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Intrahepatic cholangiocarcinoma biomarkers: Towards early detection and personalized pharmacological treatments

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ABSTRACT

Cholangiocarcinoma (CCA) is a rare malignancy originating from the biliary tree and is anatomically categorized as intrahepatic (iCCA), perihilar, and extrahepatic or distal. iCCA, the second most prevalent hepatobiliary cancer following hepatocellular carcinoma (HCC), constitutes 5–20 % of all liver malignancies, with an increasing incidence. The challenging nature of iCCA, combined with nonspecific symptoms, often leads to late diagnoses, resulting in unfavorable outcomes. The advanced phase of this neoplasm is difficult to treat with dismal results. Early diagnosis could significantly reduce mortality attributed to iCCA but remains an elusive goal. The identification of biomarkers specific to iCCA and their translation into clinical practice could facilitate diagnosis, monitor therapy response, and potentially reveal novel interventions and personalized medicine. In this review, we present the current landscape of biomarkers in each of these contexts. In addition to CA19.9, a widely recognized biomarker for iCCA, others such as A1BG, CYFRA 21–1, FAM19A5, MMP-7, RBAK, SSP411, TuM2-PK, WFA, *etc.*, as well as circulating tumor DNA, RNA, cells, and exosomes, are under investigation. Advancing our knowledge and monitoring of biomarkers may enable us to improve diagnosis, prognostication, and apply treatments dynamically and in a more personalized manner.

1. Introduction

Cholangiocarcinoma (CCA) is a rare malignancy originating from the biliary tree, categorized anatomically as intrahepatic (iCCA), perihilar (PHCC), and extrahepatic (ECC) or distal (DCCA) [1]. iCCA, the second most prevalent hepatobiliary cancer after hepatocellular carcinoma (HCC), constitutes 5–20 % of all liver malignancies, with an escalating incidence. Early nodal and vascular invasion characterizes iCCA, contributing to a bleak prognosis [2–4]. The genesis of iCCA is attributed to chronic inflammation, creating an inflammatory milieu damaging DNA and promoting cholangiocyte proliferation [5]. An alternate pathogenic theory suggests overexpression of Notch1 in hepatic progenitor cells, leading to oncologic transformation through a cholangiocellular pathway [6]. Well-established risk factors for iCCA encompass cirrhosis, viral hepatitis, primary sclerosing cholangitis, parasitic infections, carcinogen exposure, and genetic syndromes like Lynch syndrome, BRCA-associated protein-1 (BAP-1) tumor predisposition syndrome, cystic fibrosis, and biliary papillomatosis [7]. iCCA manifests in two main subtypes: proliferative and inflammatory. The proliferative subtype, marked by oncogenic signaling activation, DNA amplifications, BRAF and KRAS mutations, tends to be less differentiated and carries a poorer prognosis than the inflammatory subtype, characterized by inflammatory signaling activation and cytokine overexpression [8]. The challenging nature of the disease, coupled with nonspecific symptoms, leads to late diagnoses, particularly for iCCA, resulting in unfavorable outcomes. Since 2010, the standard care for advanced, inoperable biliary tract cancers has been gemcitabine and cisplatin combination (gem-cis).

Valle et al. demonstrated a median overall survival (OS) of 11.7 months in the ABC-02 trial with gemcitabine and cisplatin, compared to 8.1 months with single-agent gemcitabine in unresectable, recurrent, or metastatic biliary tract cancers (HR: 0.64; 95 % CI: 0.52–0.80; P <

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0.001). The response rate was 26.1 % in patients treated with gemcitabine and cisplatin compared to 15.5 % in patients treated with gemcitabine alone [9]. Subsequently, Oh et al. demonstrated in the TOPAZ-1 trial that adding durvalumab to gemcitabine and cisplatin (12.8 months) significantly improved median OS compared to placebo plus chemotherapy (11.5 months; HR: 0.80; CI: 0.66–0.97; P = 0.021) [10]. There were also improvements versus placebo plus chemotherapy in prespecified secondary endpoints, including progression-free survival. Regrettably, the observed increase of +1.3 months in median survival recorded in the TOPAZ-1 trial, although statistically significant, does not fully satisfy. The prognosis for advanced cholangiocarcinoma remains dismal, with only approximately 5 % of patients surviving at 5 years [10,11]. Thus, even though the toxicity profile of cisplatin, gemcitabine, and durvalumab is manageable, there is an urgent need to improve treatment options for the advanced phase.

However, CCA, with its diverse etiologies and clinical heterogeneity, poses a unique challenge in developing universal treatments, prompting consideration of whether targeted therapies might yield more promising results [12]. Surgical resection stands as the sole potentially curative treatment for iCCA, with liver transplantation not established as a treatment option unlike in HCC or PHCC [13,14]. However, only approximately one-third of patients exhibit resectable disease. Despite surgical resection with negative margins, early disease recurrence is prevalent. Even with curative-intent surgical resection, the 5-year overall survival (OS) remains unsatisfactory, with only 20–35 % of patients surviving. Although adjuvant chemotherapy may prolong survival for specific patients, the success of systemic therapy is constrained by drug resistance and notable toxicities, particularly in patients with a poor performance status [15]. Recent advancements in genetic analysis provide a better understanding of the distinct somatic genomic landscapes of biliary tract cancers, including iCCA. Next-generation sequencing enables personalized medicine by identifying unique mutations as potential therapeutic targets. However, it is essential to emphasize that, as in other types of solid tumors, the absolute priority remains early diagnosis. Only early diagnosis can have a significant impact on reducing mortality attributed to cholangiocarcinoma and avoiding secondary effects on patients' quality of life and healthcare costs [16]. The identification of biomarkers specific to iCCA can aid in diagnosis, monitoring therapy response, and potentially unveil novel interventions and personalized medicine [17,18]. Currently, carcinoembryonic antigen (CEA), carbohydrate antigens 19–9 (CA 19–9), and 125 (CA 125) are the only biomarkers in clinical practice, albeit with limited utility due to poor sensitivity and specificity [19]. Next-generation sequencing has begun elucidating the genetic landscape of CCA, identifying tumor biomarkers or "actionable mutations," opening avenues for targeted therapies.

This review explores potential biomarkers for iCCA and their role in diagnosis, therapy response monitoring, and the identification of novel interventions (Fig. 1).

1.1. *Utility and constraints of existing biomarkers for diagnosis in patients with iCCA*

The utility of tumor biomarkers lies in their capacity to facilitate early disease diagnosis, determine individual prognosis and relapse risk, or aid in selecting the optimal treatment modality (surgical, locoregional, or systemic). An ideal biomarker for diagnosing CCA, akin to other cancers, should be straightforward and cost-effective (readily available), highly sensitive (capable of detecting the disease at an early stage, preferably when curative therapy is viable), and specific (able to differentiate CCA from other malignant or benign conditions). Unfortunately, such an ideal biomarker for CCA has not yet been identified. Currently, CEA, CA 19–9 and CA 125 are clinically employed as serum markers for CCA; however, they exhibit low sensitivity and specificity and are inadequate for early detection. For diagnostic purposes, serum CA 19-9 lacks both high sensitivity and specificity, as moderately elevated levels of this biomarker can be observed in situations of benign bile duct obstruction. Consequently, the sensitivity and specificity of CA 19–9 for iCCA are 62 % and 63 %, respectively [20]. Additional serum biomarkers, such as cytokeratin-19 fragment (CYFRA 21–1) and CA 242, have been reported to possess higher specificity than CA 19–9 for iCCA in a limited number of studies [21], yet they are not in routine clinical use. Despite substantial efforts in recent years to identify novel, more specific, and sensitive markers for iCCA, not only in serum but also in bile and urine, none of the evaluated candidates have been deemed suitable for clinical application [22]. When assessing a liver lesion, it is crucial to differentiate iCCA from other liver tumors like HCC or metastatic disease. The primary method for diagnosing iCCA involves imaging within the appropriate clinical context, with subsequent biopsy if necessary [23–25]. However, various biomarkers have been identified to aid in the diagnostic assessment of liver lesions. Tumor markers furnish valuable diagnostic and prognostic information as supplementary confirmatory tests during the evaluation of solid tumors and in postoperative surveillance to monitor for recurrent disease. Nevertheless, it is essential to underscore that tumor markers should not be solely relied upon for diagnosis [26,27]. The diagnosis of iCCA relies primarily

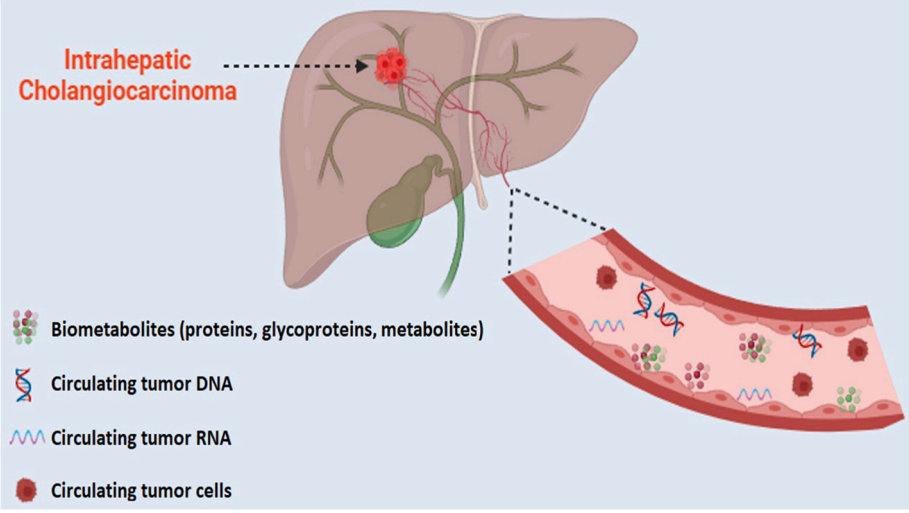


Fig. 1. Circulating biomarkers of iCCA may include proteins and metabolites released by tumor cells, as well as circulating tumor cells that migrate from primary sites. Additionally, free-floating DNA and RNA contribute to the pool of potential markers.

on imaging modalities and subsequent biopsy procedures, which are essential for distinguishing iCCA from other liver tumors, including hepatocellular carcinoma (HCC). Biomarkers detected in blood, bile, and tumor tissue play a supplementary role in the diagnostic assessment of liver lesions, as detailed in Table 1.

A crucial factor influencing the efficacy of biomarkers lies in their capacity to differentiate between HCC and iCCA. CA 19–9, a widely recognized biomarker for cholangiocarcinoma, serves as a Lewis blood group antigen produced by pancreatic, biliary ductal, gastric, and colonic epithelial cells [28]. Notably, CA19-9 is absent in the production and therefore remains undetectable in approximately 7 % of the population [29]. The association of an elevated CA19-9 is more frequently observed in iCCA, in contrast to an elevated α-fetoprotein (AFP), which leans towards suggesting HCC [30]. With a diagnostic sensitivity of 72 % and specificity of 84 % for cholangiocarcinoma, CA 19–9 is a valuable biomarker. It is imperative to recognize that CA 19–9 elevation can also occur in non-malignant conditions such as biliary obstruction, cholangitis, and primary biliary cirrhosis.

