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Combined hepatocellular-cholangiocarcinoma: biology, diagnosis, and management

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Combined hepatocellular-cholangiocarcinoma from lab to clinic

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Abstract
Background
Combined hepatocellular-cholangiocarcinoma (cHCC-iCCA) is a rare type of primary liver cancer displaying characteristics of both hepatocytic and cholangiocytic differentiation.
Summary
Because of its aggressive nature, patients with cHCC-iCCA exhibit a poorer prognosis than those with HCC. Surgical resection and liver transplantation may be considered as curative treatment approaches; however, only a minority of patients are eligible at the time of diagnosis and postoperative recurrence rates are high. For cases that are not eligible for surgery, locoregional and systemic therapy are often administered based on treatment protocols applied for HCC or iCCA. Owing to the rarity of this cancer, there are still no established standard treatment protocols; therefore, the choice of therapy is often personalized and guided by the suspected predominant component. Further, the genomic and molecular heterogeneity of cHCC-iCCA can severely compromise the efficacy of the available therapies.

Key Messages

In the present review, we summarize the latest advances in cHCC-iCCA and attempt to clarify its terminology and molecular biology. We provide an overview of the etiology of cHCC-iCCA and present new insights into the molecular pathology of this disease that could contribute to further studies aiming to improve the patient outcomes through new systemic therapies.

Introduction

Combined hepatocellular-cholangiocarcinoma (cHCC-iCCA) is a primary liver cancer (PLC) with heterogeneous phenotypes that share common characteristics of both hepatocytic and cholangiocytic differentiation [1]. cHCC-iCCA is rare, with reported incidences ranging from 0.4% to 14.2% of PLCs. The World Health Organization (WHO) estimates a similar incidence at 2%–5% of PLCs [2-5]. The diagnosis and management of cHCC-iCCA are challenging because of its ambiguous denomination and histological definition as well as the lack of an established consensus regarding its staging, diagnosis, and treatment. A report by an international group of specialists proposed a consensus that unifies the terminology of PLCs, with an aim to facilitate the diagnosis, investigation, and management of cHCC-iCCA [6]. The latest WHO classification in the recently published *WHO Classification of Tumors of the Digestive System* provides an updated definition of cHCC-iCCA and its distinction between intrahepatic cholangiocarcinoma (iCCA) and cholangiolocellular carcinoma (CLC) [7].

Although there is a general consensus that surgical resection, whenever feasible, should be attempted with curative intention [8], to the best of our knowledge, there is currently only one prospective randomized trial (NCT05211323) being conducted that is examining the systemic treatment of advanced cHCC-iCCA. Moreover, evidence regarding the clinical outcomes following resection [8-10], liver transplantation (LT) [2, 11, 12], or transarterial chemoembolization (TACE) [13-15] for localized cHCC-iCCA is limited. Clinical options that are pursued mirror the treatment for early- or intermediate-stage HCC. In patients with cHCC-iCCA, systemic treatment is often initiated on a case-by-case basis using treatment and off-label options that refer to either advanced HCC or iCCA. In the present review, we summarize and suggest solutions to current challenges regarding the clinical classification, diagnostic criteria, histogenesis, genetic background, translational research models, and clinical management of cHCC-iCCA.

Epidemiology and risk factors

Epidemiology

Analyses from large datasets indicate that approximately 0.75% of PLCs represent cHCC-iCCA, with an estimated incidence of 0.05/100,000/year [2, 5, 16]. The reported incidence varies from 2.4% to 5.3% of PLCs in independent single center studies and was estimated to be 2%–5% by the WHO in 2019 [5-7, 17, 18]. The male-to-female patient ratio for cHCC-iCCA ranges between 1.8:1 and 2.1:1 [17, 19, 20]. The median age at diagnosis ranges between 62 and 65 years of age [5, 17, 19-21]. Data from the Surveillance, Epidemiology, and End Results (SEER) Program indicated that 39.9% of patients present with a localized stage cHCC-iCCA, 25.2% with regional disease, and 25.5% with distant disease [20].

