. Biomedicine & Pharmacotherapy. 2011; 65: 22–26.
[41] Isa T, Tomita S, Nakachi A, Miyazato H, Shimoji H, Kusano T, *et al*. Analysis of microsatellite instability, K-ras gene mutation and p53 protein overexpression in intrahepatic cholangiocarcinoma. Hepato-Gastroenterology. 2002; 49: 604–608.
[42] Xie D, Ren Z, Fan J, Gao Q. Genetic profiling of intrahepatic cholangiocarcinoma and its clinical implication in targeted therapy. American Journal of Cancer Research. 2016; 6: 577–586.
[43] Robertson S, Hyder O, Dodson R, Nayar SK, Poling J, Beierl K, *et al*. The frequency of KRAS and BRAF mutations in intrahepatic cholangiocarcinomas and their correlation with clinical outcome. Human Pathology. 2013; 44: 2768–2773.

[44] Chakrabarti S, Kamgar M, Mahipal A. Targeted Therapies in Advanced Biliary Tract Cancer: An Evolving Paradigm. Cancers. 2020; 12: 2039.
[45] Takada M, Masuda N, Matsuura E, Kusunoki Y, Matui K, Nakagawa K, *et al*. Measurement of cytokeratin 19 fragments as a marker of lung cancer by CYFRA 21-1 enzyme immunoassay. British Journal of Cancer. 1995; 71: 160–165.
[46] Nakata B, Takashima T, Ogawa Y, Ishikawa T, Hirakawa K. Serum CYFRA 21-1 (cytokeratin-19 fragments) is a useful tumour marker for detecting disease relapse and assessing treatment efficacy in breast cancer. British Journal of Cancer. 2004; 91: 873–878.
[47] Nakata B, Chung YS, Kato Y, Ogawa M, Ogawa Y, Inui A, *et al*. Clinical significance of serum CYFRA 21-1 in gastric cancer. British Journal of Cancer. 1996; 73: 1529–1532.
[48] Lumachi F, Lo Re G, Tozzoli R, D'Aurizio F, Facomer F, Chiara GB, *et al*. Measurement of serum carcinoembryonic antigen, carbohydrate antigen 19-9, cytokeratin-19 fragment and matrix metalloproteinase-7 for detecting cholangiocarcinoma: a preliminary case-control study. Anticancer Research. 2014; 34: 6663–6667.
[49] Kashihara T, Ohki A, Kobayashi T, Sato T, Nishizawa H, Ogawa K, *et al*. Intrahepatic cholangiocarcinoma with increased serum CYFRA 21-1 level. Journal of Gastroenterology. 1998; 33: 447–453.
[50] Kipp BR, Voss JS, Kerr SE, Barr Fritcher EG, Graham RP, Zhang L, *et al*. Isocitrate dehydrogenase 1 and 2 mutations in cholangiocarcinoma. Human Pathology. 2012; 43: 1552–1558.
[51] Wang P, Dong Q, Zhang C, Kuan P, Liu Y, Jeck WR, *et al*. Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. Oncogene. 2013; 32: 3091–3100.
[52] Ross JS, Wang K, Gay L, Al-Rohil R, Rand JV, Jones DM, *et al*. New Routes to Targeted Therapy of Intrahepatic Cholangiocarcinomas Revealed by next-Generation Sequencing. The Oncologist. 2014; 19: 235–242.
[53] Janvilisri T, Leelawat K, Roytrakul S, Paemanee A, Tohtong R. Novel Serum Biomarkers to Differentiate Cholangiocarcinoma from Benign Biliary Tract Diseases Using a Proteomic Approach. Disease Markers. 2015; 2015: 105358.
[54] Tolek A, Wongkham C, Proungvitaya S, Silsirivanit A, Roytrakul S, Khuntikeo N, *et al*. Serum $\alpha 1\beta$-glycoprotein and afamin ratio as potential diagnostic and prognostic markers in cholangiocarcinoma. Experimental Biology and Medicine. 2012; 237: 1142–1149.
[55] Matsuda A, Kuno A, Kawamoto T, Matsuzaki H, Irimura T, Ikehara Y, *et al*. Wisteria floribunda agglutinin-positive mucin 1 is a sensitive biliary marker for human cholangiocarcinoma. Hepatology. 2010; 52: 174–182.
[56] Park JY, Park BK, Ko JS, Bang S, Song SY, Chung JB. Bile acid analysis in biliary tract cancer. Yonsei Medical Journal. 2006; 47: 817–825.
[57] Shen J, Wang W, Wu J, Feng B, Chen W, Wang M, *et al.* Comparative proteomic profiling of human bile reveals SSP411 as a novel biomarker of cholangiocarcinoma. PLoS ONE. 2012; 7: e47476.
[58] Kendall T, Verheij J, Gaudio E, Evert M, Guido M, Goeppert B, *et al*. Anatomical, histomorphological and molecular classification of cholangiocarcinoma. Liver International. 2019; 39: 7–18.