While not considered primary diagnostic genetic alterations for iCCA due to their presence in various cancer types, *KRAS* stands out as one of the most frequently mutated genes in iCCA and beyond, underscoring its potential as a promising biomarker [31–34]. It is essential to recognize that the spectrum of genetic alterations extends beyond *KRAS*. A significant discovery involves mutations in *BRAF*, identified in approximately 10 % of iCCA patients, contributing to the intricate genetic landscape of this malignancy. Moreover, a substantial proportion, around 30 %, exhibits mutations in the PI3K/PTEN/AKT/mTOR signaling pathway, highlighting the diverse genetic events driving iCCA development and progression [35]. However, the multifaceted nature of iCCA necessitates exploration into complementary biomarkers to enhance the diagnostic accuracy of genetic assessments. While more complex and not readily applicable in clinical practice, it is worth emphasizing this aspect. Specifically, it has been hypothesized that integrating genetic characterization with serum biomarkers (e.g., broader panels of serum biomarkers) could improve diagnostic precision. An intriguing investigation illustrated that the amalgamation of well-established biomarkers, namely CA19-9, CEA, MMP-7, and CYFRA 21–1, emerges as a potent diagnostic tool. This combination yielded impressive diagnostic performance with a sensitivity of 92 %, specificity of 96 %, and an overall accuracy of 94 % in the diagnosis of intrahepatic cholangiocarcinoma (iCCA) [36]. Significant is the observation that this combined biomarker panel not only has the potential to aid in refining diagnostic precision through genetic signatures but also exhibits promise in addressing particularly critical tasks, such as distinguishing intrahepatic cholangiocarcinoma (iCCA) from hepatocellular carcinoma (HCC) and pancreatic cancer [37]. Delving deeper into the genetic landscape, additional markers such as *IDH* mutations, found in 15–30 % of iCCA patients, offer insights into the molecular intricacies of this cancer. The involvement of two isoforms, IDH1 and IDH2, in the Krebs cycle further accentuates the diverse genetic alterations contributing to iCCA pathogenesis [38,39]. Complementing these findings, *FGFR* mutations are detected in 10–15 % of iCCA cases, adding yet another layer of complexity to the molecular profile of this malignancy [40]. Biomarkers play a crucial role in discerning between malignant and benign biliary diseases. Notably, FAM19A5 and RB-associated KRAB zinc-finger protein (RBAK) exhibit elevated levels in patients with CCA compared to those with benign biliary conditions, emphasizing their potential diagnostic utility [41]. Another promising marker is the serum α1b-Glycoprotein (A1BG)/afamin (AFM) ratio, with a threshold greater than 1.8, enabling the differentiation of CCA patients from healthy individuals with an impressive sensitivity of 84.4 % and specificity of 87.5 % [42]. Additionally, levels of Wisteria floribunda agglutinin (WFA) in both serum and bile present a potential differentiator between iCCA and benign biliary diseases [43]. In the realm of biliary markers, a unique diagnostic approach involves the use of bile-based indicators. Although the fluid sample is in closer proximity to the presumed tumor tissue, facilitating a potential diagnostic advantage, it is essential to acknowledge that sample collection entails an invasive procedure. Notably, patients with CCA exhibit significantly lower total bile concentrations and altered deoxycholic acid ratios compared to individuals with benign biliary diseases, reflecting changes in bile acid transport associated with the malignancy [44]. Another bile-specific marker, sperm-specific protein 411 (SSP411), demonstrates elevation in CCA patients relative to those with benign biliary disease, further underscoring its potential diagnostic significance [45]. Innovative techniques for biomarker detection, such as liquid biopsies, are currently under investigation. Liquid biopsies involve identifying markers in patient fluid samples (e.g., blood, urine, or bile) to assess disease biology. They enable the sequential detection of these markers, providing insights into changes in tumor biology. Biomarkers of interest include circulating tumor DNA (ctDNA) or RNA, circulating tumor cells (CTCs), and tumor-derived cytokines, exosomes, and proteins [46]. This expanded comprehension of the genetic and molecular features of iCCA not only deepens our understanding but also paves the way for the development of more comprehensive diagnostic and therapeutic strategies. The intricate interplay of various genetic markers opens avenues for targeted interventions, personalized treatment approaches, and a more nuanced understanding of the heterogeneity inherent in iCCA.

Table 1
Primary diagnostic biomarkers for iCCA.

Acronym (full name)	Biologic function	Source
A1BG (α1b-Glycoprotein)	Immune regulation.	B
AFM (Afamin)	Transporter for vitamins and other hydrophobic molecules.	B
CA 19–9 (Carbohydrate antigen 19–9)	Cell-cell recognition and adhesion.	B/Bi
CYFRA 21–1 (cytokeratin 19 fragments)	Part of cell cytoskeleton.	B
DKK1 (Dickkopf-1)	Inhibits Wnt signaling pathway, influencing cellular growth and differentiation.	B/T
KL-6 Mucin (Krebs von den Lungen-6 Mucin)	It is a high-molecular-weight glycoprotein expressed on the surface of type II pneumocytes in the lungs. Its main cellular function is linked to the protection and repair of the respiratory epithelium.	B
Matrix (Metalloproteinase-7)	Enzyme involved in extracellular matrix degradation, associated with tissue remodeling and repair.	B
RBAK (RB-associated KRAB zinc finger)	Transcriptional repressor playing a role in gene regulation and maintenance of genomic stability.	B
SSP411 (Sperm specific protein 411)	Involved in spermiogenesis.	B/Bi
TuM2-PK (Tumor type M2 pyruvate kinase)	Isoform of pyruvate kinase, an enzyme involved in glycolysis, is present in cancer cells. It facilitates aerobic glycolysis, known as the Warburg effect.	B/T
WFA (Wisteria floribunda agglutinin)	Involved in cell adhesion and migration.	B/Bi
14-3-3 eta (eta isoform of the 14-3-3 protein family)	Pleiotropic protein with diverse biological functions, including the regulation of the cell cycle, signal transduction, apoptosis, and stress response.	T

Bile: Bi; Blood: B; Tissue: T.

1.2. Biomarkers as predictors of outcomes in patients with iCCA

Surgical intervention in patients with iCCA represents, when possible, the main choice. However, the overall outcomes remain suboptimal, and the recurrence of the condition is frequent post-resection. Moreover, hepatectomy poses significant physiological challenges, necessitating careful patient selection for achieving operative success [47]. Consequently, there is a pressing need for biomarkers that can aid

in risk stratification and inform treatment decisions, as evidenced by the ongoing research in this field. As highlighted earlier, CA19-9 emerges as a valuable biomarker in iCCA diagnosis. Furthermore, it may carry prognostic significance for individuals with iCCA. Notably, a very interesting study has established that preoperative CA19-9 and CEA levels could prognosticate OS with a defined cutoff of 176.3 U/mL for CA19-9 and 9.6 ng/ml for CEA [48]. Indeed, elevated CA19-9 levels, surpassing the threshold of 1000 U/mL, have been associated with metastatic cholangiocarcinoma, underscoring the potential utility of this biomarker in signaling the likelihood of an advanced disease stage [49]. In 2020, Tsilimigras et al. developed a classification tree through the examination of 826 patients who underwent iCCA resection. The patients were categorized into three clusters: common, proliferative, and inflammatory based on tumor size, CA 19–9, and neutrophil-to-lymphocyte ratio (NLR). Interestingly, despite the inflammatory cluster showing the lowest CA19-9 levels, medium-sized tumors, and the highest NLR, individuals within this group experienced the poorest median OS [50]. Instead, CYFRA 21–1 which demonstrated a greater diagnostic capacity compared to both CA19-9 and CEA, showed a positive correlation with tumor stage, establishing itself as an independent predictor of decreased recurrence-free and OS [51]. Tumor type M2 pyruvate kinase (TuM2-PK) emerges as a valuable marker for distinguishing CCA from benign diseases. Elevated TuM2-PK levels in CCA patients are often proportional to the tumor burden, particularly evident in those with lymph node metastasis [52,53]. Notably, one study reported that TuM2-PK exhibited superior sensitivity and specificity for CCA compared to CA19-9, effectively discriminating CCA from healthy controls [54]. Connective tissue growth factor (CTGF) expression is potentially linked to longer disease-free survival (DFS) and OS, although the underlying mechanisms influencing tumor biology remain largely unexplored [55]. Wnt1-inducible signaling pathway protein 1 (WISP1), a component of the WNT pathways, plays a role in regulating cell proliferation, differentiation, adhesion, migration, and survival. WISP1 expression is associated with iCCA carcinogenesis, being overexpressed in 49 % of iCCA cases and correlating with a poor prognosis [56]. Malignant cholangiocytes expressing Matrix Metalloproteinase-7 or 9 (MMP-7 or 9) are indicative of an unfavorable post-operative survival [57]. Additionally, the mucin family of glycoproteins shows promise in aiding the diagnosis and prognosis of CCA [58]. Furthermore, the expression of MUC4 and MUC5AC demonstrates a discriminatory capacity between benign and malignant biliary diseases [59]. Regarding postoperative outcomes, MUC1, MUC4, and MUC16 have been identified as prognostic indicators for a poor prognosis, whereas tumors positive for MUC2 tend to exhibit a more favorable outlook [60–62]. Core 3-synthase, a pivotal component in the digestive system, has been linked to lower migratory and invasive rates, along with reduced metastatic activity when expressed in cells. Indeed, in the context of CCA, the presence of core 3 synthase, detected by the antibody G8-144, has shown an association with lower mortality rates. Conversely, the expression of 6-sulfated *N*-acetyllactosamine on extended core-1 O-glycans, identified by the antibody MECA-79, has been correlated with an unfavorable prognosis [63]. Additionally, the assessment of dual-specificity phosphatase 11 (DUSP11) in eight pairs of iCCA, PHCC, and DCCA, along with their corresponding adjacent tissues, revealed heightened DUSP11 levels in all types of CCA compared to adjacent tissues. Specifically in iCCA, elevated DUSP11 expression was linked to an advanced T stage and a poor prognosis, a correlation not observed in ECC or PHCC [64]. The presence of DKK1 expression in iCCA tumor tissue is linked to elevated levels of MMP-9 and vascular endothelial growth factor-C (VEGF-C). This association is further correlated with heightened tumor invasion and an increased likelihood of lymph node metastasis [65]. The comprehensive molecular characterization of CCA has unveiled a spectrum of variations influenced by both anatomical location and etiology. Similar to other adenocarcinomas, iCCA demonstrates a heightened prevalence of mutations in key genes, including tumor protein p53 (*tp53*), *KRAS*, and mothers against decapentaplegic homolog 4 (*SMAD4*), which may serve as potential indicators of a poorer prognosis [66]. Furthermore, these tumors frequently exhibit alterations in *FGFR2*, *IDH1*, *IDH2*, RB Transcriptional Corepressor 1 (*RB1*), Erb-B2 receptor tyrosine kinase 2 (*ERBB2*), and breast cancer-associated protein 1 (*BAP1*) [67]. In Table 2, we have delineated the key biomarkers with prognostic significance for iCCA.

