Cholangiocarcinoma

Paul J. Brindley1, Melinda Bachini2, Sumeria I. Ilyas3, Shahid A. Khan4, Alex Loukas5, Alphonse E. Sirica6, Bin Tean Teh7, Sopit Wongkham8 and Gregory J. Gores3✉

Abstract | Cholangiocarcinoma (CCA) is a highly lethal adenocarcinoma of the hepatobiliary system, which can be classified as intrahepatic, perihilar and distal. Each anatomic subtype has distinct genetic aberrations, clinical presentations and therapeutic approaches. In endemic regions, liver fluke infection is associated with CCA, owing to the oncogenic effect of the associated chronic biliary tract inflammation. In other regions, CCA can be associated with chronic biliary tract inflammation owing to choledocholithiasis, cholelithiasis, or primary sclerosing cholangitis, but most CCAs have no identifiable cause. Administration of the anthelmintic drug praziquantel decreases the risk of CCA from liver flukes, but reinfection is common and future vaccination strategies may be more effective. Some patients with CCA are eligible for potentially curative surgical options, such as resection or liver transplantation. Genetic studies have provided new insights into the pathogenesis of CCA, and two aberrations that drive the pathogenesis of non-fluke-associated intrahepatic CCA, fibroblast growth factor receptor 2 fusions and isocitrate dehydrogenase gain-of-function mutations, can be therapeutically targeted. CCA is a highly desmoplastic cancer and targeting the tumour immune microenvironment might be a promising therapeutic approach. CCA remains a highly lethal disease and further scientific and clinical insights are needed to improve patient outcomes.

Cholangiocarcinoma (CCA) is a highly lethal, epithelial cell malignancy that occurs anywhere along the biliary tree and/or within the hepatic parenchyma. CCA displays features of cholangiocyte differentiation and probably arises predominantly from the epithelial cells lining the bile ducts, which are termed cholangiocytes; however, the cancers may also develop from peribiliary glands and hepatocytes, depending on the underlying liver disease and location1–4. These cancers are heterogeneous and are best classified according to the primary, anatomic subtype as intrahepatic CCA (iCCA), perihilar CCA (pCCA) or distal CCA (dCCA)5,6 (Fig. 1). iCCA is located proximally to the second-order bile ducts within the liver parenchyma, pCCA is localized between the second-order bile ducts and the insertion of the cystic duct into the common bile duct, and dCCA is confined to the common bile duct below the cystic duct insertion. The true incidence of pCCA and iCCA is unclear owing to the extensive misclassification of pCCA as iCCA in national databases6,7. In addition, enhanced diagnostic capabilities have enabled increased clinical distinction between carcinoma of unknown primary and iCCA8,9. These factors have, in part, contributed to the reported increase in incidence of iCCA over the past two or three decades. Each of the anatomic subtypes is characterized by unique genetic aberrations, clinical presentations and management options10. However, many databases categorize both pCCA and dCCA as extrahepatic CCA. Most CCAs are adenocarcinomas and other histological subtypes, such as adenosquamous carcinoma or clear cell carcinoma, are encountered rarely11. These cancers are highly desmoplastic and are enmeshed in dense networks of inflammatory cells and matrix termed the tumour immune microenvironment12–14.

The epidemiology of these cancers varies worldwide. Infections with specific trematodes (flatworm parasites, commonly called flukes) are a major cause of CCA in some regions. For example, in Southeast Asia, the liver fluke *Opisthorchis viverrini* is the leading cause of CCA15. CCA occurring secondary to fluke infestation can arise anywhere within the biliary tree and present as any one of the three anatomic subsets. Fluke-related CCA may have a specific pathogenesis, especially genetic aberrations, but the diagnosis and management are not different from non-fluke-related CCA. In the Western world, most patients with CCA do not have an identifiable risk factor, except for some with primary sclerosing cholangitis (PSC)7,10. Further insights into the epidemiology, risk factors and biology of CCA are needed to improve its prevention and therapy.

In this Primer, we discuss the epidemiology and pathophysiological mechanisms of liver-fluke-related and non-liver-fluke-related CCA and associated risk factors and summarize diagnosis and management of...
CCA depending on aetiology and anatomic subtype. We highlight the patient experience and future directions for control and treatment of this disease. Gallbladder carcinoma and ampullary carcinoma are not discussed in this Primer, owing to differing pathogenesis, treatment options and prognosis compared with CCA.

Epidemiology

The epidemiology of CCA is diverse across the globe, and it is likely that further subsets will be identified in the future. Currently, liver flukes are a major risk factor in Southeast Asia, whereas the risk factors outside this region are largely unknown. Thus, the epidemiology of CCA can be discussed as fluke-related and non-fluke-related CCA.

Fluke-related CCA

Infections with specific trematodes are a major cause of CCA in some regions, and these flatworm parasites are group 1 biological carcinogens, that is, definite causes of cancer, including several fish-borne liver flukes of the Opisthorchidae family. The importance of fish-borne parasites Clonorchis sinensis, O. viverrini and Opisthorchis felineus to human health is due to their infection-associated morbidity and risk of CCA. Among them, only C. sinensis and O. viverrini have so far been shown to cause CCA in animal studies. The incidence of CCA associated with liver fluke infection, calculated as an age-standardized rate, varies by geographical region and other risk factors but has exceeded 100 per 100,000 in men and 40 per 100,000 in women in hotspots in northeast Thailand. At least 700 million people are at risk of infection with these liver flukes. Infection follows the consumption of undercooked freshwater cypriid fish carrying the larval parasite, termed the metacercaria. The adult liver flukes inhabit the biliary tract, from where the parasite eggs are shed into the bile and passed with the faeces to the external environment. The life cycle includes an obligate intermediate host snail, the freshwater fish and the human host. Following ingestion of infected fish flesh, gastric and intestinal juices digest the encysted metacercariae, whereupon excysted juvenile flukes migrate through the ampulla of Vater into the common bile duct and into the intrahepatic bile ducts. Here they mature, reproduce sexually, and can live for many years.

Infection caused by C. sinensis (clonorchiasis) is endemic on the Korean peninsula (particularly along the drainage of the major southern rivers, including Nakdong-gang, Seomjin-gang, Youngsan-gang and Geum-gang); in far-eastern Russia; in Taiwan and several regions of the Chinese mainland (including Liaoning, Jilin and Heilongjiang in the northeast, and Guangdong, Guanxi, Hunan and Sichuan in the south); in northern Vietnam; and occasionally in Japan. Infection with O. viverrini (opisthorchiasis) is prevalent in western Siberia, including Surgut, Tyumen and Tomsk oblast, and Kazakhstan along the drainage of the Ob and Irtysy rivers. As many as 100 million people are at risk of infection with O. viverrini in the lower Mekong River drainage, with ~10 million infected in the northern and Isan region of Thailand (including Roi Et, Yasothon, Nakhon Phanom, Mukdahan, Nong Khai and Khon Kaen, and provinces that are drained by the Mun River and Chi River), and 2 million infected in the Lao People’s Democratic Republic (mainly in the central and southern provinces of Khammouane, Savannakhet and Champasak); many infections also occur in southern Vietnam, Cambodia and Myanmar.