[59] Nakanuma Y, Kakuda Y. Pathologic classification of cholangiocarcinoma: New concepts. Best Practice & Research Clinical Gastroenterology. 2015; 29: 277–293.
[60] Cardinale V, Wang Y, Carpino G, Reid LM, Gaudio E, Alvaro D. Mucin-producing cholangiocarcinoma might derive from biliary tree stem/progenitor cells located in peribiliary glands. Hepatology. 2012; 55: 2041–2042.
[61] Igarashi S, Sato Y, Ren XS, Harada K, Sasaki M, Nakanuma Y. Participation of peribiliary glands in biliary tract pathophysiologies. World Journal of Hepatology. 2013; 5: 425–432.
[62] Nakamura H, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, *et al*. Genomic spectra of biliary tract cancer. Nature Genetics. 2015; 47: 1003–1010.
[63] Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, *et al*. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. The Oncologist. 2012; 17: 72–79.
[64] Liau J, Tsai J, Yuan R, Chang C, Lee H, Jeng Y. Morphological subclassification of intrahepatic cholangiocarcinoma: etiological, clinicopathological, and molecular features. Modern Pathology. 2014; 27: 1163–1173.
[65] Moro A, Mehta R, Sahara K, Tsilimigras DI, Paredes AZ, Farooq A, *et al*. The Impact of Preoperative CA19-9 and CEA on Outcomes of Patients with Intrahepatic Cholangiocarcinoma. Annals of Surgical Oncology. 2020; 27: 2888–2901.
[66] Shen W, Zhong W, Xu F, Kan T, Geng L, Xie F, *et al*. Clinicopathological and prognostic analysis of 429 patients with intrahepatic cholangiocarcinoma. World Journal of Gastroenterology. 2009; 15: 5976–5982.
[67] Liu S, Song Z, Hu Q, Shan D, Hu S, Li J, *et al*. Serum carbohydrate antigen (CA) 19-9 as a prognostic factor in cholangiocarcinoma: a meta-analysis. Frontiers of Medicine in China. 2010; 4: 457–462.
[68] Qiu H, Liu C, Huang M, Shen S, Wang W. Prognostic Value of Combined CA19-9 with Aspartate Aminotransferase to Lymphocyte Ratio in Patients with Intrahepatic Cholangiocarcinoma After Hepatectomy. Cancer Management and Research. 2021; 13: 5969–5980.
[69] Qiang Z, Zhang H, Jin S, Yan C, Li Z, Tao L, *et al*. The prognostic value of arginase-1 and glypican-3 expression levels in patients after surgical intrahepatic cholangiocarcinoma resection. World Journal of Surgical Oncology. 2021; 19: 316.
[70] Tsilimigras DI, Mehta R, Aldrighetti L, Poultsides GA, Maithel SK, Martel G, *et al*. Development and Validation of a Laboratory Risk Score (LabScore) to Predict Outcomes after Resection for Intrahepatic Cholangiocarcinoma. Journal of the American College of Surgeons. 2020; 230: 381–391.e2.
[71] Tsilimigras DI, Hyer JM, Paredes AZ, Diaz A, Moris D, Guglielmi A, *et al*. A Novel Classification of Intrahepatic Cholangiocarcinoma Phenotypes Using Machine Learning Techniques: an International Multi-Institutional Analysis. Annals of Surgical Oncology. 2020; 27: 5224–5232.
[72] Chen T, Jan Y, Yeh T. K-ras mutation is strongly associated with perineural invasion and represents an independent prognostic factor of intrahepatic cholangiocarcinoma after hepatectomy. Annals of Surgical Oncology. 2012; 19: S675–S681.
[73] Hoffmann A, Goekkurt E, Danenberg PV, Lehmann S, Ehninger G, Aust DE, *et al*. EGFR, FLT1 and heparanase as markers identifying patients at risk of short survival in cholangiocarcinoma. PLoS ONE. 2013; 8: e64186.
[74] Chen M, Chiang K, Cheng C, Huang S, Chen Y, Chen T, *et al*. Antitumor activity of the combination of an HSP90 inhibitor and a PI3K/mTOR dual inhibitor against cholangiocarcinoma. Oncotarget. 2014; 5: 2372–2389.
[75] Tannapfel A, Weinans L, Geissler F, Schütz A, Katalinic A, Köckerling F, *et al*. Mutations of p53 tumor suppressor gene, apoptosis, and proliferation in intrahepatic cholangiocellular carcinoma of the liver. Digestive Diseases and Sciences. 2000; 45: 317–324.
[76] Kirsch DG, Kastan MB. Tumor-suppressor p53: implications for tumor development and prognosis. Journal of Clinical Oncology.