In particular, *tp53* mutations, exemplified by their association with an aggressive tumor phenotype and resistance to traditional therapies, underscore the challenge posed by these genetic alterations in the treatment landscape. Similarly, *KRAS* mutations, pervasive in diverse cancers, play a pivotal role in fostering unregulated cell proliferation and are implicated in a dismal prognosis for iCCA [68]. Meanwhile, alterations in *FGFR2*, another prevalent mutation within iCCA, have surfaced as a promising avenue for therapeutic intervention. The exploration of inhibitors targeting FGFR signaling pathways as potential treatments highlights the potential for precision medicine in the management of iCCA, showcasing a paradigm shift towards more tailored and effective approaches [40]. Moreover, mutations in *IDH1* and *IDH2* are significant in iCCA and have implications for prognosis and treatment response. These mutations are associated with distinct molecular subtypes and may guide personalized therapeutic strategies [69]. Also the presence of *ERBB2* mutations in iCCA emphasizes the heterogeneity of this cancer type and suggests the need for tailored treatment approaches. Indeed, ERBB2-targeted therapies, proven effective in other cancers, are being investigated for their applicability in iCCA [70]. It is estimated that as many as 70 % of iCCA patients may carry mutations that are potentially targetable [71]. In conclusion, the molecular characterization of iCCA has revealed a heterogeneous landscape of mutations that play crucial roles in tumor development and progression. The identification of these biomarkers not only aids in prognostication but also opens avenues for targeted therapeutic strategies, paving the way for a more personalized and effective approach to the management of iCCA.

1.3. Biomarkers to guide therapy utility in target therapy

Tumor biomarkers not only possess diagnostic significance but may also serve to predict prognosis (such as OS and/or tumor recurrence) or guide therapeutic decisions and assess responses to potential adjuvant therapies. Despite the substantial genomic heterogeneity observed in CCA tumors, distinct genetic alterations have been documented based on the cancer subtype. Specifically, small bile duct iCCAs typically exhibit mutations in *IDH1/2* and *FGFR2* fusions, whereas large duct iCCAs, PHCCs, and DCCAs more consistently manifest *KRAS* and/or *tp53* mutations. Notably, *ELF3* mutations appear to be exclusively prevalent in patients with DCCA [72]. Genomic and transcriptomic investigations have further identified specific signatures associated with tumor development and progression, providing valuable insights into the molecular landscape of CCA. Transcriptomic examination of iCCA tumors has facilitated the categorization into two discernible subtypes: the "inflammation subtype," primarily enriched in genes associated with inflammation, and the "proliferation subtype," marked by heightened expression of oncogenes and linked to a poorer prognosis. This underscores the significance of tissue biomarkers in prognostic prediction. The distinct molecular profiles identified in these subtypes contribute valuable insights into the underlying mechanisms and potential therapeutic strategies for iCCA [73]. Nevertheless, with enhanced genetic analysis and a deeper comprehension of distinct biomarkers, targeted therapies have emerged to enhance treatment responses and OS rates [18]. *IDH1/2* mutations, *FGFR2* gene fusions, and neurotropic tyrosine kinase receptor (*NTKR*) fusions are predominantly exclusive to patients with iCCA, with prevalence rates ranging from 4.9 to 36 %, 5.5–13.6 %, and 3.5 %, respectively [74]. These molecular aberrations have garnered significant attention in the realm of drug discovery due to their potential as therapeutic targets. Despite the limited success of IDH inhibitors in the treatment of iCCA, efforts have been made to explore their

Table 2
Prognostic biomarkers for iCCA.

Acronym (full name)	Biologic function	Source	Impact on prognosis
A1BG (α1b-Glycoprotein)	Immune regulation.	B	Unfavorable
AFM (Afamin)	Transporter for vitamins and other hydrophobic molecules.	B	Unfavorable
CA 19–9 (Carbohydrate antigen 19–9)	Cell-cell recognition and adhesion.	B/Bi	Unfavorable
Core 3-synthase	Enzyme involved in the synthesis of Core 3 O-glycans, playing a role in the modification of glycoproteins and glycolipids in cells.	T	Favorable
CTGF (Connective tissue growth factor)	Regulation of cell growth, differentiation, and tissue repair.	T	Favorable
CYFRA 21–1 (Cytokeratin 19 fragments)	Part of cell cytoskeleton.	B	Unfavorable
DKK1 (Dickkopf-1)	Inhibits Wnt signaling pathway, influencing cellular growth and differentiation.	B/T	Unfavorable
DUSP11 (Dual specificity phosphatase 11)	It dephosphorylates tyrosine and serine/threonine residues within the mitogen-activated protein kinase pathways, thereby influencing cell growth, differentiation, and response to stimuli.	T	Unfavorable
ERBB2 Erb-B2 receptor tyrosine kinase 2)	Receptor tyrosine kinase involved in cell growth and differentiation.	T	Unfavorable
FGFR2 (Fibroblast growth factor receptor 2)	Receptor tyrosine kinase involved in cell growth, differentiation, and tissue repair.	T	Unfavorable
IDH 1/2 (Isocitrate dehydrogenase 1 and 2)	They catalyze the conversion of isocitrate to alpha-ketoglutarate, producing NADPH (nicotinamide adenine dinucleotide phosphate) in the tricarboxylic acid cycle.	T	Unfavorable
KL-6 Mucin (Krebs von den Lungen-6 Mucin)	It is a high-molecular-weight glycoprotein expressed on the surface of type II pneumocytes in the lungs. Its main cellular function is linked to the protection and repair of the respiratory epithelium.	T	Unfavorable
KRAS (Kirsten rat sarcoma viral oncogene homolog)	GTPase protein that plays a crucial role in cell signaling, particularly in the regulation of cell growth and differentiation.	T	Unfavorable
MMP 7/9 (Matrix metalloproteinase 7/9)	Enzymes involved in extracellular matrix degradation, associated with tissue remodeling and repair.	B	Unfavorable
MUC 1/4/16 (Mucin 1/4/16)	Transmembrane glycoprotein involved in cell adhesion and signaling.	B	Unfavorable
MUC 2 (Mucin 2)	Secreted mucin involved in the protection and lubrication of the gastrointestinal tract. It forms the mucus gel layer, contributing to the defense against pathogens and maintaining gut homeostasis.	B	Favorable
MUC5AC (Mucin5AC)	Secreted mucin primarily found in the respiratory tract and gastric mucosa. It contributes to the integrity and protection of mucosal surfaces.	B	Unfavorable
RB1 (Retinoblastoma 1)	Crucial regulator of the progression from the G1 phase to the S phase of the cell cycle. It acts by inhibiting the activity of cyclin-dependent kinases, thereby preventing the phosphorylation of key proteins involved in cell cycle progression.	T	Unfavorable
SMAD4 (Mothers against decapentaplegic homolog 4)	Transcription factor involved in the TGF-beta signaling pathway. Plays a crucial role in regulating cell growth, differentiation, and apoptosis.	T	Unfavorable
6-sulfated *N*-acetyllactosamine	Modified sugar structure with a sulfate group. Found in glycoproteins and glycolipids, playing a role in cellular recognition and interactions. Implicated in various biological processes, including cell adhesion and signaling.	T	Unfavorable
TP53 (Tumor protein 53)	Pleiotropic protein primarily involved in the regulation of cell cycle progression, apoptosis, and DNA repair. It acts as a tumor suppressor by monitoring genomic integrity.	T	Unfavorable
TuM2-PK (Tumor M2-Pyruvate kinase)	Isoform of pyruvate kinase, an enzyme involved in glycolysis, is present in cancer cells. It facilitates aerobic glycolysis, known as the Warburg effect.	B/T	Unfavorable
WISP1 (WNT1 inducible signaling pathway protein 1)	Matricellular protein involved in cell signaling and tissue repair. Associated with the Wnt signaling pathway and implicated in various biological processes, including inflammation, fibrosis, and cancer.	T	Unfavorable

Bile: Bi; Blood: B; Tissue.

efficacy. In this sense, the ClarIDHy study, a phase III randomized controlled trial (RCT), encompassed 185 patients with *IDH1*-mutated CCA. These patients were randomized to receive the IDH1 inhibitor, ivosidenib, or a placebo. Notably, individuals administered ivosidenib demonstrated significantly extended progression-free survival (PFS) compared to those on placebo (2.7 versus 1.4 months, respectively) [75]. Simultaneously, promising results have emerged in targeting *NTRK* fusions with larotrectinib [76] or entrectinib [77] for the treatment of previously treated advanced solid tumors, encompassing cases of CCA as well. In this context, the significance of FGFR2-directed therapies in iCCA patients with *FGFR2* alterations who have progressed under the initial gemcitabine-cisplatin treatment is underscored by findings from some clinical trials, showcasing encouraging outcomes. In particular, the FDA-approved FGFR inhibitor, pemigatinib, exhibited a very interesting objective response rate, yielding a median PFS of 6.9

months in the FIGHT-202 trial [78]. These results prompt consideration of the use of pemigatinib and other FGFR inhibitors as a potential first-line treatment, offering a novel perspective in comparison to the established standard of care for advanced CCA patients. The ongoing assessments across numerous clinical studies aim to provide further insights into the effectiveness of this approach. More detailed information on the referenced clinical studies is provided in Table 3, where it is evident that the majority of studies currently underway focus on *FGFR2* fusions/rearrangements.

1.4. Prospective trajectories of biomarkers in iCCA

Exploring prospective trajectories in the realm of biomarker research unveils a pivotal role in identifying and diagnosing various malignancies. Recent evidence not only underscores the significance of biomarkers in guiding patient selection for surgical interventions and systemic therapies but also highlights their potential to significantly impact survival outcomes in patients with iCCA. The quest for enhanced survival involves identifying biomarkers capable of efficiently selecting patients who stand to benefit from therapies, with a specific emphasis on identifying those at an elevated risk of recurrence post-resection. While systemic therapy presents modest benefits for a subset of iCCA patients, the challenge lies in navigating the delicate balance between therapeutic advantages and potential toxicity for individual cases. This underscores the critical importance of judicious patient selection to mitigate morbidity and minimize unnecessary exposure to potentially toxic treatments. Currently, the combination of gemcitabine with cisplatin and durvalumab stands as the standard systemic treatment for advanced iCCA [10,11]. Ongoing investigations encompass a comprehensive spectrum of studies, delving into both systemic and locoregional therapeutic modalities for iCCA, but simultaneously, the landscape of biomarker detection is evolving, with novel techniques taking center stage. Liquid biopsies, a promising avenue in this domain, involve analyzing markers present in various patient fluid samples, including blood, urine, or bile, providing valuable insights into disease biology. These biopsies offer the advantage of serial marker detection, furnishing crucial information on dynamic changes in tumor biology [80]. In upcoming research endeavors, significant emphasis is placed on discerning notable biomarkers, inclusive of ctDNA or ctRNA, CTCs, and tumor-derived exosomes, cytokines, and proteins. The trajectory of future investigations aims to delve deeper into the intricate landscape of these biomolecular entities, as their potential implications in understanding disease pathophysiology and therapeutic responsiveness continue to unfold. These biomarkers, residing in various biological

Table 3
Primary clinical studies on iCCA patients with targeted therapy-responsive mutations and their key outcomes.