Non-fluke-related CCA

In most countries, liver flukes are not endemic and CCA is a relatively rare malignancy; however, incidence and mortality rates have been consistently increasing in the past few decades. Multiple studies have shown a consistent pattern of rising rates of iCCA in the past few decades. Multiple studies have shown a consistent pattern of rising rates of iCCA and stable or decreasing rates of pCCA and/or dCCA. The most recent and comprehensive study examined WHO and Pan American Health Organization databases of 32 countries in Europe, the American continents, Asia and Oceania between 1995 and 2016 (Ref. ). Overall, global age-standardized mortality rates (ASMRs) for iCCA increased. The highest incremental changes occurred in Norway, Croatia, Denmark, the UK, Germany and Portugal among European countries; in Argentina, Chile and Brazil among American countries; and Australia. ASMRs for iCCA in men were 1–2 per 100,000 in most countries, with the highest rates in Hong Kong (2.5 per 100,000), followed by France, Austria, Spain, the UK and Australia (1.5–1.8 per 100,000). By contrast, ASMRs for pCCA and/or dCCA decreased in most countries. Between 2010 and 2014, ASMRs for pCCA and/or dCCA were <1 per 100,000 men in all countries, except Japan (2.8 per 100,000 men). The lowest ASMRs (<0.1 per 100,000) were observed in central and northern European regions, Canada, Latin American regions (Mexico, Chile, Brazil, Venezuela and Argentina), Israel and Australia. These patterns were similar to those in women but differences in the ASMRs in men and women between countries were notable. Overall CCA mortality is higher in older patients than in younger patients, in men than in women, and in Asian countries than in Western countries.

In countries with different large ethnic groups, mortality rates can vary between them. For example, in the USA, the largest increases in mortality between 2004 and 2014 occurred in individuals of African-American descent (45%), whereas the increases were 22% in those of Asian descent and 20% in white individuals.
**Controversy around iCCA and pCCA**

Interpretation of rising iCCA and declining pCCA and/or dCCA rates is hampered by the fact that historical versions of the International Classification of Diseases (ICD) did not include a separate code for pCCA, and previous versions of ICD–Oncology (ICD–O) cross-referenced pCCA to iCCA. In addition, studies refer to Klatskin tumour, hilar CCA or perihilar CCA — different terms referring to the same fundamental entity. Some have suggested that this subgroup of CCA accounts for only 1–7% of all CCAs, but this is a much smaller proportion than seen in real-world clinical practice, where the perihilar area is the commonest site of CCA. Thus, the lack of a specific code for pCCA may have led to systematic errors, particularly with miscoding of pCCA as iCCA.

In a study in the UK published in 2021, retrospective review of 625 hepatobiliary cancers from three independent regional centres by two clinicians found that only 98 of 226 (43%) CCAs that were originally coded according to ICD-10 as iCCA (C22.1) were true iCCA, whereas 76 iCCAs (34%) were actually pCCA. Conversely, 92% of those that were truly pCCA were incorrectly coded as iCCA. Thus, the lack of a specific code for pCCA may have led to systematic errors, particularly with miscoding of pCCA as iCCA.

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**Risk factors for non-fluke-related CCA**

Differences in risk factors account, at least in part, for the geographical variations in the incidence of CCA. In Western countries, primary sclerosing cholangitis is the most well-known risk factor for CCA. Several risk factors are associated with all three subtypes, whereas others are subtype-specific. For example, Caroli disease and choledochal cysts (congenital disorders of the bile ducts) have a strong association with all three CCA subtypes. By contrast, cirrhosis, non-alcoholic fatty liver disease (NAFLD) and hepatitis B have a stronger association with iCCA, whereas choledocholithiasis (bile duct stones) has a stronger correlation with pCCA and/or dCCA. The global rise in obesity and NAFLD may be contributing to the rising rates of iCCA. Although multiple risk factors for CCA exist, most CCAs do not have an identifiable risk factor.

**Mechanisms/pathophysiology**

**Genetic and epigenetic aberrations**

Extensive genomic and epigenomic studies have shown that the molecular landscapes of CCAs differ considerably by aetiology, highlighting how cancer subtypes in the same organ may arise through different extrinsic and intrinsic carcinogenic processes. The findings also illustrate the importance of conducting these molecular studies in diverse populations, as differences between their genomic and epigenomic profiles point to the need for distinct biomarkers and therapies. Among CCAs, genetic aberrations differ depending on their anatomic...
locations; for example, FGFR2 fusions are almost exclusively found in iCCAs, whereas PRKCA–PRKCB fusions are observed in pCCAs and dCCAs\(^9\). For CCAs of other causes, such as those related to herbal carcinogen aristolochic acid, primary biliary cirrhosis and choleodochal cysts, current knowledge of their molecular landscape is sparse owing to insufficient numbers of samples studied.

**Common mutations and related molecular pathways.** According to studies of ~500 fluke-related and non-fluke-related CCAs, some of the most commonly mutated genes (mutation frequencies 10–26%) in CCA include TP53, ARID1A, KRAS, SMAD4, BAP1 and APC, followed by at least another 20 genes with lower mutation frequencies of 1–6\(^%\)\(^{5,6}\). Interestingly, the mutation frequencies of driver genes differ between CCA aetiologies. For example, TP53 and ARID1A mutations are highly enriched in fluke-related CCAs, whereas BAP1 and IDH1 and IDH2 mutations are highly enriched in non-fluke-related CCAs (TABLE 1). These gene mutations are known to be associated with key cancer-related molecular pathways, such as RAS–RAF–MAPK (for example, MAP2K4 and PTEN), WNT (for example, APC and RNF43), DNA repair (for example, BRCA2 and MSH3) and epigenetic modulation (for example, ARID1A and BAP1). Some of these mutated genes may be related to the same biological pathway and their mutations are usually mutually exclusive but may occasionally occur in combination. For example, members of the Wnt signalling pathway, including APC, RNF43, AXIN1 and different forms of catenin (CTNNAA2, CTNNBD2 and CTNNB1), were found to be mutated and, when the mutations occurred in combination, they contributed to a higher percentage of CCAs than mutation of a single gene\(^1\).

Epigenetically, CCA tumours exhibit DNA hypermethylation and distinct DNA hypermethylation patterns are found that differentiate fluke-related CCA (predominantly in CpG islands) and non-fluke-related CCA (predominantly in CpG island shores)\(^3\). Integrative analysis of somatic mutations and DNA methylation led to the proposal that fluke-related CCAs are probably caused by early exposure to external carcinogenic agents that induce a chronic inflammatory milieu, which results in genome-wide epigenetic dysregulation that drives tumour development; by contrast, in non-fluke-related CCAs, an initial genetic driver mutation causes tumorigenesis and epigenetic changes occur during this process\(^5,6\).

**Fluke-related cholangiocarcinoma.** Generally, this group of CCAs exhibit substantially more somatic mutations than non-fluke-related CCAs\(^5\), probably reflecting their underlying aetiology associated with fluke-related chronic inflammation. Inactivating mutations that are more prevalent in this subgroup than in non-fluke-related CCAs include TP53, ARID1A, ARID2, BRCAl and BRC2\(^\text{(REFS 5,6,8–10)}\). Non-coding mutations in promoters associated with H3K7me3 have also been found to be enriched\(^5\). Copy number analysis also detected more frequent ERBB2 amplification in fluke-related CCAs, which may have considerable clinical implications, as these tumours may be more sensitive to ERBB2 inhibitor treatment. In addition, the expression of several genes, including TET1, encoding a DNA demethylating enzyme, and EZH2, encoding a histone methyltransferase, has been found to be aberrant, implying that these genes may have a role in the hypermethylation phenotype in CCA\(^5\). Another study identified two fluke-related CCA subtypes: the C1 subtype that is enriched with mutations in genes, such as ECT2, that lead to mitotic checkpoint defects, and the C2 subtype that is related to bile acid metabolism, T cell infiltration and obesity\(^6\).

**Non-fluke-related cholangiocarcinoma.** Inactivating mutations in PBRM1, BAP1, PIK3CA and ELF3, and gain-of-function mutations in IDH1 and IDH2 are predominantly found in this group of CCAs\(^5,6,8–10\). Chromosome translocation involving mainly FGFR and to a lesser extent PKARC is another key genetic alteration\(^2,10\). FGFR2 translocations in CCAs were first discovered through a clinical sequencing programme for advanced CCA and are rarely found in fluke-associated CCA\(^6\). PRKACA and PRKACB are part of the cAMP-dependent protein kinase signalling pathway, and are enriched in non-fluke-related CCA\(^6\). Epigenetically, this group of CCAs is dominated by hypermethylation in promoter CpG shores\(^6\), with prevalent C>T and/or G>A substitutions at CpG sites\(^6\).