1998; 16: 3158–3168.
[77] Terada T, Nakanuma Y, Sirica AE. Immunohistochemical demonstration of MET overexpression in human intrahepatic cholangiocarcinoma and in hepatolithiasis. Human Pathology. 1998; 29: 175–180.
[78] Miyamoto M, Ojima H, Iwasaki M, Shimizu H, Kokubu A, Hiraoka N, *et al*. Prognostic significance of overexpression of c-Met oncoprotein in cholangiocarcinoma. British Journal of Cancer. 2011; 105: 131–138.
[79] Chen L, Yan H, Yang W, Hu L, Yu L, Liu Q, *et al*. The role of microRNA expression pattern in human intrahepatic cholangiocarcinoma. Journal of Hepatology. 2009; 50: 358–369.
[80] Yang X, Coulombe-Huntington J, Kang S, Sheynkman GM, Hao T, Richardson A, *et al*. Widespread Expansion of Protein Interaction Capabilities by Alternative Splicing. Cell. 2016; 164: 805–817.
[81] Porazinski S, Ladomery M. Alternative Splicing in the Hippo Pathway-Implications for Disease and Potential Therapeutic Targets. Genes. 2018; 9: 161.
[82] Dong S, Lu L. An alternative splicing signature model for predicting hepatocellular carcinoma-specific survival. Journal of Gastrointestinal Oncology. 2020; 11: 1054–1064.
[83] Xiong Y, Yang G, Wang K, Riaz M, Xu J, Lv Z, *et al.* Genome-Wide Transcriptional Analysis Reveals Alternative Splicing Event Profiles in Hepatocellular Carcinoma and Their Prognostic Significance. Frontiers in Genetics. 2020; 11: 879.
[84] Luo D, Zhao D, Zhang M, Hu C, Li H, Zhang S, *et al.* Alternative Splicing-Based Differences Between Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma: Genes, Immune Microenvironment, and Survival Prognosis. Frontiers in oncology. 2021; 11: 731993.
[85] Thanan R, Oikawa S, Yongvanit P, Hiraku Y, Ma N, Pinlaor S, *et al*. Inflammation-induced protein carbonylation contributes to poor prognosis for cholangiocarcinoma. Free Radical Biology & Medicine. 2012; 52: 1465–1472.
[86] Shirota T, Ojima H, Hiraoka N, Shimada K, Rokutan H, Arai Y, *et al*. Heat Shock Protein 90 is a Potential Therapeutic Target in Cholangiocarcinoma. Molecular Cancer Therapeutics. 2015; 14: 1985–1993.
[87] Eigenbrodt E, Kallinowski F, Ott M, Mazurek S, Vaupel P. Pyruvate kinase and the interaction of amino acid and carbohydrate metabolism in solid tumors. Anticancer Research. 1998; 18: 3267–3274.
[88] Li YG, Zhang N. Clinical significance of serum tumour M2-PK and CA19-9 detection in the diagnosis of cholangiocarcinoma. Digestive and Liver Disease. 2009; 41: 605–608.
[89] Suzuki H, Komuta M, Bolog A, Yokobori T, Wada S, Araki K, *et al*. Relationship between 18-F-fluoro-deoxy-D-glucose uptake and expression of glucose transporter 1 and pyruvate kinase M2 in intrahepatic cholangiocarcinoma. Digestive and Liver Disease. 2015; 47: 590–596.
[90] Gardini A, Corti B, Fiorentino M, Altimari A, Ercolani G, Grazi GL, *et al*. Expression of connective tissue growth factor is a prognostic marker for patients with intrahepatic cholangiocarcinoma. Digestive and Liver Disease. 2005; 37: 269–274.
[91] Chen LM, Xiang L, Sun WJ, Zhai YJ, Gao S, Fan YC, *et al.* Diagnostic Value of the Hypomethylation of the WISP1 Promoter in Patients with Hepatocellular Carcinoma Associated with Hepatitis B Virus. The Tohoku Journal of Experimental Medicine. 2020; 252: 297–307.
[92] Tanaka S, Sugimachi K, Kameyama T, Maehara S, Shirabe K, Shimada M, *et al*. Human WISP1v, a member of the CCN family, is associated with invasive cholangiocarcinoma. Hepatology. 2003; 37: 1122–1129.