Risk factors

Because cHCC-iCCA is a tumor with coexisting HCC and iCCA components, certain risk factors, etiologies, and oncogenic agents may predispose an individual to the development of this disease, including cirrhosis, chronic hepatitis, alcohol consumption, and metabolic syndrome, with varying weights between Eastern and Western populations [21]. The etiology of cHCC-iCCA varies and is largely dependent upon data derived from different regions or patient populations.

Although, the reported frequency of cHCC-iCCA cases occurring in patients with liver cirrhosis differs between studies, most report that about half of the cases are diagnosed in patients with underlying liver cirrhosis [1, 3, 17, 18, 22, 23] and that the etiological factors causing chronic liver disease reflect the regional prevalence of risk factors, such as hepatitis B (HBV) in Asian countries [5, 6, 8, 18, 24]

and hepatitis C (HCV) and chronic alcohol intake in Western countries [5, 17, 25]. Liver cirrhosis is associated with the vast majority of patients with HCC (approximately 80%) and has also been identified as a risk factor for iCCA (odds ratio: 15.32) [17, 18, 26, 27]; however, cHCC-iCCA may occur in both cirrhotic and noncirrhotic livers [17, 28].

Histological classification

Early classification

cHCC-iCCA was first described in a case report by Wells in 1903 **(Figure 1)** [29]. In 1949, Allen and Lisa reported five cases of combined liver cell and bile duct carcinoma [30]. They proposed that such tumors should be classified into three subcategories based on their distribution—type 1: separate nodules of hepatocellular and bile duct carcinoma; type 2: contiguous or intermingling areas of hepatocellular and bile duct carcinoma; and type 3: intimate association owing to origin from the same focus **(Figure 2)** [30]. In 1957, Popper and Schaffner postulated that with careful examination, the majority of primary hepatic carcinomas exhibit ductal elements [31]; however, Edmondson stated that these ductal elements originate from hepatocyte-like tumor cells and actually reflect a variant of HCC [32]. Moreover, Anthony *et al.* [33] suggested that most of the ductal elements described previously may reflect pseudoglands within the HCC but are not the classical mucin-producing glandular structures observed in cHCC-iCCA [33]. In 1985, Goodman *et al.* published the first systematic review of 24 cases of cHCC-iCCA [34]. Goodman *et al.* classified these tumors into three subtypes—type I or "collision tumors" in which both HCC and iCCA coincidentally occur in the same liver; type II or "transitional tumors" in which areas of intermediate differentiation and identifiable transition between HCC and iCCA are present; and type III or "fibrolamellar tumors," which resemble the fibrolamellar variant of HCC but also contain mucin-producing pseudoglands **(Figure 2)** [34].

WHO classification

Both the type 1 according to Allen and Lisa [30] and the type I described by Goodman *et al.* [34] refer to a collision type of tumor (separate HCC and iCCA that coincidentally coexist in the same liver) that is not classified as cHCC-iCCA by the WHO staging system [7]. Collision tumors comprise two independent neoplastic clones that happen to develop in proximity without exhibiting any histological transition [22]. Currently, the intimate intermingling subtype of the HCC and iCCA components (defined as type 3 by Allen and Lisa and type II by Goodman *et al.*) are recognized as cHCC-iCCA **(Figure 1+2)**.

In the 2010 (4th) edition of its cHCC-iCCA classification **(Figure 2)**, WHO subdivided cHCC-iCCA into two subtypes: (i) classical cHCC-iCCA and (ii) cHCC-iCCA with stem cell features (typical, intermediate cell, and cholangiolocellular) [35]. Different subtypes of cHCC-iCCA may coexist in a percentage-pattern because a careful examination may reveal more than one of these subtypes in a given cHCC-iCCA. However, in the latest 2019 (5th) edition of its cHCC-iCCA classification, WHO omits the "stem cell features" subcategorization and solely defines cHCC-iCCA as a PLC that is characterized by the unequivocal presence of the characteristics of both hepatocytic and cholangiocytic differentiation within the same tumor. This is a description that matches that of the 3rd edition released in 2000 [7, 36]. This adjustment was mainly driven by the fact that "stem cell features" may potentially be detected in all forms of cHCC-iCCA [4, 37]. In addition, CLC was removed from cHCC-iCCA and is now classified as part of small duct type iCCA [7]. Notably, Komuta indicated that cHCC-iCCA may contain small duct type iCCA features as part of its tumor heterogeneity, thereby suggesting that cHCC-iCCA may exhibit features of the small duct phenotype [38]. Recently, an international consensus paper suggested that it is not necessary to subtype cHCC-iCCA; however, it also recommended that a description of any "stem/progenitor cell features" be provided when observed [6].