**Table 1 | Genomic and epigenomic aberrations of CCA**

| Alteration                  | Enriched in fluke-related CCA                                                                 | Enriched in non-fluke-related CCA  | Found in both CCA types |
|-----------------------------|-------------------------------------------------------------------------------------------------|---------------------------------|-------------------------|
| Mutations                   | ACVR1B, ARID1A, BRCAl, FBXW7, H3K27me3-associated promoter mutations, MAP2K4, MSH3, PTEN, SMAD4, TP53 | BAP1, IDH1, IDH2                | ACVR2A, APC, ARID2, ASXL1, BRAF, BRCAl, BRC2, CDKN1B, CTNNB1, ELF3, KRAS, NCO1, NRS, P4HTM, PBRM1, PIK3R1, RASA1, RIB1, RNF43, SF3B1, STK11, TGFB2 |
| Copy number alterations     | ERBB2 amplification                                                                           | NA                              | NA                      |
| Gene fusions                | NA                                                                                             | FGF2, FGF3, PRKACA, PRKACB       | NA                      |
| Epigenetic phenotype        | CpG island hypermethylation                                                                   | CpG shore hypermethylation       | Hypermethylation         |

CCA, cholangiocarcinoma; NA, not applicable.
Liver fluke infection is thought to drive CCA via multiple distinct but interacting pathways: mechanical damage to the bile duct epithelium caused by adult flukes grazing on the resident cells, notably cholangiocytes; immunopathology driven by chronic infection–related inflammation; and effects of parasite excretory–secretory molecules, including secreted vesicles, proteins and small molecules\(^6\). The interplay of these mechanisms, in addition to a traditional diet in disease-endemic areas that is rich in nitrosamine-containing foods, such as fermented fish contaminated with liver fluke metacercariae, is in keeping with current knowledge of carcinogenesis (FIG. 2).

The attachment of liver flukes to the biliary wall results in ulceration and formation of precancerous lesions\(^6\). This process is accompanied by immune cell infiltration and persistent secretion of inflammatory cytokines, such as IL-6, which is well known as a link between inflammation and carcinogenesis, particularly in liver tissue\(^6\). Elevated IL-6 levels are strongly associated with advanced and persistent periductal fibrosis in \textit{O. viverrini} infections, which is thought to contribute to the pathogenesis of fluke-induced CCA\(^7\). Similar to other helminth infections, flukes induce local recruitment of type 2 macrophages, eosinophils, mast cells and T cells. Parasite-specific B lymphocyte and T lymphocyte responses occur to a diverse array of antigens\(^7\); however, despite this robust response, sterile immunity does not develop, and older people living in endemic areas are often heavily infected\(^7\), necessitating a vaccine that induces long-term anti-parasite immunity and protects against the onset of fluke-induced CCA \textquotesingle BOX 1\textquotesingle.

The hamster model of liver fluke-induced CCA is a powerful tool for investigating the aetiology and immunopathogenesis of fluke-infection-associated liver pathologies. Hamsters infected with \textit{O. viverrini} fed a diet high in nitrosamines develop CCA within 6 months\(^8\). Soon after flukes arrive in the biliary tree, proliferating cholangiocytes can be detected, highlighting a process of constant wounding and repair that occurs over decades in infected people. \textit{Opisthorchis} spp. secrete several molecular entities that contribute to this process, including a glutathione-dependent prostaglandin synthase that drives formation of precancerous lesions\(^9\), a granulin-like growth factor\(^8\) and extracellular vesicles (EVs)\(^7\), the latter two of which drive cholangiocyte proliferation and IL-6 secretion in vitro. Indeed, \textit{O. viverrini} flukes that had undergone CRISPR–Cas9-induced editing of the granulin gene still protected against the onset of fluke-induced CCA \textquotesingle BOX 1\textquotesingle.

Molecular biology of progression and invasion
CCA cells gradually adopt invasive phenotypes to metastasize, for example by changing to a mesenchymal-like phenotype, which increases their migratory and invasion capabilities, and eventually deposit at distant sites. Various alterations related to cancer hallmarks occur to gain these invasive properties, including those during the invasion process\(^7\), and in angiogenesis and lymphangiogenesis\(^9\).

### Fluke pathophysiology
Chronic liver fluke infection is associated with numerous hepatobiliary diseases, including inflammation of the gallbladder and bile ducts (cholecystitis and cholangitis, respectively), periductal fibrosis and, ultimately, CCA.

**Fig. 2 | Pathophysiology of liver-fluke infection and CCA.**

- **a** | Resected liver from a patient with cholangiocarcinoma (CCA) showing numerous adult \textit{Opisthorchis viverrini} flukes (arrows) in the bile ducts (BD). A CCA tumour mass can be seen in the upper left part of the image.
- **b** | Transverse section of an \textit{O. viverrini} fluke (Ov) attached via its oral sucker (S) to the bile duct epithelium (E) of an experimentally infected hamster. Cellular infiltrate in response to the feeding fluke is evident.
- **c** | Proliferating cholangiocytes in the biliary epithelium (BE) producing 8-oxo-deoxyguanosine (arrows, brown stain) as a marker of DNA damage in the bile duct of a hamster infected with an \textit{O. viverrini} fluke (Ov).
- **d** | Uptake of \textit{O. viverrini}-secreted extracellular vesicles (green dots) by human H69 primary cholangiocytes in culture.
- **e** | Thickened and disordered biliary epithelium (BE) in a hamster infected with \textit{O. viverrini} in which \textit{Ov}-\textit{grn-1}, which encodes a secreted granulin-like growth factor, has been edited using CRISPR–Cas9 (right panel); editing of \textit{Ov}-\textit{grn-1} has resulted in substantial reduction of biliary hyperplasia as highlighted by the square brackets. L, liver.
- **f** | Resected liver from a patient with cholangiocarcinoma (CCA) showing numerous adult \textit{Opisthorchis viverrini} flukes (arrows) in the bile ducts (BD). A CCA tumour mass can be seen in the upper left part of the image.
- **g** | Transverse section of an \textit{O. viverrini} fluke (Ov) attached via its oral sucker (S) to the bile duct epithelium (E) of an experimentally infected hamster. Cellular infiltrate in response to the feeding fluke is evident.
- **h** | Proliferating cholangiocytes in the biliary epithelium (BE) producing 8-oxo-deoxyguanosine (arrows, brown stain) as a marker of DNA damage in the bile duct of a hamster infected with an \textit{O. viverrini} fluke (Ov).
- **i** | Uptake of \textit{O. viverrini}-secreted extracellular vesicles (green dots) by human H69 primary cholangiocytes in culture.
- **j** | Thickened and disordered biliary epithelium (BE) in a hamster infected with \textit{O. viverrini} in which \textit{Ov}-\textit{grn-1}, which encodes a secreted granulin-like growth factor, has been edited using CRISPR–Cas9 (right panel); editing of \textit{Ov}-\textit{grn-1} has resulted in substantial reduction of biliary hyperplasia as highlighted by the square brackets. L, liver.

Of note, the distribution of mutations between non-fluke subtypes seems to vary according to the anatomic site of the CCA; for example, \textit{FGFR2} translocations are exclusively found in iCCAs\(^{50,56,63}\).