[93] Uenishi T, Yamazaki O, Tanaka H, Takemura S, Yamamoto T, Tanaka S, *et al*. Serum Cytokeratin 19 Fragment (CYFRA21-1) as a Prognostic Factor in Intrahepatic Cholangiocarcinoma. Annals of Surgical Oncology. 2008; 15: 583–589.
[94] Shirabe K, Shimada M, Kajiyama K, Hasegawa H, Gion T, Ikeda Y, *et al*. Expression of matrix metalloproteinase-9 in surgically resected intrahepatic cholangiocarcinoma. Surgery. 1999; 126: 842–846.
[95] Xu H, Inagaki Y, Tang W, Guo Q, Wang F, Seyama Y, *et al*. Elevation of serum KL-6 mucin levels in patients with cholangiocarcinoma. Hepato-Gastroenterology. 2008; 55: 2000–2004.
[96] Matull WR, Andreola F, Loh A, Adiguzel Z, Deheragoda M, Qureshi U, *et al*. MUC4 and MUC5AC are highly specific tumour-associated mucins in biliary tract cancer. British Journal of Cancer. 2008; 98: 1675–1681.
[97] Sawanyawisuth K, Silsirivanit A, Kunlabut K, Tantapotinan N, Vaeteewoottacharn K, Wongkham S. A novel carbohydrate antigen expression during development of Opisthorchis viverrini-associated cholangiocarcinoma in golden hamster: a potential marker for early diagnosis. Parasitology International. 2012; 61: 151–154.
[98] Silsirivanit A, Araki N, Wongkham C, Pairojkul C, Narimatsu Y, Kuwahara K, *et al*. A novel serum carbohydrate marker on mucin 5AC: values for diagnostic and prognostic indicators for cholangiocarcinoma. Cancer. 2011; 117: 3393–3403.
[99] Sasaki M, Nakanuma Y, Kim YS. Characterization of apomucin expression in intrahepatic cholangiocarcinomas and their precursor lesions: an immunohistochemical study. Hepatology. 1996; 24: 1074–1078.
[100] Matsumura N, Yamamoto M, Aruga A, Takasaki K, Nakano M. Correlation between expression of MUC1 core protein and outcome after surgery in mass-forming intrahepatic cholangiocarcinoma. Cancer. 2002; 94: 1770–1776.
[101] Higashi M, Yonezawa S, Ho JJ, Tanaka S, Irimura T, Kim YS, *et al*. Expression of MUC1 and MUC2 mucin antigens in intrahepatic bile duct tumors: its relationship with a new morphological classification of cholangiocarcinoma. Hepatology. 1999; 30: 1347–1355.
[102] Shibahara H, Tamada S, Higashi M, Goto M, Batra SK, Hollingsworth MA, *et al*. MUC4 is a novel prognostic factor of intrahepatic cholangiocarcinoma-mass forming type. Hepatology. 2004; 39: 220–229.
[103] Higashi M, Yamada N, Yokoyama S, Kitamoto S, Tabata K, Koriyama C, *et al*. Pathobiological implications of MUC16/CA125 expression in intrahepatic cholangiocarcinoma-mass forming type. Pathobiology. 2012; 79: 101–106.
[104] Boonla C, Sripa B, Thuwajit P, Cha-On U, Puapairoj A, Miwa M, *et al*. MUC1 and MUC5AC mucin expression in liver fluke-associated intrahepatic cholangiocarcinoma. World Journal of Gastroenterology. 2005; 11: 4939–4946.
[105] Boottanun P, Ino Y, Shimada K, Hiraoka N, Angata K, Narimatsu H. Association between the expression of core 3 synthase and survival outcomes of patients with cholangiocarcinoma. Oncology Letters. 2021; 22: 760.
[106] Xu L, Wang P, Zhang W, Li W, Liu T, Che X. Dual-Specificity Phosphatase 11 Is a Prognostic Biomarker of Intrahepatic Cholangiocarcinoma. Frontiers in Oncology. 2021; 11: 757498.
[107] Shi R, Yang X, Shen Q, Yang L, Xu Y, Qiu S, *et al*. High expression of Dickkopf-related protein 1 is related to lymphatic metastasis and indicates poor prognosis in intrahepatic cholangiocarcinoma patients after surgery. Cancer. 2013; 119: 993–1003.
[108] Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, *et al*. Cisplatin plus Gemcitabine versus Gemcitabine for Biliary Tract Cancer. New England Journal of Medicine. 2010; 362: 1273–1281.
[109] Abou-Alfa GK, Macarulla T, Javle MM, Kelley RK, Lubner