Current consensus

cHCC-iCCA is characterized as a PLC comprising the characteristics of both unequivocal hepatocytic and cholangiocytic differentiation with transitional features within the same tumor. This description is fundamentally different from a collision tumor shaped by separate HCC and iCCA. Typical cHCC-

iCCA exhibits unequivocal HCC and iCCA, where the two components intimately intermingle with each other, and accounts for approximately 17% of the 54 cases reported in one study [4], whereas HCC- or iCCA-dominant phenotypes exhibited various levels of heterogeneity with different areas of differentiation. Notably, the ductular configuration is often detected in CLC and cord-like structures are typically observed in intermediate cell carcinomas. The WHO classification indicated that a tumor comprising >80% ductular reaction-like structure should be considered indicative of a CLC [7] in the absence of HCC components [7, 39].

Differentiation from other PLCs

Heterogeneous entity per se

Currently, the definition and diagnosis of cHCC-iCCA are based on the histopathological recognition of unequivocal hepatocytic and cholangiocytic differentiation [6, 7]. No cut-off defines the percentage of each differentiation that is required to establish a diagnosis [6, 7, 19]. HCC and iCCA components may intermingle with each other [21, 26] through a sharp or poorly defined transition [19]. However, some cHCC-iCCA cases may exhibit no discernable interface; therefore, a diagnosis may be challenging [21]. Furthermore, the distinction between cHCC-iCCA and HCC or iCCA may be overlooked because of the heterogeneous nature of the initial and sampling-associated misdiagnoses. Typically, epithelial cell adhesion molecule (*EpCAM*), *MOC31*, epithelial membrane antigen (*EMA*), *CK7*, and *CK19* staining exhibits positive results in cases of iCCA, whereas hepatocyte paraffin 1 (*HepPar-1*), *arginase-1*, alpha fetoprotein (*AFP*), and *CD10* expression indicate [21]. Notably, the interface may stain positive for *CK7* and *CK19* as well as for *HepPar-1* and *arginase-1* [21]. In addition, HCC can express cholangiocellular markers with atypical features of fibrous stroma [40-42]. *EpCAM*, a "stemness marker", was found to be positive in >90% of the iCCA areas and in 10%–20% of the HCC areas within cHCC-iCCA tumors [40], whereas it was positive in 35% of the HCC cases [41].

Cytokeratin 19 (CK19)-positive HCC

CK19^{+} HCC is a specific type of HCC devoid of glandular structures that exhibits variable membranous positivity for *CK19*. The overall survival (OS) of patients with *CK19*$^{+}$ HCC was not different compared with that of patients with cHCC-iCCA; however, significantly lower and higher compared with that of patients with *CK19*$^{-}$ HCC and those with iCCA, respectively [43]. Typically, *CK19* expression suggests the presence of iCCA; however, approximately 10% of HCC cases were reported to be positive for *CK19* [44, 45]. By contrast, positive *CK19* staining results are observed in the majority (44–100%) of iCCA cases and is typically indicative of iCCA [44]. For cHCC-iCCA, *CK19* expression was present in the majority of cases (83%) within the transition area and in the glandular areas [46] and exhibited a monotonous cytoplasmic expression inside the iCCA areas of cHCC-iCCA [47]. Therefore, *CK19*$^{+}$ HCC reflects a potential differential diagnosis for cHCC-iCCA.

CLC

CLC is defined as a tumor consisting of >80% ductular reaction-like structure with abundant fibrous stroma [7, 48, 49]. In the 4th edition of the WHO cHCC-iCCA classification, CLC was classified as a cHCC-iCCA with stem cell features (cholangiolocellular type) based on morphological and molecular features [50]. However, in its 5th edition, CLC was categorized as a small duct type iCCA [7]. Molecular analysis revealed that CLC represents a distinct biliary-derived entity that is independent of HCC [51]. Based on the current consensus, the diagnosis of CLC can only be made in the absence of HCC components [39].