Study type, Ref	NCT	Phase	No. of patients Biomarker	Study Arms	Primary Endpoint	Outcomes
Interventional (ClarIDHy) [75] Interventional [76] Interventional	NCT02989857	III	187 IDH1-mutated	Active Comparator: AG-120 Placebo Comparator: Placebo Experimental: After Cross over to AG-120	PFS	PFS was significantly improved with AG-120 compared with placebo (median 2·7 months [95 % CI 1·6–4·2] vs 1·4 months [1·4–1·6]; hazard ratio 0·37; 95 % CI 0·25–0·54; one-sided p < 0·0001).
Interventional (STARTRK-2) [77]	NCT02122913	I	75 NTRK fusion	Larotrectinib (Dose excalation)	MTD RR	No results posted
	NCT02568267	II	534 NTRK fusion	Oral RXDX-101	ORR	No results posted (still active)
Interventional (FIGHT-202) [78]	NCT02924376	II	147 FGFR2 translocation (Rearrangements or Fusions)	Oral Pemigatinib	ORR	The median follow-up was 17·8 months (IQR 11·6–21·3). 38 (35·5 % [95 % CI 26·5–45·4]) patients with FGFR2 fusions or rearrangements achieved an objective response (three complete responses and 35 partial responses).
Interventional (FIGHT-302) [79]	NCT03656536	III	434 FGFR2 rearrangement or fusion	Experimental: Pemigatinib Active Comparator: Cisplatin + Gemcitabine	PFS	No results posted (The study is currently in recruiting)
Interventional (FOENIX-CCA3) [80]	NCT04093362	III	216 FGFR2 rearrangement	Experimental: Oral TAS-120 Active Comparator: Cisplatin + Gemcitabine	PFS	No results posted (still active)
Interventional (from CT.gov)	NCT05565794	II	20 FGFR2 rearrangement or fusion	Experimental: Pemigatinib	ORR	No results posted (The study is currently in recruiting)
Interventional (REFOCUS) (from CT.gov)	NCT04526106	I/II	550 FGFR2 rearrangement or fusion	Oral RLY-4008 (dose escalation)	MTD/ ORR	No results posted (The study is currently in recruiting)
Interventional (from CT.gov)	NCT05174650	II	37 FGFR2 rearrangement or fusion	Combined treatment: Atezolizumab + Derazantinib	ORR	No results posted (The study is currently in recruiting)
Interventional (from CT.gov)	NCT05678270	II	64 FGFR2-Rearranged	Experimental: Oral ICP-192	ORR	No results posted (The study is currently in recruiting)
Interventional (from CT.gov)	NCT05242822	I	120 FGFR2 and/or FGFR3 gene alterations	Experimental: Part A - dose escalation (Drug KIN-3248) Experimental: Part B - dose expansion (Drug KIN-3248)	DLTs AEs ORR DCR DOR PFS	No results posted (The study is currently in recruiting)

AG-120: Ivosidenib; PFS: progression-free survival; MTD: Maximum tolerated dose; RR: Response rate; RXDX-101: Entrectinib; ORR: Objective Response Rate; TAS-120: Futibatinib; CT.gov: ClinicalTrials.gov; RLY-4008: lirafugratinib; DLTs: Dose limiting toxicities; AEs: Adverse events; DCR: Disease control rate; DOR: duration of response.

fluids, offer a comprehensive view of the dynamic molecular milieu associated with malignancies. As we navigate this evolving field, the integration of advanced technologies and methodologies promises to unveil novel insights, shaping the landscape of personalized diagnostics and targeted therapies. Indeed, the potential of ctDNA as an indicator of systemic therapeutic response has already been emphasized in recent published research. For instance, a 92 % concordance rate was observed between tumor tissue and corresponding ctDNA samples in iCCAs [81–85]. In a very recent study conducted by Choi et al. [86], the current evidence and future perspectives on the use of ctDNA in the diagnosis, management, and understanding of the prognosis of patients with iCCA undergoing surgery are presented. Specifically, the authors outline the application of liquid biopsies or ctDNA in various capacities [1]: characterizing the molecular profile of the tumor to inform the selection of molecularly targeted therapy in the neoadjuvant setting [2], functioning as a surveillance tool to identify minimal residual disease or detect cancer recurrence post-surgery, and [3] aiding in the diagnosis and screening for early detection of iCCA in populations at elevated risk.

CtDNA serves as a valuable DNA source for tumor genetic assessment, playing a pivotal role in ensuring the clinical validity and utility of assays in a laboratory setting, following evolving guidelines [87]. While elaborating on the limitations of this technique exceeds the scope of this work, it is crucial to emphasize the importance of ctDNA, especially in iCCA. In fact, its non-invasive and repeatable nature is noteworthy, particularly in situations where the available tumor quantity may be inadequate for a comprehensive tumor DNA analysis. In iCCA, conducting a comprehensive gene panel analysis before initiating treatment is advisable. This panel should ideally include multigene NGS assessments, incorporating at least IDH1 for ivosidenib treatment and FGFR2 fusions for pemigatinib therapy. The therapeutic implications of other mutations (ARID1A, KRAS, PIK3CA, tp53, etc.) in CCA remain unclear. Importantly, ctDNA and tumor DNA demonstrate good concordance in detecting actionable mutations [88–90].

Furthermore, the variant allele frequency in ctDNA showed correlation with tumor load and PFS. Investigations into CTCs by Yang et al. [91] unveiled their association with disease extent, aggressiveness, and survival prediction in CCA patients. Additionally, studies on circulating microRNA as a bile-derived biomarker in CCA, led by Han et al. [92], demonstrated the ability of microRNA to mirror the oncogenic characteristics of CCA tissue. Emerging on the biomarker horizon is the identification of extracellular vesicles (EVs) as potential indicators for CCA. Released by tumor cells, EVs play a vital role in regulating cellular microenvironments by transporting biologic material [93]. Implications for tumor-induced inflammation and chemoresistance add further intrigue to the potential of EVs as biomarkers [94,95]. In a recent study, Xu et al. [96] identified a CCA-associated circular RNA, circ-CCAC1, upregulated in bile EVs and tissues, suggesting its potential role as both a biomarker and therapeutic target. Ongoing trials investigating the role of circular RNA (circRNA) in iCCA are underway, with studies indicating decreased expression of circSMARCA5 in iCCA tissue, negatively correlated with advanced disease stage [97,98]. Additionally, circACTN4 has been identified as a promoter of tumor cell growth by regulating Wnt signaling pathways, indicating its potential as a therapeutic target [99]. Strategies targeting the miRNA response elements carried by circRNAs, such as target site blockers (TSBs), hold promise for reducing the pro-oncogenic activity of circRNA [100,101].

One of the most useful and pragmatic approaches in the field of biomarkers in CCA might involve assessing biomarkers capable of predicting the development of CCA in at-risk patients (obesity, sclerosing cholangitis, opisthorchiasis infection, chronic viral infections, and cholelithiasis). This is undoubtedly an extremely challenging and complex task, pursued in the context of all solid tumors. In the scientific literature, there are very few relevant data, often stemming from studies in which a prospective design is not clearly identifiable, and the studies are underpowered. In Table 4, we present studies that investigate the role of soluble biomarkers in predicting the progression to CCA from benign conditions [102–107].

Table 4
Studies reporting on the role of biomarkers in predicting the development of CCA from predisposing conditions.

Author, Year	Risk factor	Biomarker	Predictive power
Lempinen M, 2007	PSC	Serum trypsinogen-2	Median: 66 mg/L, IQR: 39–131 for patients with PSC developing CCA. AUC: 0.804.
Halme L, 2012	PSC	Aneuploid DNA content on biopsy	Aneuploidy (yes vs no). Positive predictive value 92 %, negative predictive value 53 %.
Jamnongkan W, 2012	Opisthorchiasis-associated CCA	Serum oxidized alpha-1 antitrypsin	Cut-off OD value 0.12. AUC: 0.818. Sensitivity and specificity of 81 and 80 %, respectively.
Prakobwong S, 2012	Opisthorchiasis-associated CCA	Serum hydroxyproline	Hydroxyproline >41.0 mg/ml vs < 41.0; OR: 3.69; 95 % CI: 1.198–11.382; *p*: 0.020.
Jo JH, 2013	Hepatolithiasis	Serum CA19-9 level	CA19-9>22 U/mL. AUC: 0.799; OR: 1.001. The sensitivity and specificity of 73.9 and 68.4 %, respectively.
Lapitz A, 2023	PSC	CRP/PIGR/VWF from serum extracellular vesicles	Concomitant CRP>14,000 ng/ml/ PIGR>750 ng/ml/ VWF>8500 ng/ml. AUC: 0.992; OR: 387.5. The sensitivity and specificity were 92.6 and 96.9 %, respectively.

AUC: Area Under the Curve (it represents the overall performance of the test in distinguishing between patients at risk developing CCA and those who will not develop CCA. 1 indicates complete accuracy and 0.5 no discriminatory power); CI: Confidence Intervals; CRP: *C*-Reactive Protein; IQR: Inter-Quartile Range; OD: Optical Density; OR: Odds Ratio (it represents the likelihood of having a high biomarker level among patients who will develop CCA compared to the likelihood among those who will not); PlGR: Alpha-enolase plasminogen receptor; PSC: primary sclerosing cholangitis; VWF: Von Willebrand Factor.

Finally, it is worth discussing that the exploration of immunohistochemistry biomarkers for prediction of CCA in patients predisposed to the disease, encounters formidable challenges, highlighting an existing unmet need in the field. Firstly, the inherent heterogeneity of CCA poses a significant hurdle. The intricate interplay of genetic, epigenetic, and microenvironmental factors contributes to the diversity within CCA tumors, leading to variations in biomarker expression patterns. Secondly, a significant proportion of the identified biomarkers are associated with a phenotype of CCA that is fully malignant. Given the biological aggressiveness of the neoplasm, this association lacks clinical-prognostic utility as it does not allow for the implementation of impactful preventive measures against disease progression. Moreover, the challenge is compounded by the scarcity of large, prospective, and well-defined cohorts of patients at risk of developing CCA.

2. Conclusions and perspectives

Cholangiocarcinoma represents an exceptionally intriguing and paradigmatic model in oncology due to its high genetic and molecular heterogeneity. This heterogeneity, with the advancement of technology and our ability to dynamically characterize predictive biomarkers for response and prognosis, could potentially become its “Achilles’ heel” in the future. The enhancement of knowledge and monitoring of

biomarkers may, in fact, allow us to apply treatments dynamically and in a personalized manner, increasingly based on specific targets, thereby combating the disease more effectively. This approach has the potential to improve the time-to-outcome and the quality of life for patients.

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CRediT authorship contribution statement

Maurizio Capuozzo: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Mariachiara Santorsola:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Francesco Ferrara:** Investigation, Data curation, Conceptualization. **Claudia Cinque:** Writing – original draft, Investigation. **Stefania Farace:** Writing – original draft, Investigation. **Renato Patrone:** Writing – original draft, Investigation. **Vincenza Granata:** Methodology, Investigation. **Andrea Zovi:** Writing – review & editing, Investigation. **Guglielmo Nasti:** Writing – original draft, Validation, Conceptualization. **Alessandro Ottaiano:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

[1] S. Massironi, L. Pilla, A. Elvevi, R. Longarini, R.E. Rossi, P. Bidoli, P. Invernizzi, New and emerging systemic therapeutic options for advanced cholangiocarcinoma, Cells 9 (3) (2020 Mar 11) 688, https://doi.org/10.3390/cells9030688.