SJ, Adeva J, *et al*. Ivosidenib in IDH1-mutant, chemotherapy-refractory cholangiocarcinoma (ClarIDHy): a multicentre, randomised, double-blind, placebo-controlled, phase 3 study. The Lancet Oncology. 2020; 21: 796–807.

[110] Andersen JB, Spee B, Blechacz BR, Avital I, Komuta M, Barbour A, *et al*. Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. Gastroenterology. 2012; 142: 1021–1031.e15.

[111] Philip PA, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, *et al*. Phase II study of erlotinib in patients with advanced biliary cancer. Journal of Clinical Oncology. 2006; 24: 3069–3074.

[112] Lubner SJ, Mahoney MR, Kolesar JL, Loconte NK, Kim GP, Pitot HC, *et al*. 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. Journal of Clinical Oncology. 2010; 28: 3491–3497.

[113] Kim ST, Jang K, Lee SJ, Jang H, Lee J, Park SH, *et al*. Tumour shrinkage at 6 weeks predicts favorable clinical outcomes in a phase III study of gemcitabine and oxaliplatin with or without erlotinib for advanced biliary tract cancer. BMC Cancer. 2015; 15: 530.

[114] Abou-Alfa GK, Sahai V, Hollebecque A, Vaccaro G, Melisi D, Al-Rajabi R, *et al*. Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study. The Lancet Oncology. 2020; 21: 671–684.

[115] Acher AW, Paro A, Elfadaly A, Tsilimigras D, Pawlik TM. Intrahepatic Cholangiocarcinoma: A Summative Review of Biomarkers and Targeted Therapies. Cancers. 2021; 13: 5169.

[116] Rizzo A, Ricci AD, Brandi G. Futibatinib, an investigational agent for the treatment of intrahepatic cholangiocarcinoma: evidence to date and future perspectives. Expert Opinion on Investigational Drugs. 2021; 30: 317–324.

[117] Goyal L, Kongpetch S, Crolley VE, Bridgewater J. Targeting FGFR inhibition in cholangiocarcinoma. Cancer Treatment Reviews. 2021; 95: 102170.

[118] Chen X, Wang D, Liu J, Qiu J, Zhou J, Ying J, *et al*. Genomic alterations in biliary tract cancer predict prognosis and immunotherapy outcomes. Journal for ImmunoTherapy of Cancer. 2021; 9: e003214.

[119] Tsilimigras DI, Hyer JM, Paredes AZ, Moris D, Sahara K, Guglielmi A, *et al.* Tumor Burden Dictates Prognosis Among Patients Undergoing Resection of Intrahepatic Cholangiocarcinoma: A Tool to Guide Post-Resection Adjuvant Chemotherapy? Annals of Surgical Oncology. 2021; 28: 1970–1978.

[120] Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, *et al*. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017; 357: 409–413.