Intermediate cell carcinoma

Intermediate cell carcinoma is a rare and unique PLC that purely comprises cells with an intermediate phenotype between HCC and iCCA [6, 7, 52]. This cHCC-iCCA subtype contains monomorphic tumor cells that are typically smaller than normal hepatocytes but larger than hepatic progenitor cells and exhibits features of both hepatocytes and cholangiocytes **(Figure 3)** [40, 53]. It may exhibit an invasive pattern similar to both HCC (intravascular and intrabiliary) and iCCA (lymphatic and perineural) [6]. It usually presents on a background of chronic hepatitis or cirrhosis with a

simultaneous immunoreactivity of hepatocytic and cholangiocytic markers by the same tumor cells [53].

Clinical presentation and diagnosis

HCC can be diagnosed based on radiological features alone. The absence of histological confirmation may lead to under-reporting of the CCA component of cHCC-iCCAs which bear similar radiological features. *Vice versa*, cHCC-iCCAs may be underdiagnosed in situations where biopsy samples capture only one of the tumor's components.

Imaging

The radiographical features of cHCC-iCCA largely depend on the proportion of the HCC and the iCCA components [54, 55]; however, radiologically distinguishing small duct type iCCA itself from HCC is difficult [56]. Because the radiological criteria alone are considered sufficient for the diagnosis of HCC on the basis of the typical features of contrast medium kinetics in cirrhotic livers, some cHCC-iCCA cases may be diagnosed as HCC based only on radiological criteria. Therefore, cHCC-iCCA cannot be radiologically diagnosed, which may lead to radiological misdiagnosis. cHCC-iCCA cases can only be diagnosed histologically following surgical resection or biopsy. In a study involving patients with cHCC-iCCA, the consistency of the diagnosis between imaging and histology was found to be only 66.7% [55]. Imaging alone has a limited diagnostic value, with a sensitivity of only 48% and a specificity of 81%, whereas the combination of imaging and biopsy can increase sensitivity (60%) and specificity (82%) [57]. Furthermore, by combining imaging, biopsy, and immunohistochemical markers, one can improve the diagnostic performance and achieve a 12% increase in sensitivity [57].

Diagnosis

The identification of both hepatocytic and cholangiocytic differentiation is essential for the diagnosis of cHCC-iCCA [1, 6]. It is useful to combine histomorphology with immunohistochemistry to support or confirm hepatocytic and cholangiocytic differentiation. In addition, HCC cases that are positive for biliary and/or stem cell markers should not be overdiagnosed as cHCC-iCCA, because a fraction of HCC cases (29.3%–37%) exhibits positivity for biliary markers, such as *CK7*, *CK19*, and *AE1/AE3* [58, 59]. Some studies have suggested that *nestin* may serve as a biomarker for the diagnosis and prognosis of cHCC-iCCA, because it is a marker of bipotent progenitor oval cells [60-62]. Xue *et al.* concluded that high *nestin* expression is an important feature for cHCC-iCCA and it may be the basis for its observed bilinear differentiation and high cellular plasticity [60]. In addition, Malvi *et al.* found that the intermediate areas of cHCC-iCCA were positive for *nestin* in 92.3% of the 13 examined cases, although the differentiated HCC and iCCA components of these cHCC-iCCA cases were found to be negative for *nestin* expression [62]. This was recently supported by Calderaro *et al.,* who conducted a large multicenter analysis on the prognostic value of *nestin* in cHCC-iCCA [63]. Although an association of *nestin* expression for the differentiation of cHCC-iCCA from HCC with an AUC of 0.85 was established [63], its diagnostic value was lower for cases that were distinct from iCCA. This is consistent with an observation by Sasaki *et al*, who found that *nestin* expression can be detected in a significant proportion of patients with small duct type iCCA (40.9%). This was significantly more often positive compared with that observed in large duct type iCCA (5%) or HCC (2.9%) [64]. However, cHCC-iCCAs were also more frequently positive for *nestin* (66.7%) compared with large duct type iCCA or HCC [64]. Therefore, *nestin* may also represent a diagnostic marker for small duct type iCCA and cHCC-iCCA [64].