[2] J. Morawitz, N.M. Bruckmann, K. Jannusch, J. Kirchner, G. Antoch, S. Loosen, T. Luedde, C. Roderburg, P. Minko, Update on locoregional therapies for cholangiocellular carcinoma, Cancers 15 (8) (2023 Apr 19) 2368, https://doi.org/10.3390/cancers15082368.

[3] L. Wu, D.I. Tsilimigras, A.Z. Paredes, R. Mehta, J.M. Hyer, K. Merath, K. Sahara, F. Bagante, E.W. Beal, F. Shen, T.M. Pawlik, 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 J. Surg. 43 (7) (2019 Jul) 1777–1787, https://doi.org/10.1007/s00268-019-04966-4.

[4] A.A. Florio, J. Ferlay, A. Znaor, D. Ruggieri, C.S. Alvarez, M. Laversanne, F. Bray, K.A. McGlynn, J.L. Petrick, Global trends in intrahepatic and extrahepatic cholangiocarcinoma incidence from 1993 to 2012, Cancer 126 (11) (2020 Jun 1) 2666–2678, https://doi.org/10.1002/cncr.32803.

[5] D. Høgdall, C.J. O'Rourke, A. Taranta, D.V. Oliveira, J.B. Andersen, Molecular pathogenesis and current therapy in intrahepatic cholangiocarcinoma, Dig. Dis. 34 (4) (2016) 440–451, https://doi.org/10.1159/000444562.

[6] S. Aishima, Y. Oda, Pathogenesis and classification of intrahepatic cholangiocarcinoma: different characters of perihilar large duct type versus peripheral small duct type, J Hepatobiliary Pancreat Sci 22 (2) (2015 Feb) 94–100, https://doi.org/10.1002/jhbp.154.

[7] S.A. Khan, S. Tavolari, G. Brandi, Cholangiocarcinoma: epidemiology and risk factors, Liver Int. 39 (Suppl 1) (2019 May) 19–31, https://doi.org/10.1111/liv.14095.

[8] A. Moeini, D. Sia, Z. Zhang, G. Camprecios, A. Stueck, H. Dong, R. Montal, L. Torrens, I. Martinez-Quetglas, M.I. Fiel, K. Hao, A. Villanueva, S.N. Thung, M. E. Schwartz, J.M. Llovet, Mixed hepatocellular cholangiocarcinoma tumors: cholangiolocellular carcinoma is a distinct molecular entity, J. Hepatol. 66 (5) (2017 May) 952–961, https://doi.org/10.1016/j.jhep.2017.01.010.

[9] J. Valle, H. Wasan, D.H. Palmer, D. Cunningham, A. Anthoney, A. Maraveyas, S. Madhusudan, T. Iveson, S. Hughes, S.P. Pereira, M. Roughton, J. Bridgewater, ABC-02 Trial Investigators. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer, N. Engl. J. Med. 362 (14) (2010 Apr 8) 1273–1281, https://doi.org/10.1056/NEJMoa0908721.

[10] D.Y. Oh, A.R. He, S. Qin, L.T. Chen, T. Okusaka, A. Vogel, J.W. Kim, For the TOPAZ-1 investigators. Durvalumab plus gemcitabine and cisplatin in advanced biliary tract cancer, NEJM Evid (8) (2022 Jun 1) 1, https://doi.org/10.1056/EVIDoa2200015.

[11] S. Fung, Y.Y. Syed, Durvalumab: a review in advanced biliary tract cancer, Targeted Oncol. 18 (6) (2023 Nov) 965–972, https://doi.org/10.1007/s11523-023-01007-y.

[12] R.T. Shroff, M.M. Javle, L. Xiao, A.O. Kaseb, G.R. Varadhachary, R.A. Wolff, K.P. S. Raghav, M. Iwasaki, P. Masci, R.K. Ramanathan, D.H. Ahn, T.S. Bekaii-Saab, M. J. Borad, Gemcitabine, cisplatin, and nab-paclitaxel for the treatment of advanced biliary tract cancers: a phase 2 clinical trial, JAMA Oncol. 5 (6) (2019 Jun 1) 824–830, https://doi.org/10.1001/jamaoncol.2019.0270.

[13] D. Moris, M. Palta, C. Kim, P.J. Allen, M.A. Morse, M.E. Lidsky, Advances in the treatment of intrahepatic cholangiocarcinoma: an overview of the current and future therapeutic landscape for clinicians, CA A Cancer J. Clin. 73 (2) (2023 Mar) 198–222, https://doi.org/10.3322/caac.21759.

[14] A. Lauterio, R. De Carlis, L. Centonze, V. Buscemi, N. Incarbone, I. Vella, L. De Carlis, Current surgical management of peri-hilar and intra-hepatic cholangiocarcinoma, Cancers 13 (15) (2021 Jul 21) 3657, https://doi.org/10.3390/cancers13153657.

[15] E.W. Beal, J.M. Cloyd, T.M. Pawlik, Surgical treatment of intrahepatic cholangiocarcinoma: current and emerging principles, J. Clin. Med. 10 (1) (2020 Dec 30) 104, https://doi.org/10.3390/jcm10010104.

[16] H. Bourien, C.C. Pircher, B. Guiu, A. Lamarca, J.W. Valle, M. Niger, J. Edeline, Locoregional treatment in intrahepatic cholangiocarcinoma: which treatment for which patient? Cancers 15 (17) (2023 Aug 23) 4217, https://doi.org/10.3390/cancers15174217.

[17] D. Crosby, S. Bhatia, K.M. Brindle, L.M. Coussens, C. Dive, M. Emberton, S. Esener, R.C. Fitzgerald, S.S. Gambhir, P. Kuhn, T.R. Rebbeck, S. Balasubramanian, Early detection of cancer, Science 375 (6586) (2022 Mar 18) eaay9040, https://doi.org/10.1126/science.aay9040.

[18] J.W. Valle, J. Furuse, M. Jitlal, S. Beare, N. Mizuno, H. Wasan, J. Bridgewater, T. Okusaka, Cisplatin and gemcitabine for advanced biliary tract cancer: a meta-analysis of two randomised trials, Ann. Oncol. 25 (2) (2014 Feb) 391–398, https://doi.org/10.1093/annonc/mdt540.

[19] M. Capuozzo, M. Santorsola, L. Landi, V. Granata, F. Perri, V. Celotto, O. Gualillo, G. Nasti, A. Ottaiano, Evolution of treatment in advanced cholangiocarcinoma: old and new towards precision oncology, Int. J. Mol. Sci. 23 (23) (2022 Dec 1) 15124, https://doi.org/10.3390/ijms232315124.

[20] A.A. Rahnemai-Azar, A. Weisbrod, M. Dillhoff, C. Schmidt, T.M. Pawlik, Intrahepatic cholangiocarcinoma: molecular markers for diagnosis and prognosis, Surg Oncol 26 (2) (2017 Jun) 125–137, https://doi.org/10.1016/j.suronc.2016.12.009.

[21] R.I.R. Macias, J.M. Banales, B. Sangro, J. Muntané, M.A. Avila, E. Lozano, M. J. Perugorria, F.J. Padillo, L. Bujanda, J.J.G. Marin, The search for novel diagnostic and prognostic biomarkers in cholangiocarcinoma, Biochim. Biophys. Acta, Mol. Basis Dis. 1864 (4 Pt B) (2018 Apr) 1468–1477, https://doi.org/10.1016/j.bbadis.2017.08.002.

[22] J.A. Marrero, Biomarkers in cholangiocarcinoma, Clin. Liver Dis. 3 (5) (2014 May 27) 101–103, https://doi.org/10.1002/cld.345.

[23] L. Huang, W. Chen, P. Liang, W. Hu, K. Zhang, S. Shen, J. Chen, Z. Zhang, B. Chen, Y. Han, F. Meng, S. DeMorrow, X. Yin, J. Lai, L. Liang, Serum CYFRA 21-1 in biliary tract cancers: a reliable biomarker for gallbladder carcinoma and intrahepatic cholangiocarcinoma, Dig. Dis. Sci. 60 (5) (2015 May) 1273–1283, https://doi.org/10.1007/s10620-014-3472-0.

[24] R.I. Macias, Cholangiocarcinoma: biology, clinical management, and pharmacological perspectives, ISRN Hepatol (2014) 828074, https://doi.org/10.1155/2014/828074, 2014 Feb 16.

[25] A. Kupietzky, A. Ariche, Surgical aspects of intrahepatic cholangiocarcinoma, Cancers 14 (24) (2022 Dec 19) 6265, https://doi.org/10.3390/cancers14246265.

[26] K. Fábrega-Foster, M.A. Ghasabeh, T.M. Pawlik, I.R. Kamel, Multimodality imaging of intrahepatic cholangiocarcinoma, Hepatobiliary Surg. Nutr. 6 (2) (2017 Apr) 67–78, https://doi.org/10.21037/hbsn.2016.12.10.

[27] L.H. Liu, M.L. Wang, F. Jiang, L.L. Chen, Y. Ji, M.S. Zeng, Distinct radiological features of lymphoepithelioma-like intrahepatic cholangiocarcinoma: comparison