**Fig. 2 | Pathophysiology of liver-fluke infection and CCA.**

**a** | Resected liver from a patient with cholangiocarcinoma (CCA) showing numerous adult \textit{Opisthorchis viverrini} flukes (arrows) in the bile ducts (BD). A CCA tumour mass can be seen in the upper left part of the image. **b** | Transverse section of an \textit{O. viverrini} fluke (Ov) attached via its oral sucker (S) to the bile duct epithelium (E) of an experimentally infected hamster. Cellular infiltrate in response to the feeding fluke is evident. **c** | Proliferating cholangiocytes in the biliary epithelium (BE) producing 8-oxo-deoxyguanosine (arrows, brown stain) as a marker of DNA damage in the bile duct of a hamster infected with an \textit{O. viverrini} fluke (Ov). **d** | Uptake of \textit{O. viverrini}-secreted extracellular vesicles (green dots) by human H69 primary cholangiocytes in culture. **e** | Thickened and disordered biliary epithelium (BE) in a hamster infected with wild-type \textit{O. viverrini} flukes (Ov; left panel) compared with the BE in a hamster infected with \textit{O. viverrini} in which \textit{Ov}-\textit{grn-1}, which encodes a secreted granulin-like growth factor, has been edited using CRISPR–Cas9 (right panel); editing of \textit{Ov}-\textit{grn-1} has resulted in substantial reduction of biliary hyperplasia as highlighted by the square brackets. L, liver. **Part a** courtesy of B. Sripa, Khon Kaen University. **Part b** reprinted with permission from REF.\(^{20}\), Royal Society of Chemistry. **Part d** courtesy of S. Chaiyadet, Khon Kaen University. **Part e** reprinted from REF.\(^{28}\), CC BY 4.0.
An integrated and in-depth understanding of the molecular mechanisms in CCA progression could aid in developing precision therapy for advanced CCA. Several pathways are dysregulated and represent potential therapeutic targets, including the inflammation-related IL-6–JAK–STAT3 pathway, oestrogen and oestrogen receptors, epithelial–mesenchymal transition, EGFR activating the MAPK–ERK pathway, hepatocyte growth factor–AKT–ERK signalling and others. Further transcription factors involving morphogenetic signalling pathways, for example, Hedgehog, Wnt, and Notch, as well as microRNAs are dysregulated to support the invasiveness of CCA. The PI3K–AKT–mTOR, HIF1α and MYC pathways are stimulated to support metabolic shifts in CCA cells.

Post-translational modifications, O-GlcNAcylation and glycosylation have also been shown to mediate CCA invasiveness (Fig. 5). O-GlcNAcylation is a reversible process, in which a single GlcNAc residue is added to proteins, modulating protein function, stability and localization with or without coordinating phosphorylation. In human CCA tissues, high levels of O-GlcNAcylation have been observed and are associated with a poor prognosis. Upregulation of O-GlcNAcylation enhances the stability of the structural protein vimentin and increases the nuclear translocation of proteins that activate expression of downstream genes involved in epithelial–mesenchymal transition, cell migration and invasion. Epidemiological studies have indicated that diabetes mellitus is a risk factor and possibly a promoting factor for CCA. Increased levels of O-GlcNAcylation and STAT3 activation have been reported to be key processes in the aggressiveness of CCA cells enhanced by high glucose levels. Increased initial O-GalNAcylation and terminal fucosylation have been implicated in CCA development in the hamster model and in tissues from inpatients with CCA. Modulation of either process substantially affects the metastatic potential of CCA cells. Upregulation of specific high-mannose N-glycans facilitates the progression of highly metastatic CCA cells, some of which can be detected in the serum from patients with CCA. The collective evidence suggests that certain glycans and/or enzymes involved in glycan synthesis might serve as new biomarkers and targets to manage CCA metastasis.

Pathology, inflammation and tumour microenvironment

CCA has three predominant macroscopic growth patterns: mass-forming lesions; periductal infiltrating lesions; and intraductal papillary lesions. The histopathology may also be classified as small bile duct type (which may derive from septal and interlobular bile ducts, progenitor cells, and possibly hepatocytes), large bile duct type (potentially arising from segmental bile ducts or associated peribiliary glands), and rare variants. For iCCA, patients with mass-forming and periductal infiltrating subtypes have the poorest prognosis, whereas those with intraductal papillary lesions have the most favourable outcomes following curative surgical resection.

The mass-forming growth pattern is most common in iCCA and is generally seen at presentation as a single, nodular solid mass. Advanced mass-forming iCCA may also have satellite or multifocal tumour growth within the liver. The periductal infiltrating growth pattern of CCA does not form a nodular mass but grows longitudinally along the walls of the large bile ducts and spreads along the portal tracts, resulting in strictures of the affected bile ducts and dilation of the smaller proximal bile ducts. This is the growth pattern most frequently observed in pCCA. The intraductal papillary type of CCA is seen at presentation as a slow-growing polyoid or papillary tumour growing within the lumen of a dilated bile duct.

Histologically, 90–95% of CCAs are adenocarcinomas, which may be well, moderately or poorly differentiated. They can be small bile duct or large bile duct type lesions. The small bile duct types usually show no or minimal mucin production, whereas the large bile duct types are mucin-producing adenocarcinomas. pCCA, dCCA and large bile duct iCCA share similar pathological and molecular features. Whether precursor lesions for mass-forming iCCA exist is unknown, but the presence of premalignant dysplastic precursor lesions for pCCA, dCCA and large bile duct type iCCA has been established.

Unique rare variants of CCA include intestinal-type CCA, combined hepatocellular carcinoma and CCA (cHCC–CCA), and lymphoepithelioma-like CCA. Cholangiolocellular carcinoma (CLC) is a rare primary liver cancer in which the epithelial component resembles cholangiocytes (canals of Hering). It is recommended that CLC is categorized as a histological subtype of well-differentiated iCCA.

Unlike conventional HCCs, CCAs frequently have a prominent desmoplastic microenvironment
characterized by a dense collagen-fibre-enriched tumour stroma and matricellular proteins (for example, periostin and tenascin C), an abundance of cancer-associated fibroblasts (Fig. 4) and, to a lesser extent, tumour-associated macrophages and varying numbers of innate immune cells\(^{107}\). CCAs are also dissimilar to HCC by often being hypovascular\(^{108}\), although CCAs formed in cirrhosis can display increased vascularity\(^{109}\).

This evolving and complex desmoplastic tumour microenvironment has a pre-eminent role in promoting CCA progression, therapeutic resistance and immnosuppression\(^{14,110–112}\). Cancer-associated myofibroblasts are a major source of secreted stromal components, including multiple growth factors, cytokines, metabolites, extracellular matrix (ECM) proteins and modifying enzymes that facilitate CCA growth and invasiveness, cell survival, ECM remodelling, and metabolic reprogramming. Indeed, in preclinical models, deletion of cancer-associated fibroblasts limits tumour progression\(^{113}\). Stromal matrix stiffness in CCA can trigger signalling pathways regulating malignant behaviour and mechanically collapse blood microvessels, causing hypoxia and limiting drug and immune cell bioavailability\(^{114}\).

**Diagnosis, screening and prevention**

**Diagnosis**

Diagnosis and management of CCA vary by anatomic subtype of disease (TABLE 2).

**iCCA.** iCCA is often seen at presentation as an intrahepatic mass and is incidentally found in 25–30% of patients\(^{115}\). Patients with iCCA are often asymptomatic during early disease stages and develop symptoms or signs, such as abdominal pain or less commonly jaundice, during disease progression to an advanced stage. Carbohydrate antigen 19-9 (CA19-9) is the primary serum biomarker used in CCA diagnosis, although it has subpar specificity and can be elevated in various conditions, such as biliary obstruction or pancreatic cancer. Nonetheless, a levels of CA19-9 >1,000 U/ml are concerning for the presence of metastatic CCA\(^{116}\). Imaging modalities used for iCCA diagnosis include conventional ultrasonography,
periostin (POSTN), produced by associated fibroblasts (CAFs) accumulated within the desmoplastic stroma of CCA are differentiation, whereas cholangiocarcinoma cells (CC) do not show fibres (predominantly comprising collagen type I).