Importantly, the combination of elevated tumor markers and contrast enhancement patterns upon imaging suggests the presence of cHCC-iCCA under the following conditions [65]: (i) imaging features of both HCC and iCCA, regardless of markers levels, (ii) elevation of both *AFP* and *CA19-9*, regardless of the imaging patterns, or (iii) discordance between imaging and tumor marker elevation (typical HCC pattern for imaging with elevated *CA19-9* or atypical HCC enhancement pattern with elevated *AFP*). Although serum markers alone are inadequate for the diagnosis of cHCC-iCCA, an incompatible level of *CA19-9* and *AFP* along with the identification of contrast enhancement patterns may indicate the presence of this particular tumor type [66]. In fact, one study showed that the tumor markers

CA19-9 or *AFP* were significantly increased in cHCC-iCCA, with mean values of 35.33 U/mL and 294.26 ng/mL, respectively [65].

Biopsy

Despite improvements in serological and radiological techniques, liver biopsy remains the most reliable way to assess hepatic nodules [67]. Biopsy should be performed in all patients with atypical radiologic findings and/or in those with elevated *CA19-9* levels when no primary surgical approach is followed [68]. However, the indication for a liver biopsy should be determined on a case-by-case basis and considered judiciously if cirrhosis is present [67, 69]. The diagnosis of HCC can be established through imaging in cirrhotic livers, and biopsy can be used in this setting as recommended, for example, by German guidelines [69, 70]. Notably, the indications for biopsy differ between the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) guidelines. The EASL guidelines consider a biopsy to be obligatory for lesions ranging from 1 to 2 cm in size [71], whereas the AASLD guidelines recommend no biopsy for lesions larger than 1 cm, provided that two different imaging studies have yielded clear and concordant findings [72, 73]. Similar to the EASL guidelines, the Asian Pacific Association for the Study of the Liver recommends that a biopsy be considered for nodules of 1 cm or larger to distinguish between early HCC and a dysplastic nodule [74].

If there is no liver cirrhosis present, a biopsy should be performed despite the typical imaging features for HCC being present [70], which can result in the diagnosis of cHCC-iCCA based on our clinical experience. Another mass lesion that should be distinguished in the setting of the underlying liver disease is CCA. This tumor typically arises in the presence of a chronic biliary tract disease involving either the biliary hilum or the hepatic parenchyma [75]. If surgical resection is feasible, then a preoperative biopsy is typically not necessary; however, in the context of LT, the risk of seeding should be considered before a biopsy is pursued. However, a biopsy under image guidance is generally recommended, particularly to rule out metastasis for non-liver carcinomas [72].

If the biopsy confirms a biphenotypic pattern, the patient is preliminarily recommended for surgery following discussion in a multidisciplinary tumor board. In a pre-surgical study, biopsy exhibited an estimated 48% sensitivity and 100% specificity for the diagnosis of cHCC-iCCA [57]. Importantly, cHCC-iCCA can be overlooked or misdiagnosed as HCC in patients undergoing a biopsy alone [5, 76]. Therefore, a two-step strategy is recommended by combining imaging as the initial step followed by biopsy to improve the diagnostic performance of cHCC-iCCA [57]. Finally, sampling variability or error is considered to be a major limitation of liver biopsy, to the extent that the absence of key histological findings does not necessarily rule out a suspected diagnosis [72].

Prognosis

The prognosis for cHCC-iCCA may be similar to that of iCCA but is worse compared with that of HCC [53, 77], with a reported 1- and 3-year OS of 81.9% and 47%, respectively, compared with 47.3% and 18.3% for iCCA and 92.4% and 77.1% for HCC following surgery [77, 78]. Vascular invasion and lymph node metastasis tend to be more frequent in cHCC-iCCA compared with HCC [79]. The median OS from the SEER regarding distant, regional, and localized cHCC-iCCA is 4 months, 7 months, and 20 months, respectively [20]. Another large dataset revealed that the median OS is 28.6 months for stage I, 24.2 months for stage II, 7.5 months for stage III, and 3.1 months for stage IV cHCC-iCCA [80].