with classical intrahepatic cholangiocarcinoma, Abdom Radiol (NY) 48 (6) (2023 Jun) 2038–2048, https://doi.org/10.1007/s00261-023-03890-5.
[28] R. El-Diwany, T.M. Pawlik, A. Ejaz, Intrahepatic cholangiocarcinoma, Surg. Oncol. Clin. 28 (4) (2019 Oct) 587–599, https://doi.org/10.1016/j.soc.2019.06.002.
[29] H. Haga, T. Patel, Molecular diagnosis of intrahepatic cholangiocarcinoma, J Hepatobiliary Pancreat Sci 22 (2) (2015 Feb) 114–123, https://doi.org/10.1002/jhbp.156.
[30] D. Viterbo, V. Gausman, T. Gonda, Diagnostic and therapeutic biomarkers in pancreaticobiliary malignancy, World J. Gastrointest. Endosc. 8 (3) (2016 Feb 10) 128–142, https://doi.org/10.4253/wjge.v8.i3.128.
[31] L.Y. Tao, L. Cai, X.D. He, W. Liu, Q. Qu, Comparison of serum tumor markers for intrahepatic cholangiocarcinoma and hepatocellular carcinoma, Am. Surg. 76 (11) (2010 Nov) 1210–1213.
[32] D. Xie, Z. Ren, J. Fan, Q. Gao, Genetic profiling of intrahepatic cholangiocarcinoma and its clinical implication in targeted therapy, Am. J. Cancer Res. 6 (3) (2016 Feb 15) 577–586.
[33] Z. Gao, J.F. Chen, X.G. Li, Y.H. Shi, Z. Tang, W.R. Liu, X. Zhang, A. Huang, X. M. Luo, Q. Gao, G.M. Shi, A.W. Ke, J. Zhou, J. Fan, X.T. Fu, Z.B. Ding, KRAS acting through ERK signaling stabilizes PD-L1 via inhibiting autophagy pathway in intrahepatic cholangiocarcinoma, Cancer Cell Int. 22 (1) (2022 Mar 19) 128, https://doi.org/10.1186/s12935-022-02550-w.
[34] A. Ottaiano, F. Sabbatino, F. Perri, M. Cascella, R. Sirica, R. Patrone, M. Capuozzo, G. Savarese, M. Ianniello, N. Petrillo, L. Circelli, V. Granata, M. Berretta, M. Santorsola, G. Nasti, KRAS p.G12C mutation in metastatic colorectal cancer: prognostic implications and advancements in targeted therapies, Cancers 15 (14) (2023 Jul 12) 3579, https://doi.org/10.3390/cancers15143579.
[35] S. Chakrabarti, M. Kamgar, A. Mahipal, Targeted therapies in advanced biliary tract cancer: an evolving paradigm, Cancers 12 (8) (2020 Jul 24) 2039, https://doi.org/10.3390/cancers12082039.
[36] F. Lumachi, G. Lo Re, R. Tozzoli, F. D'Aurizio, F. Facomer, G.B. Chiara, S. M. Basso, 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 Res. 34 (11) (2014 Nov) 6663–6667.
[37] S. Pavicevic, S. Reichelt, D. Uluk, I. Lurje, C. Engelmann, D.P. Modest, U. Pelzer, F. Krenzien, N. Raschzok, C. Benzing, I.M. Sauer, S. Stintzing, F. Tacke, W. Schöning, M. Schmelzle, J. Pratschke, G. Lurje, Prognostic and predictive molecular markers in cholangiocarcinoma, Cancers 14 (4) (2022 Feb 17) 1026, https://doi.org/10.3390/cancers14041026.
[38] L. Su, X. Zhang, L. Zheng, M. Wang, Z. Zhu, P. Li, Mutation of isocitrate dehydrogenase 1 in cholangiocarcinoma impairs tumor progression by inhibiting isocitrate metabolism, Front. Endocrinol. 11 (2020 Apr 21) 189, https://doi.org/10.3389/fendo.2020.00189.
[39] E. Aguado-Fraile, A. Tassinari, Y. Ishii, C. Sigel, M.A. Lowery, L. Goyal, C. Gliser, L. Jiang, S.S. Pandya, B. Wu, N. Bardeesy, S. Choe, V. Deshpande, Molecular and morphological changes induced by ivosidenib correlate with efficacy in mutant-IDH1 cholangiocarcinoma, Future Oncol. 17 (16) (2021 Jun) 2057–2074, https://doi.org/10.2217/fon-2020-1274.
[40] A. Vogel, O. Segatto, A. Stenzinger, A. Saborowski, FGFR2 inhibition in cholangiocarcinoma, Annu. Rev. Med. 74 (2023 Jan 27) 293–306, https://doi.org/10.1146/annurev-med-042921-024707.
[41] T. Janvilisri, K. Leelawat, S. Roytrakul, A. Paemanee, R. Tohtong, Novel serum biomarkers to differentiate cholangiocarcinoma from benign biliary tract diseases using a proteomic approach, Dis. Markers 2015 (2015) 105358, https://doi.org/10.1155/2015/105358.
[42] A. Tolek, C. Wongkham, S. Proungvitaya, A. Silsirivanit, S. Roytrakul, N. Khuntikeo, S. Wongkham, Serum α1β-glycoprotein and afamin ratio as potential diagnostic and prognostic markers in cholangiocarcinoma, Exp. Biol. Med. 237 (10) (2012 Oct) 1142–1149, https://doi.org/10.1258/ebm.2012.012215.
[43] Z. Tang, Y. Yang, X. Wang, W. Meng, X. Li, Meta-analysis of the diagnostic value of Wisteria floribunda agglutinin-sialylated mucin1 and the prognostic role of mucin1 in human cholangiocarcinoma, BMJ Open 9 (1) (2019 Jan 29) e021693, https://doi.org/10.1136/bmjopen-2018-021693.
[44] K. Miyabe, V. Chandrasekhara, N. Wongjarupong, J. Chen, L. Yang, S. Johnson, N. Chia, M. Walther-Antonio, J.Z. Yao, S.C. Harrington, C.K. Nordyke, J.E. Eaton, A.A. Gossard, S. Oli, H.A. Ali, S. Lavu, N.H. Giama, F.A. Hassan, H.M. Ali, F. T. Enders, S.I. Ilyas, G.J. Gores, M.D. Topazian, P.C. Kashyap, L.R. Roberts, Potential role of inflammation-promoting biliary microbiome in primary sclerosing cholangitis and cholangiocarcinoma, Cancers 14 (9) (2022 Apr 24) 2120, https://doi.org/10.3390/cancers14092120.
[45] J. Shen, W. Wang, J. Wu, B. Feng, W. Chen, M. Wang, J. Tang, F. Wang, F. Cheng, L. Pu, Q. Tang, X. Wang, X. Li, Comparative proteomic profiling of human bile reveals SSP411 as a novel biomarker of cholangiocarcinoma, PLoS One 7 (10) (2012) e47476, https://doi.org/10.1371/journal.pone.0047476.
[46] M. Capuozzo, F. Ferrara, M. Santorsola, A. Zovi, A. Ottaiano, Circulating tumor cells as predictive and prognostic biomarkers in solid tumors, Cells 12 (2023) 2590, https://doi.org/10.3390/cells12222590.
[47] J.H. Jiang, D.Z. Fang, Y.T. Hu, Influence of surgical margin width on survival rate after resection of intrahepatic cholangiocarcinoma: a systematic review and meta-analysis, BMJ Open 13 (5) (2023 May 8) e067222, https://doi.org/10.1136/bmjopen-2022-067222.
[48] A. Moro, R. Mehta, K. Sahara, D.I. Tsilimigras, A.Z. Paredes, A. Farooq, J.M. Hyer, I. Endo, F. Shen, A. Guglielmi, L. Aldrighetti, M. Weiss, T.W. Bauer, S. Alexandrescu, G.A. Poultsides, S.K. Maithel, H.P. Marques, G. Martel, C. Pulitano, O. Soubrane, B.G. Koerkamp, K. Sasaki, T.M. Pawlik, The impact of preoperative CA19-9 and CEA on outcomes of patients with intrahepatic cholangiocarcinoma, Ann. Surg Oncol. 27 (8) (2020 Aug) 2888–2901, https://doi.org/10.1245/s10434-020-08350-8.
[49] S. Rizvi, J. Eaton, J.D. Yang, V. Chandrasekhara, G.J. Gores, Emerging technologies for the diagnosis of perihilar cholangiocarcinoma, Semin. Liver Dis. 38 (2) (2018 May) 160–169, https://doi.org/10.1055/s-0038-1655775.
[50] D.I. Tsilimigras, J.M. Hyer, A.Z. Paredes, A. Diaz, D. Moris, A. Guglielmi, L. Aldrighetti, M. Weiss, T.W. Bauer, S. Alexandrescu, G.A. Poultsides, S. K. Maithel, H.P. Marques, G. Martel, C. Pulitano, F. Shen, O. Soubrane, B. G. Koerkamp, I. Endo, T.M. Pawlik, A novel classification of intrahepatic cholangiocarcinoma phenotypes using machine learning techniques: an international multi-institutional analysis, Ann. Surg Oncol. 27 (13) (2020 Dec) 5224–5232, https://doi.org/10.1245/s10434-020-08696-z.
[51] L. Huang, W. Chen, P. Liang, W. Hu, K. Zhang, S. Shen, J. Chen, Z. Zhang, B. Chen, Y. Han, F. Meng, S. DeMorrow, X. Yin, J. Lai, L. Liang, Serum CYFRA 21-1 in biliary tract cancers: a reliable biomarker for gallbladder carcinoma and intrahepatic cholangiocarcinoma, Dig. Dis. Sci. 60 (5) (2015 May) 1273–1283, https://doi.org/10.1007/s10620-014-3472-0.
[52] H. Suzuki, M. Komuta, A. Bolog, T. Yokobori, S. Wada, K. Araki, N. Kubo, A. Watanabe, M. Tsukagoshi, H. Kuwano, Relationship between 18-F-fluoro-deoxy-D-glucose uptake and expression of glucose transporter 1 and pyruvate kinase M2 in intrahepatic cholangiocarcinoma, Dig. Liver Dis. 47 (7) (2015 Jul) 590–596, https://doi.org/10.1016/j.dld.2015.03.017.
[53] K. Pant, S. Richard, E. Peixoto, S.A. Gradilone, Role of glucose metabolism reprogramming in the pathogenesis of cholangiocarcinoma, Front. Med. 7 (2020 Apr 3) 113, https://doi.org/10.3389/fmed.2020.00113.
[54] G. Tshering, P.W. Dorji, W. Chaijaroenkul, K. Na-Bangchang, Biomarkers for the diagnosis of cholangiocarcinoma: a systematic review, Am. J. Trop. Med. Hyg. 98 (6) (2018 Jun) 1788–1797, https://doi.org/10.4269/ajtmh.17-0879.
[55] F. Zhao, C. Li, Y. Wu, J. Xia, M. Zeng, T. Li, K. Xie, Connective tissue growth factor in digestive system cancers: a review and meta-analysis, BioMed Res. Int. (2020) 8489093, https://doi.org/10.1155/2020/8489093, 2020 Dec 27.
[56] L.M. Chen, L. Xiang, W.J. Sun, Y.J. Zhai, S. Gao, Y.C. Fan, K. Wang, Diagnostic value of the hypomethylation of the WISP1 promoter in patients with hepatocellular carcinoma associated with hepatitis B virus, Tohoku J. Exp. Med. 252 (4) (2020 Dec) 297–307, https://doi.org/10.1620/tjem.252.297.
[57] T. Hirashita, Y. Iwashita, M. Ohta, Y. Komori, H. Eguchi, K. Yada, S. Kitano, Expression of matrix metalloproteinase-7 is an unfavorable prognostic factor in intrahepatic cholangiocarcinoma, J. Gastrointest. Surg. 16 (4) (2012 Apr) 842–848, https://doi.org/10.1007/s11605-011-1813-2.
[58] A. Kasprzak, A. Adamek, Mucins: the old, the new and the promising factors in hepatobiliary carcinogenesis, Int. J. Mol. Sci. 20 (6) (2019 Mar 14) 1288, https://doi.org/10.3390/ijms20061288.
[59] W.R. Matull, F. Andreola, A. Loh, Z. Adiguzel, M. Deheragoda, U. Qureshi, S. K. Batra, D.M. Swallow, S.P. Pereira, MUC4 and MUC5AC are highly specific tumour-associated mucins in biliary tract cancer, Br. J. Cancer 98 (10) (2008 May 20) 1675–1681, https://doi.org/10.1038/sj.bjc.6604364.
[60] Y. Lan, W. Ni, G. Tai, Expression of MUC1 in different tumours and its clinical significance, Mol Clin Oncol 17 (6) (2022) 161, https://doi.org/10.3892/mco.2022.2594, submitted for publication.
[61] Y.R. Pan, C.E. Wu, S.M. Jung, S.C. Huang, S.H. Lin, W.C. Chou, Y.C. Chang, M. H. Chen, T.H. Hung, A.L. Yu, W.K. Huang, C.N. Yeh, Mucin 4 confers gemcitabine resistance and an unfavorable prognosis in patients with cholangiocarcinoma via AKT activation, Int. J. Biol. Sci. 19 (9) (2023 May 21) 2772–2786, https://doi.org/10.7150/ijbs.79126.
[62] S.Y. Park, S.J. Roh, Y.N. Kim, S.Z. Kim, H.S. Park, K.Y. Jang, M.J. Chung, M. J. Kang, D.G. Lee, W.S. Moon, Expression of MUC1, MUC2, MUC5AC and MUC6 in cholangiocarcinoma: prognostic impact, Oncol. Rep. 22 (3) (2009 Sep) 649–657, https://doi.org/10.3892/or_00000485.
[63] P. Boottanun, Y. Ino, K. Shimada, N. Hiraoka, K. Angata, H. Narimatsu, Association between the expression of core 3 synthase and survival outcomes of patients with cholangiocarcinoma, Oncol. Lett. 22 (5) (2021 Nov) 760, https://doi.org/10.3892/ol.2021.13021.
[64] L. Xu, P. Wang, W. Zhang, W. Li, T. Liu, X. Che, Dual-specificity phosphatase 11 is a prognostic biomarker of intrahepatic cholangiocarcinoma, Front. Oncol. 11 (2021 Sep 29) 757498, https://doi.org/10.3389/fonc.2021.757498.
[65] S.R. Kim, H.S. Won, J.H. Yang, S. Sun, K. Yim, M. Hong, S.A. Hong, J.S. Yoon, S. H. Chun, K.H. Kim, Y.H. Ko, Prognostic value of Dickkopf-1 and ß-catenin expression according to the antitumor immunity of CD8-positive tumor-infiltrating lymphocytes in biliary tract cancer, Sci. Rep. 12 (1) (2022 Feb 4) 1931, https://doi.org/10.1038/s41598-022-05914-4.
[66] M.H. Storandt, P.C. Kurniali, A. Mahipal, Z. Jin, Targeted therapies in advanced cholangiocarcinoma, Life 13 (10) (2023 Oct 16) 2066, https://doi.org/10.3390/life13102066.
[67] J. Lin, Y. Cao, X. Yang, G. Li, Y. Shi, D. Wang, J. Long, Y. Song, J. Mao, F. Xie, Y. Bai, L. Zhang, X. Yang, X. Wan, A. Wang, M. Guan, L. Zhao, K. Hu, J. Pan, L. Huo, X. Lu, Y. Mao, X. Sang, H. Zhang, K. Wang, X. Wang, H. Zhao, Mutational spectrum and precision oncology for biliary tract carcinoma, Theranostics 11 (10) (2021 Mar 4) 4585–4598, https://doi.org/10.7150/thno.56539.
[68] J. Peng, S. Fang, M. Li, Y. Liu, X. Liang, Z. Li, G. Chen, L. Peng, N. Chen, L. Liu, X. Xu, W. Dai, Genetic alterations of KRAS and TP53 in intrahepatic cholangiocarcinoma associated with poor prognosis, Open Life Sci. 18 (1) (2023 Jul 17) 20220652, https://doi.org/10.1515/biol-2022-0652.