[121] Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, *et al*. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. The New England Journal of Medicine. 2015; 372: 2509–2520.

[122] Rizzo A, Ricci AD, Brandi G. PD-L1, TMB, MSI, and Other Predictors of Response to Immune Checkpoint Inhibitors in Biliary Tract Cancer. Cancers. 2021; 13: 558.

[123] Bezu L, Gomes-de-Silva LC, Dewitte H, Breckpot K, Fucikova J, Spisek R, *et al*. Combinatorial strategies for the induction of immunogenic cell death. Frontiers in Immunology. 2015; 6: 187.

[124] Fujiwara Y, Koyama T, Helwig C, Watanabe M, Doi T. M7824 (MSB0011359C), a bifunctional fusion protein targeting PD-L1 and TGF-β, in Asian patients with advanced solid tumors. Journal of Clinical Oncology. 2018; 36: 762–762.

[125] Kim RD, Chung V, Alese OB, El-Rayes BF, Li D, Al-Toubah TE, *et al*. A Phase 2 Multi-institutional Study of Nivolumab for Patients with Advanced Refractory Biliary Tract Cancer. JAMA Oncology. 2020; 6: 888.

[126] Piha-Paul SA, Oh DY, Ueno M, Malka D, Chung HC, Nagrial A, *et al.* Efficacy and safety of pembrolizumab for the treatment of advanced biliary cancer: Results from the KEYNOTE-158 and KEYNOTE-028 studies. International Journal of Cancer. 2020; 147: 2190–2198.

[127] Lwin Z, Gomez-Roca C, Saada-Bouzid E, Yanez E, Muñoz FL, Im S, *et al*. LBA41 LEAP-005: Phase II study of lenvatinib (len) plus pembrolizumab (pembro) in patients (pts) with previously treated advanced solid tumours. Annals of Oncology. 2020; 31: S1170.

[128] Fontugne J, Augustin J, Pujals A, Compagnon P, Rousseau B, Luciani A, *et al*. PD-L1 expression in perihilar and intrahepatic cholangiocarcinoma. Oncotarget. 2017; 8: 24644–24651.

[129] Reck M, Rodríguez–Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, *et al*. Updated Analysis of KEYNOTE-024: Pembrolizumab Versus Platinum-Based Chemotherapy for Advanced Non–Small-Cell Lung Cancer with PD-L1 Tumor Proportion Score of 50

[130] Rizzo A, Mollica V, Ricci AD, Maggio I, Massucci M, Rojas Limpe FL, *et al*. Third- and later-line treatment in advanced or metastatic gastric cancer: a systematic review and meta-analysis. Future Oncology. 2020; 16: 4409–4418.

[131] Powles T, Walker J, Andrew Williams J, Bellmunt J. The evolving role of PD-L1 testing in patients with metastatic urothelial carcinoma. Cancer Treatment Reviews. 2020; 82: 101925.

[132] Pilié PG, Tang C, Mills GB, Yap TA. State-of-the-art strategies for targeting the DNA damage response in cancer. Nature Reviews Clinical Oncology. 2019; 16: 81–104.

[133] Reisländer T, Groelly FJ, Tarsounas M. DNA Damage and Cancer Immunotherapy: a STING in the Tale. Molecular Cell. 2020; 80: 21–28.

[134] Saisho T, Okusaka T, Ueno H, Morizane C, Okada S. Prognostic factors in patients with advanced biliary tract cancer receiving chemotherapy. Hepato-Gastroenterology. 2005; 52: 1654–1658.

[135] Goydos JS, Brumfield AM, Frezza E, Booth A, Lotze MT, Carty SE. Marked elevation of serum interleukin-6 in patients with cholangiocarcinoma: validation of utility as a clinical marker. Annals of Surgery. 1998; 227: 398–404.

[136] Cheon YK, Cho YD, Moon JH, Jang JY, Kim YS, Kim YS, *et al*. Diagnostic utility of interleukin-6 (IL-6) for primary bile duct cancer and changes in serum IL-6 levels following photodynamic therapy. The American Journal of Gastroenterology. 2007; 102: 2164–2170.

[137] Bonney GK, Craven RA, Prasad R, Melcher AF, Selby PJ, Banks RE. Circulating markers of biliary malignancy: opportunities in proteomics? The Lancet. Oncology. 2008; 9: 149–158.