Molecular biology

Cells of origin

The concept that cHCC-iCCA originates from hepatic progenitor cells (HPCs) was adopted by the 4th edition of the WHO classification of this disease [35]. This consensus is based on studies that suggest a common clonal derivation of the HCC and iCCA components with respect to origin, based on histological [4] and molecular analyses [51, 81]. cHCC-iCCA with stem cell features exhibits the molecular characteristics of undifferentiated PLC and *CK19*$^{+}$ HCC, thereby implying a single bi-potential clonal origin [82]. *In vivo* studies have highlighted the role of HPCs in the carcinogenesis of cHCC-iCCA through the activation of the *Bmi1* and the *Wnt/β-catenin* pathways that disrupts HPC

self-renewal [83]. In addition, the liver-specific knockouts of *GRP94* or conditional mutations of *Sav1* and *mst1/2* during *Hippo* signaling have been shown to induce significant hyperproliferation of HPCs, thus resulting in the development of cHCC-iCCA [84, 85]. Furthermore, the development of cHCC-iCCA may be triggered by the direct differentiation of HPCs into HCC and iCCA *via* the *p53*-dependent *nestin* regulation [61] or through the activation of *KRAS (G12D)* and the deletion of *p53*, which induces an iCCA-dominant cHCC-iCCA [86]. In summary, cHCC-iCCA may be derived from HPCs that express markers of both the hepatic and biliary lineages [87]; however, HPCs may not be the sole cell type of origin for this disease **(Figure 4)** [82].

In another study, $p53^{-/-}$ murine hepatoblasts generated cHCC-iCCA both *in situ* and within metastases following a syngeneic injection [88]. This raises the question of whether hepatoblasts also contribute to the formation of cHCC-iCCA. Another potential origin of cHCC-iCCA could lie with mature hepatocytes that retain phenotypic plasticity for differentiating into cholangiocytes. Some studies have suggested that cHCC-iCCA may originate from hepatocytes rather than HPCs [89, 90]. A lineage-tracing study revealed that iCCA in mice could originate from hepatocytes following a dual-activation of the *AKT* and *Notch* signaling pathways [91]. Similarly, Mu *et al.* have reported that hepatocytes represent the cell of origin for HCC and that a progenitor signature does not reflect progenitor origin but rather the dedifferentiation of hepatocyte-derived tumor cells [90]. This suggestion supports the hypothesis of hepatocyte-derived cHCC-iCCA formation [89]. Moreover, other studies have shown that hepatocytes can dedifferentiate back into HPCs and may undergo malignant transformation into cHCC-iCCA **(Figure 4)** [92, 93].

In summary, current data indicate that the cellular origin of cHCC-iCCA may be HPCs and/or hepatocytes **(Figure 4)** [19, 94]; however, whether HPCs, hepatocytes, or hepatoblasts are the actual cells of origin remains unclear.

Tumor microenvironment

The tumor microenvironment may impact the formation of cHCC-iCCA **(Figure 4)** [89, 95]. This is based on the plasticity of hepatobiliary cells and on their role in guiding lineage differentiation [89, 96-98]. In HCC, the tumor microenvironment can transdifferentiate the tumor into cHCC-iCCA by modifying oncogenes and signaling pathways [19, 98]. For example, by blocking the nuclear factor kappa B (*NF-κB*) signaling, one can modify a *MYC*-driven HCC phenotype toward cHCC-iCCA [98]. Interestingly, it appears that a necroptotic microenvironment may promote hepatocyte-derived iCCA [96], whereas an apoptotic environment may trigger the formation of hepatocyte-derived HCC [89]. In addition, mutant *β-catenin* was found to be associated with microenvironment remodeling and transforming growth factor beta (*TGF-β*) activation in cHCC-iCCA samples [87]. Furthermore, genomic and transcriptomic profiles revealed that multiple pathways are associated with the immune microenvironment in cHCC-iCCA, including the migration of leukocytes, regulation of lymphocytes, and regulation of T cell activation. Interestingly, the presence of these pathways may be indicative of the potential response of the tumor to immune therapies [60]. Future studies to identify the molecular mechanisms underlying the role of the microenvironment in directing the liver cancer phenotype are required.