[69] T. Boerner, E. Drill, L.M. Pak, B. Nguyen, C.S. Sigel, A. Doussot, P. Shin, D. A. Goldman, M. Gonen, P.J. Allen, V.P. Balachandran, A. Cercek, J. Harding, D. B. Solit, N. Schultz, R. Kundra, H. Walch, M.I. D'Angelica, R.P. DeMatteo, J. Drebin, N.E. Kemeny, T.P. Kingham, A.L. Simpson, J.F. Hechtman, E. Vakiani, M.A. Lowery, J.N.M. Ijzermans, S. Buettner, B.G. Koerkamp, M. Doukas, R. Chandwani, W.R. Jarnagin, Genetic determinants of outcome in intrahepatic cholangiocarcinoma, Hepatology 74 (3) (2021 Sep) 1429–1444, https://doi.org/10.1002/hep.31829.

[70] J. Li, F.G. Rocha, S.C. Mayo, Past, present, and future management of localized biliary tract malignancies, Surg. Oncol. Clin. 32 (1) (2023 Jan) 83–99, https://doi.org/10.1016/j.soc.2022.07.007.

[71] M. Tsujie, T. Iwai, S. Kubo, T. Ura, E. Hatano, D. Sakai, Y. Takeda, M. Kaibori, T. Kobayashi, A. Katanuma, Y. Katayose, K. Fukase, Fibroblast growth factor receptor 2 (FGFR2) fusions in Japanese patients with intrahepatic cholangiocarcinoma, Jpn. J. Clin. Oncol. 51 (6) (2021 May 28) 911–917, https://doi.org/10.1093/jjco/hyab029.

[72] S. Rizvi, G.J. Gores, Emerging molecular therapeutic targets for cholangiocarcinoma, J. Hepatol. 67 (3) (2017 Sep) 632–644, https://doi.org/10.1016/j.jhep.2017.03.026.

[73] D. Sia, Y. Hoshida, A. Villanueva, S. Roayaie, J. Ferrer, B. Tabak, J. Peix, M. Sole, V. Tovar, C. Alsinet, H. Cornella, B. Klotzle, J.B. Fan, C. Cotsoglou, S.N. Thung, J. Fuster, S. Waxman, J.C. Garcia-Valdecasas, J. Bruix, M.E. Schwartz, R. Beroukhim, V. Mazzaferro, J.M. Llovet, Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes, Gastroenterology 144 (4) (2013 Apr) 829–840, https://doi.org/10.1053/j.gastro.2013.01.001.

[74] J.S. Ross, K. Wang, L. Gay, R. Al-Rohil, J.V. Rand, D.M. Jones, H.J. Lee, C. E. Sheehan, G.A. Otto, G. Palmer, R. Yelensky, D. Lipson, D. Morosini, M. Hawryluk, D.V. Catenacci, V.A. Miller, C. Churi, S. Ali, P.J. Stephens, New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing, Oncol. 19 (3) (2014 Mar) 235–242, https://doi.org/10.1634/theoncologist.2013-0352.

[75] G.K. Abou-Alfa, T. Macarulla, M.M. Javle, R.K. Kelley, S.J. Lubner, J. Adeva, J. M. Cleary, D.V. Catenacci, M.J. Borad, J. Bridgewater, W.P. Harris, A.G. Murphy, D.Y. Oh, J. Whisenant, M.A. Lowery, L. Goyal, R.T. Shroff, A.B. El-Khoueiry, B. Fan, B. Wu, C.X. Chamberlain, L. Jiang, C. Gliser, S.S. Pandya, J.W. Valle, A. X. Zhu, Ivosidenib in IDH1-mutant, chemotherapy-refractory cholangiocarcinoma (ClarIDHy): a multicentre, randomised, double-blind, placebo-controlled, phase 3 study, Lancet Oncol. 21 (6) (2020 Jun) 796–807, https://doi.org/10.1016/S1470-2045(20)30157-1.

[76] A. Drilon, T.W. Laetsch, S. Kummar, S.G. DuBois, U.N. Lassen, G.D. Demetri, M. Nathenson, R.C. Doebele, A.F. Farago, A.S. Pappo, B. Turpin, A. Dowlati, M. S. Brose, L. Mascarenhas, N. Federman, J. Berlin, W.S. El-Deiry, C. Baik, J. Deeken, V. Boni, R. Nagasubramanian, M. Taylor, E.R. Rudzinski, F. Meric-Bernstam, D.P.S. Sohal, P.C. Ma, L.E. Raez, J.F. Hechtman, R. Benayed, M. Ladanyi, B.B. Tuch, K. Ebata, S. Cruickshank, N.C. Ku, M.C. Cox, D.S. Hawkins, D.S. Hong, D.M. Hyman, Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children, N. Engl. J. Med. 378 (8) (2018 Feb 22) 731–739, https://doi.org/10.1056/NEJMoa1714448.

[77] R.C. Doebele, A. Drilon, L. Paz-Ares, S. Siena, A.T. Shaw, A.F. Farago, C. M. Blakely, T. Seto, B.C. Cho, D. Tosi, B. Besse, S.P. Chawla, L. Bazhenova, J. C. Krauss, Y.K. Chae, M. Barve, I. Garrido-Laguna, S.V. Liu, P. Conkling, T. John, M. Fakih, D. Sigal, H.H. Loong, G.L. Buchschacher Jr., P. Garrido, J. Nieva, C. Steuer, T.R. Overbeck, D.W. Bowles, E. Fox, T. Riehl, E. Chow-Maneval, B. Simmons, N. Cui, A. Johnson, S. Eng, T.R. Wilson, G.D. Demetri, Trial investigators. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1-2 trials, Lancet Oncol. 21 (2) (2020 Feb) 271–282, https://doi.org/10.1016/S1470-2045(19)30691-6.

[78] G.K. Abou-Alfa, V. Sahai, A. Hollebecque, G. Vaccaro, D. Melisi, R. Al-Rajabi, A. S. Paulson, M.J. Borad, D. Gallinson, A.G. Murphy, D.Y. Oh, E. Dotan, D. V. Catenacci, E. Van Cutsem, T. Ji, C.F. Lihou, H. Zhen, L. Féliz, A. Vogel, Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study, Lancet Oncol. 21 (5) (2020 May) 671–684, https://doi.org/10.1016/S1470-2045(20)30109-1.

[79] T.S. Bekaii-Saab, J.W. Valle, E. Van Cutsem, L. Rimassa, J. Furuse, T. Ioka, D. Melisi, T. Macarulla, J. Bridgewater, H. Wasan, M.J. Borad, G.K. Abou-Alfa, P. Jiang, C.F. Lihou, H. Zhen, E. Asatiani, L. Féliz, A. Vogel, FIGHT-302: first-line pemigatinib vs gemcitabine plus cisplatin for advanced cholangiocarcinoma with FGFR2 rearrangements, Future Oncol. 16 (30) (2020 Oct) 2385–2399, https://doi.org/10.2217/fon-2020-0429.

[80] S.M. Ruff, S. Roychowdhury, T.M. Pawlik, The future of fibroblast growth factor receptor inhibitors and mechanisms of resistance for cholangiocarcinoma, Expet Opin. Pharmacother. 24 (7) (2023 May) 779–788, https://doi.org/10.1080/14656566.2023.2202814.

[81] G. Rompianesi, M. Di Martino, A. Gordon-Weeks, R. Montalti, R. Troisi, Liquid biopsy in cholangiocarcinoma: current status and future perspectives, World J. Gastrointest. Oncol. 13 (5) (2021 May 15) 332–350, https://doi.org/10.4251/wjgo.v13.i5.332.

[82] J.C. Stadler, Y. Belloum, B. Deitert, M. Sementsov, I. Heidrich, C. Gebhardt, L. Keller, K. Pantel, Current and future clinical applications of ctDNA in immuno-oncology, Cancer Res. 82 (3) (2022 Feb 1) 349–358, https://doi.org/10.1158/0008-5472.CAN-21-1718.