Histological features of the desmoplastic microenvironment in human iCCA.

**a** Masson trichrome staining of a moderately to poorly differentiated small duct type mass-forming intrahepatic cholangiocarcinoma (iCCA) largely comprising prominent desmoplastic stroma strongly stained for collagen (blue staining). Arrows point to representative small clusters of cholangiocarcinoma cells (CC) do not show SMA staining.

**b** | Picrosirius red (PR) staining for collagen (orange staining under polarized light)
| Masson trichrome staining of a moderately to poorly differentiated small duct type iCCA with arterial contrast enhancement. Arrows point to representative small clusters of cholangiocarcinoma cells (CC) do not show SMA staining.

**c** | Immunostaining for matricellular peristin (POSTN), produced by aSMA+ CAFs, which has a binding site for collagen, is exclusively localized to the desmoplastic stroma of CCA. It is now generally believed that aSMA+ CAFs in iCCA are principally derived from activated portal fibroblasts and hepatic stellate cells and are phenotypically and functionally heterogeneous. High expression levels of SMA and POSTN, both of which are induced by transforming growth factor-β, have been associated with poor iCCA prognosis following surgical resection.

**d** \[PR POSTN\]作画

CT, MRI and contrast-enhanced ultrasonography (CEUS). MRI may provide enhanced assessment of the primary mass, whereas CT imaging has superior detection of vascular enhancement and is, therefore, important in determining resectability. In patients with cirrhosis, HCC resection may facilitate earlier iCCA diagnosis. However, distinguishing HCC and iCCA can be difficult in this patient population. iCCAs are characterized by an initial arterial contrast enhancement at the tumour periphery and progressive homogeneous contrast enhancement. HCCs are characterized by arterial hyper-enhancement and washout in the portal venous phase or delayed phase. In a cirrhotic liver, gadolinium-enhanced MRI has an increased specificity, but lower sensitivity, for diagnosing HCC and distinguishing it from iCCA when portal venous phase washout rather than conventional delayed phase washout is used. Compared with CT or MRI, CEUS is more likely to misdiagnose iCCA as HCC. In CEUS imaging, iCCAs have an earlier contrast washout from the vascularized portions of the lesions, whereas HCCs have delayed portal venous washout. Hence, CEUS is not reliable as the sole imaging technique to differentiate iCCA from HCC but may be useful in scenarios with inconclusive CT or MRI. PET scanning is typically not used in the diagnosis of iCCA owing to limited accuracy. 18F-Fluorodeoxyglucose (18F-FDG) PET imaging has reasonable performance in detection of lymph node and distant metastasis and, therefore, may have a role in CCA staging. Histopathological analysis of a biopsy specimen remains the mainstay for confirmation of an iCCA diagnosis. If a patient is eligible for resection, then a biopsy need not be performed.

**pCCA and dCCA.** Patients with pCCA and dCCA typically present with painless jaundice owing to underlying biliary obstruction. Following initial CT that may be concerning for pCCA and/or dCCA, a specific type of MRI termed magnetic resonance cholangiopancreato- (MRCP) is employed for CCA detection. The sensitivity and specificity of MRCP to distinguish benign and malignant causes of hilar obstruction are 87% and 85%, respectively. MRCP can also delineate the biliary anatomy before endoscopic intervention with endoscopic retrograde cholangiography (ERC). ERC has a diagnostic and therapeutic role in pCCA and dCCA as it enables detection of malignant strictures and acquisition of biliary brushings for cytology and fluorescence in situ hybridization (FISH) analysis. Biliary cytology or a biopsy sample positive for adenocarcinoma are diagnostic of CCA. However, the sensitivity of biliary cytology for pCCA detection is low (43% according to one meta-analysis). FISH analysis has a higher sensitivity than cytology (65% versus 19% in one series) and similar specificity for detection of CCA. Endoscopic techniques such as cholangioscopy and confocal laser endomicroscopy can be used to visualize indeterminate biliary strictures. However, whether these advanced techniques can improve tissue diagnostic yield through targeted biliary biopsies remains unclear.

Endoscopic ultrasonography (EUS) is useful in the diagnosis and staging of pCCA, as it enables a detailed visual assessment of the extrahepatic bile duct as well as tissue acquisition via fine-needle aspiration (FNA). EUS–FNA has a higher sensitivity for detection of dCCA than detection of pCCA (81% versus 59%). In addition, EUS-guided tissue acquisition of pCCA is controversial owing to the potential risk of tumour dissemination. PET scanning is typically not utilized in the diagnosis of pCCA owing to limited accuracy. 18F-FDG PET has subpar performance for detection of the primary tumour but may have a role in the assessment of lymph node and distant metastasis.

**Prevention and screening of fluke-related CCA.**

Populations at high risk of clonorchiasis and opisthorchiasis include those who prefer meals that include cooked, fermented or dried freshwater fish in endemic areas. Current or past infection with liver fluke can be diagnosed through faecal, blood and/or urine examinations for the presence of the eggs, fluke antigens, antibodies and/or nucleic acids. Infection is treated and
cured with the anthelmintic drug praziquantel taken by mouth\(^5\). For prevention, health education is necessary and increasingly employed and supported by public health authorities; for example, the Lawa model used in northeast Thailand aims to change food consumption behaviours away from dishes that include raw fish, which are important elements of local and traditional culture\(^5\). Health education for schoolchildren and the wider community in endemic regions addresses the biology and modes of infection, highlights freshwater fish as the infection source, safe handling of food, safe cooking procedures, and improved personal and community sanitation to avoid the entry of unprocessed sewage into the local freshwater environment. Preventive measures also address screening, diagnosis and treatment, in particular with praziquantel, at both the individual and population level\(^5\).

Given the high prevalence of liver fluke infection in diverse geographical regions\(^5\), large-scale screening has been undertaken, involving stool examination for fluke ova coupled with abdominal ultrasonography or other radiological imaging\(^5\). Serological assays are used to supplement stool examination as a screening tool in the clinic for the diagnosis of opisthorchiasis and clonorchiasis, and may be useful in screening populations at risk of CCA associated with liver fluke infection\(^5\). Imaging can reveal periductal, fibrosis-induced, chronic inflammation, which can progress to CCA\(^5\). Although praziquantel adequately treats the infection, the periductal fibrosis rarely resolves following parasite clearance\(^5\). Regarding population-level public health screening, the CCA screening and care programme at Khon Kaen University, Thailand, is noteworthy in its population reach and impact, reporting the screening of $\geq 200,000$ at-risk residents, with radiological diagnosis indicating a $\sim 1\%$ incidence of ICCA. The programme incorporates teleconsultation ultrasonography in its follow-up protocols\(^5\).

### Surveillance in primary sclerosing cholangitis

PSC is a premalignant biliary tract disease that confers a considerable risk of CCA development, usually pCCA. The incidence of hepatobiliary pancreatic malignancy, of which most are CCA, is $1.43$ cases per $100$ patient years, and the cumulative incidence at $20$ years is $20–25\%$\(^5\). Although the incidence of CCA is high in patients with PSC, surveillance strategies in asymptomatic patients are not universally endorsed because of several diagnostic and therapeutic concerns\(^5\). First, the diagnosis of CCA in PSC is challenging because inflammation-related dominant biliary tract strictures mimic CCA on radiography. Second, conventional cytology and advanced cytological techniques, such as FISH for polysomy, lack sensitivity and require invasive endoscopic procedures\(^5\). Finally, curative treatment options rely on the availability of liver transplantation and neoadjuvant chemoradiation therapy\(^5\). Despite these caveats, retrospective data from a multicentre review suggest that surveillance of asymptomatic patients with PSC and annual MRCP and CA19–9 assessment is life-saving\(^5\). In patients with PSC and asymptomatic CCA, MRI detection was associated with a reduction in mortality compared with ultrasonography (HR $0.10$, $95\%$ CI $0.01–0.96$). Whether these data can be confirmed by others remains to be seen.