[138] Chen Y, Ma L, He Q, Zhang S, Zhang C, Jia W. TGF-β1 expression is associated with invasion and metastasis of intrahepatic cholangiocarcinoma. Biological Research. 2015; 48: 26.

[139] Yan X, Zhang W, Zhang B, Liang H, Zhang W, Chen X. Inactivation of Smad4 is a prognostic factor in intrahepatic cholangiocarcinoma. Chinese Medical Journal. 2013; 126: 3039–3043.

[140] Chuang S, Lee K, Tsai K, Sheen P, Nagai E, Mizumoto K, *et al*. Immunohistochemical study of DPC4 and p53 proteins in gallbladder and bile duct cancers. World Journal of Surgery. 2004; 28: 995–1000.

[141] Job S, Rapoud D, Dos Santos A, Gonzalez P, Desterke C, Pascal G, *et al*. Identification of Four Immune Subtypes Characterized by Distinct Composition and Functions of Tumor Microenvironment in Intrahepatic Cholangiocarcinoma. Hepatology. 2020; 72: 965–981.

[142] Tran E, Turcotte S, Gros A, Robbins PF, Lu Y, Dudley ME, *et al*. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science. 2014; 344: 641–645.

[143] Rompianesi G, Di Martino M, Gordon-Weeks A, Montalti R,

Troisi R. Liquid biopsy in cholangiocarcinoma: Current status and future perspectives. World Journal of Gastrointestinal Oncology. 2021; 13: 332–350.

[144] Ettrich TJ, Schwerdel D, Dolnik A, Beuter F, Blätte TJ, Schmidt SA, *et al*. Genotyping of circulating tumor DNA in cholangiocarcinoma reveals diagnostic and prognostic information. Scientific Reports. 2019; 9: 13261.

[145] Yang JD, Campion MB, Liu MC, Chaiteerakij R, Giama NH, Ahmed Mohammed H, *et al*. Circulating tumor cells are associated with poor overall survival in patients with cholangiocarcinoma. Hepatology. 2016; 63: 148–158.

[146] Han J, Ahn KS, Kim YH, Kim T, Baek W, Suh S, *et al*. Circulating microRNAs as biomarkers in bile-derived exosomes of cholangiocarcinoma. Annals of Surgical Treatment and Research. 2021; 101: 140.

[147] Liu Y, Gu Y, Cao X. The exosomes in tumor immunity. Oncoimmunology. 2015; 4: e1027472.

[148] Zhang H, Jiang L, Hou J, Zhong S, Zhu L, Wang D, *et al*. Exosome: a novel mediator in drug resistance of cancer cells. Epigenomics. 2018; 10: 1499–1509.

[149] Umezu T, Ohyashiki K, Kuroda M, Ohyashiki JH. Leukemia cell to endothelial cell communication via exosomal miRNAs. Oncogene. 2013; 32: 2747–2755.

[150] Xu Y, Leng K, Yao Y, Kang P, Liao G, Han Y, *et al*. A Circular RNA, Cholangiocarcinoma-Associated Circular RNA 1, Contributes to Cholangiocarcinoma Progression, Induces Angiogenesis, and Disrupts Vascular Endothelial Barriers. Hepatology. 2021; 73: 1419–1435.

[151] Louis C, Leclerc D, Coulouarn C. Emerging roles of circular RNAs in liver cancer. JHEP Reports. 2022; 4: 100413.

[152] Lu Q, Fang T. Circular RNA SMARCA5 correlates with favorable clinical tumor features and prognosis, and increases chemotherapy sensitivity in intrahepatic cholangiocarcinoma. Journal of Clinical Laboratory Analysis. 2020; 34: e23138.

[153] Chen Q, Wang H, Li Z, Li F, Liang L, Zou Y, *et al*. Circular RNA ACTN4 promotes intrahepatic cholangiocarcinoma progression by recruiting YBX1 to initiate FZD7 transcription. Journal of Hepatology. 2022; 76: 135–147.

[154] Gilot D, Migault M, Bachelot L, Journé F, Rogiers A, Donnou-Fournet E*, et al.* A non-coding function of TYRP1 mRNA promotes melanoma growth. Nature Cell Biology. 2017; 19: 1348–1357.