[83] A. Tivey, M. Church, D. Rothwell, C. Dive, N. Cook, Circulating tumour DNA - looking beyond the blood, Nat. Rev. Clin. Oncol. 19 (9) (2022 Sep) 600–612, https://doi.org/10.1038/s41571-022-00660-y.

[84] B. Cena, E. Melloul, N. Demartines, O. Dormond, I. Labgaa, Basic science with preclinical models to investigate and develop liquid biopsy: what are the available data and is it a fruitful approach? Int. J. Mol. Sci. 23 (10) (2022 May 10) 5343, https://doi.org/10.3390/ijms23105343.

[85] S. Gonvers, P. Tabrizian, E. Melloul, O. Dormond, M. Schwartz, N. Demartines, I. Labgaa, Is liquid biopsy the future commutator of decision-making in liver transplantation for hepatocellular carcinoma? Front. Oncol. 12 (2022 Aug 11) 940473 https://doi.org/10.3389/fonc.2022.940473.

[86] W.J. Choi, T. Ivanics, A. Gravely, S. Gallinger, G. Sapisochin, G.M. O'Kane, Optimizing circulating tumour DNA use in the perioperative setting for intrahepatic cholangiocarcinoma: diagnosis, screening, minimal residual disease detection and treatment response monitoring, Ann. Surg Oncol. 30 (6) (2023 Jun) 3849–3863, https://doi.org/10.1245/s10434-023-13126-x.

[87] F. Mosele, J. Remon, J. Mateo, C.B. Westphalen, F. Barlesi, M.P. Lolkema, N. Normanno, A. Scarpa, M. Robson, F. Meric-Bernstam, N. Wagle, A. Stenzinger, J. Bonastre, A. Bayle, S. Michiels, I. Bièche, E. Rouleau, S. Jezdic, J.Y. Douillard, J.S. Reis-Filho, R. Dienstmann, F. André, Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group, Ann. Oncol. 31 (11) (2020 Nov) 1491–1505, https://doi.org/10.1016/j.annonc.2020.07.014.

[88] J.E. Berchuck, F. Facchinetti, D.F. DiToro, I. Baiev, U. Majeed, S. Reyes, C. Chen, K. Zhang, R. Sharman, P.L.S. Uson Junior, J. Maurer, R.T. Shroff, C.C. Pritchard, M.J. Wu, D.V.T. Catenacci, M. Javle, L. Friboulet, A. Hollebecque, N. Bardeesy, A. X. Zhu, J.K. Lennerz, B. Tan, M. Borad, A.R. Parikh, L.A. Kiedrowski, R.K. Kelley, K. Mody, D. Juric, L. Goyal, The clinical landscape of cell-free DNA alterations in 1671 patients with advanced biliary tract cancer, Ann. Oncol. 33 (12) (2022 Dec) 1269–1283, https://doi.org/10.1016/j.annonc.2022.09.150.

[89] M.A. Israel, N. Danziger, K.A. McGregor, K. Murugesan, O. Gjoerup, E.S. Sokol, H. Tukachinsky, R. Kurzrock, S. Kato, J.K. Sicklick, H.S. Nimeiri, G.R. Oxnard, J. S. Ross, Comparative genomic analysis of intrahepatic cholangiocarcinoma: biopsy type, ancestry, and testing patterns, Oncol. 26 (9) (2021 Sep) 787–796, https://doi.org/10.1002/onco.13844.

[90] S.L. Csoma, J. Bedekovics, G. Veres, A. Árokszállási, C. András, G. Méhes, A. Mokánszki, Circulating cell-free DNA-based comprehensive molecular analysis of biliary tract cancers using next-generation sequencing, Cancers 14 (1) (2022 Jan 4) 233, https://doi.org/10.3390/cancers14010233.

[91] J.D. Yang, M.B. Campion, M.C. Liu, R. Chaiteerakij, N.H. Giama, H. Ahmed Mohammed, X. Zhang, C. Hu, V.L. Campion, J. Jen, S.K. Venkatesh, K.C. Halling, B.R. Kipp, L.R. Roberts, Circulating tumor cells are associated with poor overall survival in patients with cholangiocarcinoma, Hepatology 63 (1) (2016 Jan) 148–158, https://doi.org/10.1002/hep.27944.

[92] J.Y. Han, K.S. Ahn, Y.H. Kim, T.S. Kim, W.K. Baek, S.I. Suh, K.J. Kang, Circulating microRNAs as biomarkers in bile-derived exosomes of cholangiocarcinoma, Ann Surg Treat Res 101 (3) (2021 Sep) 140–150, https://doi.org/10.4174/astr.2021.101.3.140.

[93] V. Tamasi, K. Németh, M. Csala, Role of extracellular vesicles in liver diseases, Life 13 (5) (2023 Apr 30) 1117, https://doi.org/10.3390/life13051117.

[94] R. Muñoz-Hernández, Á. Rojas, S. Gato, J. Gallego, A. Gil-Gómez, M.J. Castro, J. Ampuero, M. Romero-Gómez, Extracellular vesicles as biomarkers in liver disease, Int. J. Mol. Sci. 23 (24) (2022 Dec 19) 16217, https://doi.org/10.3390/ijms232416217.

[95] A. Eguchi, M. Iwasa, H. Nakagawa, Extracellular vesicles in fatty liver disease and steatohepatitis: role as biomarkers and therapeutic targets, Liver Int. 43 (2) (2023 Feb) 292–298, https://doi.org/10.1111/liv.15490.

[96] Y. Xu, K. Leng, Y. Yao, P. Kang, G. Liao, Y. Han, G. Shi, D. Ji, P. Huang, W. Zheng, Z. Li, J. Li, L. Huang, L. Yu, Y. Zhou, X. Jiang, H. Wang, C. Li, Z. Su, S. Tai, X. Zhong, Z. Wang, Y. Cui, A circular RNA, cholangiocarcinoma-associated circular RNA 1, contributes to cholangiocarcinoma progression, induces angiogenesis, and disrupts vascular endothelial barriers, Hepatology 73 (4) (2021 Apr) 1419–1435, https://doi.org/10.1002/hep.31493.

[97] C. Louis, D. Leclerc, C. Coulouarn, Emerging roles of circular RNAs in liver cancer, JHEP Rep 4 (2) (2021 Nov 27) 100413, https://doi.org/10.1016/j.jhepr.2021.100413.

[98] W. Liao, J. Du, L. Li, X. Wu, X. Chen, Q. Feng, L. Xu, X. Chen, M. Liao, J. Huang, K. Yuan, Y. Zeng, CircZNF215 promotes tumor growth and metastasis through inactivation of the PTEN/AKT pathway in intrahepatic cholangiocarcinoma, J. Exp. Clin. Cancer Res. 42 (1) (2023 May 18) 125, https://doi.org/10.1186/s13046-023-02699-w.

[99] Q. Chen, H. Wang, Z. Li, F. Li, L. Liang, Y. Zou, H. Shen, J. Li, Y. Xia, Z. Cheng, T. Yang, K. Wang, F. Shen, Circular RNA ACTN4 promotes intrahepatic cholangiocarcinoma progression by recruiting YBX1 to initiate FZD7 transcription, J. Hepatol. 76 (1) (2022 Jan) 135–147, https://doi.org/10.1016/j.jhep.2021.08.027.

[100] W. Liao, Q. Feng, H. Liu, J. Du, X. Chen, Y. Zeng, Circular RNAs in cholangiocarcinoma, Cancer Lett. 553 (2023 Jan 28) 215980, https://doi.org/10.1016/j.canlet.2022.215980.

[101] D. Gilot, M. Migault, L. Bachelot, F. Journé, A. Rogiers, E. Donnou-Fournet, A. Mogha, N. Mouchet, M.L. Pinel-Marie, B. Mari, T. Montier, S. Corre, A. Gautron, F. Rambow, P. El Hajj, R. Ben Jouira, S. Tartare-Deckert, J.C. Marine, B. Felden, G. Ghanem, M.D. Galibert, A non-coding function of TYRP1 mRNA promotes melanoma growth, Nat. Cell Biol. 19 (11) (2017 Nov) 1348–1357, https://doi.org/10.1038/ncb3623.

[102] M. Lempinen, H. Isoniemi, H. Mäkisalo, A. Nordin, L. Halme, J. Arola, K. Höckerstedt, U.H. Stenman, Enhanced detection of cholangiocarcinoma with serum trypsinogen-2 in patients with severe bile duct strictures, J. Hepatol. 47 (5) (2007 Nov) 677–683, https://doi.org/10.1016/j.jhep.2007.05.017.

[103] L. Halme, J. Arola, K. Numminen, L. Krogerus, H. Mäkisalo, M. Färkkilä, Biliary dysplasia in patients with primary sclerosing cholangitis: additional value of DNA ploidity, Liver Int. 32 (5) (2012 May) 783–789, https://doi.org/10.1111/j.1478-3231.2011.02672.x.

[104] W. Jamnongkan, A. Techasen, R. Thanan, K. Duenngai, P. Sithithaworn, E. Mairiang, W. Loilome, N. Namwat, C. Pairojkul, P. Yongvanit, Oxidized alpha-1 antitrypsin as a predictive risk marker of opisthorchiasis-associated cholangiocarcinoma, Tumour Biol 34 (2) (2013 Apr) 695–704, https://doi.org/10.1007/s13277-012-0597-7.

[105] S. Prakobwong, L. Charoensuk, Y. Hiraku, P. Pinlaor, C. Pairojkul, E. Mairiang, P. Sithithaworn, P. Yongvanit, N. Khuntikeo, S. Pinlaor, Plasma hydroxyproline, MMP-7 and collagen I as novel predictive risk markers of hepatobiliary disease-associated cholangiocarcinoma, Int. J. Cancer 131 (4) (2012 Aug 15) E416–E424, https://doi.org/10.1002/ijc.26443.

[106] J.H. Jo, M.J. Chung, J.Y. Park, S. Bang, S.W. Park, K.S. Kim, W.J. Lee, S.Y. Song, J.B. Chung, High serum CA19-9 levels are associated with an increased risk of cholangiocarcinoma in patients with intrahepatic duct stones: a case-control study, Surg. Endosc. 27 (11) (2013 Nov) 4210–4216, https://doi.org/10.1007/s00464-013-3025-1.

[107] A. Lapitz, M. Azkargorta, P. Milkiewicz, P. Olaizola, E. Zhuravleva, M. M. Grimsrud, C. Schramm, A. Arbelaiz, C.J. O'Rourke, A. La Casta, M. Milkiewicz, T. Pastor, M. Vesterhus, R. Jimenez-Agüero, M.T. Dill, A. Lamarca, J.W. Valle, R.I. R. Macias, L. Izquierdo-Sanchez, Y. Pérez Castaño, F.J. Caballero-Camino, I. Riaño, M. Krawczyk, C. Ibarra, J. Bustamante, L.M. Nova-Camacho, J. M. Falcon-Perez, F. Elortza, M.J. Perugorria, J.B. Andersen, L. Bujanda, T. H. Karlsen, T. Folseraas, P.M. Rodrigues, J.M. Banales, Liquid biopsy-based protein biomarkers for risk prediction, early diagnosis, and prognostication of cholangiocarcinoma, J. Hepatol. 79 (1) (2023 Jul) 93–108, https://doi.org/10.1016/j.jhep.2023.02.027.