### Pathogenesis and management of CCA subtypes

| CCA subtype | Risk factors | Genetic aberrations | Clinical presentation | Diagnosis | Surgical treatment options | Systemic treatment options |
|-------------|-------------|---------------------|----------------------|-----------|---------------------------|---------------------------|
| iCCA        | Cirrhosis, viral hepatitis, liver fluke, alcohol consumption, non-alcoholic fatty liver disease, type 2 diabetes mellitus, cigarette smoking, obesity | ARID1A, BAP1, EPHX2, FGFR2, IDH1, IDH2, KRAS, MCL1, PTEN, PTPN3, TP53 | Abdominal pain, asymptomatic (incidental $25–30\%$ of cases), malaise, weight loss | MRI abdomen, CT abdomen, ultrasonography, contrast-enhanced ultrasonography, percutaneous biopsy | Surgical resection (hepatectomy with regional lymphadenectomy), liver transplantation (tumour size $\leq 3\, \text{cm}$) | Chemotherapy: gemcitabine plus cisplatin |
|             |             |                     |                      |           |                           | Targeted therapies: FGFR inhibitors (pemigatinib FDA-approved), IDH inhibitors |
|             |             |                     |                      |           |                           | Immunotherapy: immune checkpoint blockade |
| pCCA        | Caroli disease, primary sclerosing cholangitis, cholelithiasis, liver fluke, cigarette smoking, obesity | ARID1B, BAP1, ERBB2, PRKACA, PRKACB | Painless jaundice, abdominal pain, malaise, weight loss, pruritus | MRI and MRCP, ERC with biliary brushings (cytology and FISH), CT abdomen | Surgical resection (hepatectomy with en-bloc resection of the extrahepatic bile duct and regional lymphadenectomy), neoadjuvant chemoradiation followed by liver transplantation | Possibly gemcitabine plus cisplatin* |
| dCCA        | Primary sclerosing cholangitis, chronic pancreatitis, cholelithiasis, liver fluke, cigarette smoking, obesity | ERBB2, ERBB3, ELF3, PRKACA, PRKACB | Painless jaundice, abdominal pain, malaise, weight loss, pruritus | MRI and MRCP, CT abdomen, ERC with biliary brushings (cytology and FISH), endoscopic ultrasonography | Pancreatocoduodenectomy | Possibly gemcitabine plus cisplatin* |

CCA, cholangiocarcinoma; dCCA, distal CCA; ERC, endoscopic retrograde cholangiography; FGFR, fibroblast growth factor receptor; FISH, fluorescence in situ hybridization; iCCA, intrahepatic CCA; IDH, isocitrate dehydrogenase; MRCP, magnetic resonance cholangiopancreatography; pCCA, perihilar CCA. *A subgroup analysis of the ABC-02 trial suggested a lack of benefit in pCCA and dCCA\(^5\).
In the meantime, societal guidelines remain ambiguous regarding surveillance for CCA in patients with PSC. Management Like diagnosis, the management of CCA varies by anatomic subtype of disease (TABLE 2).

**iCCA**

Surgical resection. Surgery with intent to attain a margin-negative (R0) resection is a potentially curative treatment option for iCCA. However, most patients with iCCA present with large, unresectable tumours. Staging laparoscopy is recommended particularly in patients with high-risk features, such as multicentric disease, high CA19-9 levels, suspicion of vascular invasion or peritoneal disease. Diagnostic laparoscopy can detect occult metastatic disease not apparent on preoperative cross-sectional staging imaging. In patients undergoing surgical resection, a positive resection margin (R1) is associated with an increased risk of recurrence and shortened overall survival. Multifocal disease is a prognostic factor that is associated with poor long-term outcomes. Another important prognostic factor in patients undergoing surgical resection is lymph node status. Accordingly, porta hepatis lymphadenectomy is performed routinely during surgical resection of iCCA. Patients with lymph node metastasis have worse diseasespecific survival. Moreover, the median survival in patients with lymph node metastasis undergoing surgical resection is similar to that in patients who have been treated with chemotherapy alone, indicating that resection in these patients does not offer a survival benefit.

Liver transplantation. Emerging data suggest that liver transplantation may be an option in patients with iCCA with small tumours. In a multicentre retrospective study, the 5-year actuarial survival in patients with cirrhosis and small (<2 cm), incidental iCCA tumours was 65% following liver transplantation. These patients probably underwent liver transplantation for presumed HCC; it is uncommon to diagnose small iCCA tumours unless a patient with liver cirrhosis is undergoing surveillance for HCC. In a follow-up study in a larger, multinational cohort of patients with cirrhosis and incidental iCCA, the 1-year, 3-year and 5-year actuarial survival in patients with very early iCCA (≤2 cm) was 93%, 84% and 65%, respectively. Factors associated with tumour recurrence were presence of microvascular invasion and poor tumour differentiation. Liver transplantation in patients with large iCCA tumours is associated with a high risk of recurrence.

Locoregional therapy. Locoregional therapy is an option in patients with locally advanced iCCA who are not eligible for surgical options. A multicentre retrospective analysis assessed the efficacy of intra-arterial therapy (conventional transarterial chemoembolization, drug-eluting beads or transarterial bland embolization) in 198 patients with locally advanced iCCA (median tumour size 8.1 cm; 47.5% of patients with a solitary lesion). The median overall survival was 13.2 months and the type of intra-arterial therapy did not affect patient responses. A phase II clinical trial of hepatic arterial infusion of fluorouracil in combination with systemic gemcitabine and oxaliplatin in 38 patients with locally advanced iCCA is ongoing.

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**Fig. 5** | Screening for liver fluke infection and associated hepatobiliary disease including CCA. a | Egg of *Opisthorchis viverrini* in human stool (egg dimensions 19–30 µm long and 10–20 µm wide). b | Suspected cholangiocarcinoma (CCA) imaged using abdominal sonography during community screening in liver fluke endemic regions of northeast Thailand; the ultrasonographic image shows a mass (green circle), calcification (thin arrow) and dilated intrahepatic duct (large arrow). c | Immunochromatographic device for the serodiagnosis of opisthorchiasis and clonorchiasis. Part a adapted from REF.211, CC BY 4.0. Part b reprinted with permission from REF.142, Elsevier. Part c reprinted with permission from REF.135, The American Journal of Tropical Medicine and Hygiene.
advanced iCCA demonstrated promising efficacy\(^{165}\). The median overall survival was 25.0 months and 1-year overall survival was 89.5%. Advanced external beam radiation therapy (EBRT) techniques, such as charged-particle (proton or carbon) beam techniques, can be used to deliver high-dose EBRT to patients while sparing adjacent non-malignant tissues. A single-arm, phase II, multicentre study evaluated the efficacy and safety of high-dose, hypofractionated proton beam therapy in 92 patients with HCC or iCCA (37 with iCCA only)\(^{166}\). Proton beam therapy achieved a local disease control rate of 94.1% at 2 years.

**pCCA and dCCA**

**Surgical resection.** Surgery is an option in patients who present with early-stage pCCA or dCCA. Staging laparoscopy before planned resection for pCCA can detect radiologically occult metastatic disease. In one series including 116 patients, staging laparoscopy had an all-cause yield of 27.2% for the detection of unresectable disease\(^{67}\). Although the surgical approach has become more aggressive over time with more patients with locally advanced pCCA undergoing resection, long-term survival following resection has also increased\(^{164,169}\). The incidence of major hepatectomy, bile duct resection, and vascular resection and/or reconstruction has increased over time\(^{76,175}\), whereas operative complications have decreased. In a series of 574 patients with pCCA undergoing surgical resection, the 5-year disease-specific survival was 32.5%\(^{165}\). Over the period 2001–2010, 243 patients with R0 disease and no lymph node metastasis after resection had a 5-year survival of 67.1%. The tumours of patients with locally advanced pCCA with bilateral involvement of the second-order bile ducts have traditionally been considered unresectable. However, advances in surgical techniques have enabled resection of locally advanced tumours, and patients with bilateral involvement of the second-order bile ducts who undergo resection have an improved overall survival compared with those who do not undergo resection (5-year survival 32.8% versus 1.5% in one series)\(^{172}\). Unilateral and main hepatic artery involvement are independent poor prognostic factors, as patients with these features do not have a survival benefit following resection\(^{173}\). Lymph node metastasis is associated with poor prognosis in patients undergoing resection for pCCA\(^{169}\).

Surgical resection of dCCA typically involves a pancreaticoduodenectomy or Whipple procedure with removal of the gallbladder and bile duct, the head of the pancreas, and the first part of the duodenum. The 5-year survival of patients with dCCA following surgical resection ranges from 20% to 40% depending on disease extent\(^{167,174}\). Predictors of survival following resection of dCCA include resection margin status, lymph node status, tumour size and degree of tumour differentiation\(^6\).

**Liver transplantation.** Liver transplantation following neoadjuvant chemoradiation therapy is an option in patients with early-stage, unresectable pCCA or dCCA in the setting of PSC regardless of resectability\(^{170,175,176}\). The treatment protocol includes EBRT with concomitant 5-fluorouracil followed by bile duct brachytherapy, and subsequent capecitabine maintenance treatment until the time of transplantation. Operative staging is performed before transplantation. Data from 12 large-volume centres in the USA showed that patients with early-stage, unresectable pCCA who underwent neoadjuvant chemoradiation followed by liver transplantation had a 5-year post-transplant recurrence-free survival of 65%, and an intent-to-treat survival of 53%\(^{166}\). Predictors of a patient dropping out before liver transplantation, for example owing to disease progression include CA19-9 levels of ≥500 U/ml, malignant brushing or biopsy, and a model for end-stage liver disease (a measure of liver disease severity) score of ≥20 (REF.\(^{177}\)). Predictors of recurrence following liver transplantation include high pretransplant CA19-9 levels of ≥500 U/ml, portal vein encasement and residual tumour on explant.

**Systemic therapy for CCA**

**Cytotoxic therapy.** In patients with advanced-stage CCA who are ineligible for surgical or locoregional options, chemotherapy with first-line cisplatin and gemcitabine is an option. The ABC-02 study demonstrated a median overall survival of 11.7 months for gemcitabine in combination with cisplatin compared with 8.1 months for gemcitabine alone\(^{178}\). In patients whose disease has progressed on gemcitabine and cisplatin, the ABC-06 study demonstrated benefit of folinic acid, 5-fluorouracil and oxaliplatin (FOLFOX) in the second-line setting\(^{179}\). FOLFOX has become the standard second-line therapy for advanced CCA. A phase III randomized trial of gemcitabine plus S-1 (also known as Teysonu, an oral combination of the 5-fluorouracil prodrug tegafur with gimeracil and oteracil to increase efficacy and reduce adverse effects) demonstrated non-inferiority of this combination to gemcitabine and cisplatin\(^{180}\). Preliminary results from an open-label, randomized phase II–III trial show that the combination of 5-fluorouracil, oxaliplatin and irinotecan (FOLFIRINOX) does not result in improved progression-free survival compared with gemcitabine and cisplatin\(^{181}\). Chemotherapy combinations currently under investigation as first-line therapy for CCA include gemcitabine plus cisplatin combined with albumin-bound paclitaxel\(^{182}\).

In patients with advanced disease, well-designed, stratified studies examining specifically systemic therapy, targeted therapy or immunotherapy in those with iCCA, pCCA or dCCA are lacking. In those studies with post hoc analyses, the numbers of patients with dCCA have been insufficient to draw conclusions. In the original trial that investigated cisplatin plus gemcitabine the confidence intervals for pCCA crossed 1, suggesting a lack of benefit\(^{178}\). Indeed, post hoc analysis of the ABC-01, ABC-02 and ABC-03 studies demonstrated that patients with iCCA had a longer overall survival than those with non-iCCA biliary cancers (HR 0.58, 95% CI 0.35–0.95; P = 0.03)\(^{14}\).

**Targeted therapy.** Each CCA subtype has a distinct genetic landscape. iCCAs are characterized by mutations of IDH1 and IDH2 (~15% of iCCAs), which encode isocitrate dehydrogenase, genetic alterations in fibroblast growth factor receptors, BAP1 mutations...
and others. Early results of treatment with inhibitors of IDH or fibroblast growth factor receptor (FGFR) in human CCA were promising. In a phase III multicentre, double-blind, randomized controlled trial with a crossover design in 185 patients with chemotherapy-refractory iCCA with IDH1 mutations, there was a statistically significant improvement in progression-free survival in patients receiving ivosidenib, an inhibitor of mutant IDH1, compared with those receiving placebo (2.7 months versus 1.4 months; HR 0.37, 95% CI 0.25–0.54). Several FGFR inhibitors have been investigated in human CCA. In a phase II trial, ~35% of patients with CCA and FGFR2 gene fusions or rearrangements had an objective response to pemigatinib, a potent selective inhibitor of FGFR1–3. Pemigatinib subsequently received accelerated FDA approval for the treatment of adults with previously treated, unresectable, locally advanced or metastatic CCA with FGFR2 gene fusions or rearrangements. Of note, pemigatinib treatment may not be suitable for fluke-related CCA, owing to the low incidence of FGFR2 fusions found in these tumours. BRAF mutations occur in 3–5% of CCAs, primarily in iCCAs. A phase II, open-label, single-arm basket trial of dabrafenib, a BRAF inhibitor, and trametinib, a MEK inhibitor, demonstrated promising efficacy of this combination in patients with CCA with BRAFV600E mutations.

Immunotherapy. Emerging clinical data of immune-directed therapies, such as immune checkpoint blockade, suggest modest efficacy in CCA. In KEYNOTE-158, a phase II trial in patients with advanced biliary tract cancer, the objective response rate with pembrolizumab, a PD1 inhibitor, was a subpar 5.8%. Although the data are limited in CCA, pembrolizumab has enhanced activity against mismatch repair-deficient tumours. Two subsequent trials of immune checkpoint blockade in patients with CCA have demonstrated improved efficacy. In a phase II trial in 54 patients with previously treated biliary tract cancer, treatment with the PD1 inhibitor nivolumab resulted in an objective response rate of 22%. In the intention-to-treat population, median overall survival was 14 months. Phase II trial data for the combination of nivolumab and ipilimumab, a cytotoxic T lymphocyte associated protein 4 (CTLA4) inhibitor, demonstrated an overall survival of 5.7 months and progression-free survival of 2.9 months; notably, the responses were exclusively observed in patients with iCCA or gallbladder CCA. Characterization of the immune landscape of each CCA subtype will be essential in the effort to develop effective immune-directed therapies. A phase III clinical trial of pembrolizumab in combination with gemcitabine and cisplatin (KEYNOTE-966) is currently ongoing.

Adjuvant therapy. Overall survival following surgical resection for CCA remains suboptimal with a high risk of recurrent disease even with R0 resection, prompting interest in prospective trials of adjuvant therapies. The PRODIGE trial of adjuvant gemcitabine and oxaliplatin in all biliary tract cancers did not demonstrate a clear benefit of adjuvant therapy. In addition, adjuvant gemcitabine following resection of pCCA or dCCA did not demonstrate a benefit in the BCAT trial. The BILCAP study, a phase III randomized controlled trial of capecitabine versus observation following surgical resection, included 447 patients with biliary tract cancer of whom 43 patients had iCCA and 65 patients had pCCA. Although a protocol-specified sensitivity analysis adjusted for nodal status, disease grade and patient sex showed a significant difference in overall survival with capecitabine (HR 0.71, 95% CI 0.55–0.92), the unadjusted intention-to-treat analysis did not demonstrate a significant benefit in overall survival (HR 0.81, 95% CI 0.63–1.04). On the basis of these data, practice guidelines recommend capecitabine for 6 months following surgical resection of CCA.

Quality of life

Robust studies and data on the quality of life of patients with CCA are sparse. This paucity of information is due to the low incidence of the disease, its high lethality, the limited number of patients enrolling in clinical trials until very recently, and the lack of dedicated quality-of-life measurement tools. Quality of life following liver resection surgery or a pancreaticoduodenectomy has been well documented. Also, quality of life is highly dependent on the systemic therapy regimen employed. In the absence of robust data, the voice of the patient is a powerful narrative that can help us reflect on and guide our research mission. The patient's experience is unique in its highly successful outcome given the high lethality of advanced CCA, but the medical odyssey described, including the decision to undergo invasive procedures and engage in clinical trials, is a shared experience common to many patients with CCA.

Perspective of a patient and advocate

As an 11-year survivor of advanced stage iCCA, I have experienced multiple surgeries, disease recurrence, various chemotherapy regimens, numerous adverse effects, and participated in a clinical trial. I found my perspective and approach to this journey similar to other patients with CCA who I have interacted with during the past 10 years. I resonate with other patients and advocates I have connected and collaborated with through various forums sponsored by the Cholangiocarcinoma Foundation (CCF). This foundation sponsors an annual meeting attended by equal numbers of patients and professionals (physicians and scientists) with expertise in CCA. Patients share their stories from the podium, which is beneficial to patients, caregivers, physicians and scientists alike. This relationship is a powerful incentive to continue enhancing the collaborative network of CCA stakeholders and improving the lives of patients with CCA.

Surgery is a potentially curative treatment option for patients with tumours that have not grown too large. Surgeries may be performed to remove both the primary and recurrent disease and to obtain malignant tissue to guide other therapies. However, surgery can affect many organs, and serious complications can occur. I had a total of five operations and I would have another if it were necessary for my treatment plan.
Imaging showed disease recurrence 3 months after my initial liver resection; however, it is likely that metastases were already present at the time of surgery. Even so, the surgical resection bought me time to find the clinical trial that has kept me alive and healthy for 11 years. The four other surgeries consisted of three lung wedges with video-assisted thoracoscopic surgery (VATS) procedures and one thoracotomy. The first VATS was to confirm metastasis, whereas the others were to remove tumours to harvest tumour-infiltrating lymphocytes for treatment in a clinical trial. The thoracotomy was to remove malignant tissue again for the clinical trial and to guide next steps. All of these surgeries were worth the risk and contributed to my survival time. Other patients have also confirmed their willingness to undergo invasive surgical procedures to extend survival or buy time to explore additional therapies.

Especially for treatments that are not surgical, molecular profiling is an essential and necessary part of treatment plans. Patients with CCA are proactive about their medical care and want their treatments personalized. I was willing to undergo treatments with severe adverse effects for 3–12 months. Because there is no proven treatment and minimal options for CCA, patients are willing to try treatments that have no guarantee of working. I enrolled in a clinical trial with the thought that I had nothing to lose and everything to gain, especially as there were no other options for me. All patients, including myself, desire to increase our survival time. However, I came to a point in my journey in which quality of life became more important than the quantity of life, and my fellow patients agree with this sentiment. I came to this decision because my quality of life declined after 2 years of undergoing chemotherapy, and I had no desire to continue. I did not want my children to think I was giving up, but I wished to spend what time I had left with a good quality of life.

Clinical trials are a significant component of our treatment plan. I knew that to survive this diagnosis, I would have to find a clinical trial. My oncologist found a clinical trial for me as first-line therapy. Unfortunately, my insurance at that time would not cover the standard-of-care costs that go along with being in a clinical trial. I chose not to join the trial because I did not want to put my family in financial distress. Two years later, after chemotherapy failed to control my disease, I stumbled upon the clinical trial that successfully prolonged my life. My husband and I made phone calls to be evaluated for this trial and ultimately did all the work to be enrolled. Most patients struggle with understanding how to find and qualify for a clinical trial. They rely on their oncologist to provide the information.

Access to clinical trials is the primary barrier patients face to enrolling and researchers face in accruing participants. Patients most likely will have to travel to be able to participate in a trial and that, in turn, causes other issues, such as financial burden. Patients with a diagnosis as grim as CCA are deterred from clinical trials with a placebo arm unless there is an opportunity for crossover after a reasonable amount of time. There is no time to waste on a placebo. In addition to improved access, technologies are needed to help patients identify clinical trials and determine whether they match the inclusion and exclusion criteria. Furthermore, data on patient-reported outcomes should be better documented in these trials. For example, quantitative information from the patient’s perspective on adverse effects, reasons for foregoing and dropping out of trials, and the overall patient experience need to be incorporated into the clinical trial design. It is imperative to consider the patient perspective when planning future studies. Patient-reported outcomes and quality of life instruments provide information that is critical in optimizing research and therapeutic endeavours. As we move into an era of virtual visits and remote monitoring, I am hopeful that engaging in clinical trials will become less burdensome.

**Outlook**

Despite the recent progress in our knowledge of CCA mechanisms and management, many critical scientific and clinical questions remain, and prevention and treatment of this devastating disease still need to be better developed and defined, including identification of preventable risk factors. From an experimental perspective, preclinical models of pCCA and dCCA are still lacking. These models will be required if we are to better understand and develop rational therapies for these diseases. Although several preclinical models of iCCA have been developed205, many of the mutations commonly present in human iCCA, such as ARID1A, BAP1 and PBRM1, have not been examined as oncogenic drivers in these preclinical models. Given the unpredictability of extrapolating the information obtained from animal models, the use of human CCA organoids and patient-derived xenografts needs to be more deeply explored for disease mechanisms and response to new therapeutic agents. The relationships between coding and non-coding genetic and epigenetic alterations will require intense investigation and bioinformatics interrogation.

Even in the clinic, better diagnostic modalities are necessary for pCCA and dCCA, including molecular diagnostics in bile and blood206. Chemopreventive strategies in populations at high risk of CCA require further study, such as the use of statins207. In addition, vaccine development for liver-fluke-associated CCA requires extensive work before these agents can be used in humans.

The tumour immune microenvironment is a potential target for the treatment of CCA. Targeting cancer-associated fibroblasts, T cells and myeloid-derived suppressor cells are emerging therapeutic strategies with a high potential for the treatment of this highly desmoplastic cancer208,209. Although exciting, the effectivity of targeted therapies with pemigatinib in patients with FGFFR2 fusion aberrations and ivosidenib in patients with isocitrate dehydrogenase gain-of-function mutations is hampered by primary treatment resistance and a short durability of response185,207. Combinatorial therapies will be necessary to improve outcomes in these patient populations.

The advances in identifying targetable genetic aberrations in CCA highlight the emerging role of precision medicine for this disease. Analysis of circulating cell-free DNA will probably aid in this approach186 and
constitutes an important advance that will continue to be refined. The use of neoadjuvant and adjuvant therapeutic strategies in combination with surgery and loco-regional therapies to improve outcomes is also an active area of clinical investigation. Finally, selection of patients who will benefit from surgery in the long term remains a challenge and further insight into prognostic biomarkers is needed. Thus, considerably more information on this enigmatic cancer is required if we are to adequately minimize its current devastating human impact.

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