Genetic landscape

The molecular profiles of cHCC-iCCA tumors indicate heterogeneity and remain inconclusive because only a limited number of studies have examined this rare tumor. This contrasts with the more extensive number of studies that have explored the genetic characteristics of HCC and iCCA. A better genetic understanding of cHCC-iCCA may contribute to the underlying nature of its etiology, carcinogenesis, treatment response, and prognosis and can identify novel therapeutic targets **(Figure 5)**.

The genetic landscape of cHCC-iCCA exhibits features of both the HCC and iCCA [99]. The mutational and transcriptional landscapes associated with cHCC-iCCA and iCCA are different, particularly with regard to the two distinct subtypes of PLCs involved [100, 101]. Genetic alterations in the *TERT* promoter, *TP53*, *ARID1A*, and *ARID2* have been noted in cHCC-iCCA, whereas mutations in *PBRM1*, *KRAS*, *IDH1/2*, and *FGFR2* have frequently been observed in iCCA **(Table 1, Figure 5)** [5, 100, 101].

Moreover, *CTNNB1* and *p53*-related pathways exhibit alterations in both HCC and cHCC-iCCA cases **(Table 1, Figure 5)** [5].

IDH1/2 mutations were detected in HCC cases exhibiting iCCA features, thereby suggesting that *IDH1/2* may mediate a switch to the biliary phenotype [99, 102]. cHCC-iCCA appears to be genetically closer to iCCA because of similar molecular profiles that were shown to be different from those of HCC [28, 87]. A high level of chromosome instability was observed in both the cHCC-iCCA and iCCA, and a loss of heterozygosity was identified at chromosomes *3p* and *14q* in >50% of cHCC-iCCA and iCCA cases; by contrast, these chromosomal deletions were detected in <10% of the HCC cases examined [28]. A genome-wide transcriptional analysis revealed that cHCC-iCCA exhibits a decreasing program of hepatocyte differentiation while being committed to a biliary lineage [87].

Some studies have suggested that even within the iCCA component, cHCC-iCCA is genetically distinct from iCCA but similar to HCC [5, 103]. Joseph *et al.* found that cHCC-iCCA contained alterations in the *TERT* promoter (80%), *TP53* (80%), cell cycle genes (40%), tyrosine kinase genes (55%), chromatin regulators (20%), and *Wnt* pathway-associated genes (20%), which are all closely related to HCC [103].

Typically, cHCC-iCCA harbors mutations in the *TERT* promoter and *TP53* **(Table 1)** [51]. *TERT* promoter mutations may initiate early carcinogenesis in cHCC-iCCA because its mutations have consistently been identified in both the HCC and iCCA components [103]. Furthermore, *TP53* mutations have been found in both components in over half of the *TP53*-altered cases, thereby suggesting that a *TP53* mutation may contribute to intratumoral heterogeneity [103]. Some specific genetic features in cHCC-iCCA, such as the *TERT* promoter mutations, are associated with chronic hepatitis [99, 104], whereas mutations in *ARID1A* are associated with alcoholic liver disease [95]. Although oncogenic drivers remain poorly understood in cHCC-iCCA, the activation of the *TGF-β*, *Wnt*, *AKT*, *N-RAS*, *Notch*, and Hedgehog signaling pathways as well as the inactivation of the *NF-kB* pathway have been associated with carcinogenesis [82, 105] and may represent potential molecular targets of therapeutic interest **(Figure 5)**.

A study by Xue *et al.* [60] established genetic profiles for 133 cases of separate, mixed, and combined cHCC-iCCA along with HCC and iCCA specimens. Their results indicated that separate cHCC-iCCA cases were present, which comprised both mono- and multicellular origin, whereas combined and mixed cHCC-iCCA cases were exclusively monocellular [60]. Of the two monocellular subtypes, a molecular analysis revealed that the combined subtype exhibited features closer to those of iCCA, whereas the mixed type exhibited HCC-like features. Notably, *TP53* mutation (a well-known oncogenic event in both HCC and iCCA) was shown to be more significantly dysregulated in cHCC-iCCA compared with HCC or iCCA. Although *TP53* mutation is commonly observed in cHCC-iCCA, the infrequent mutations of *CTNNB1* and *KRAS* may indicate a certain molecular pattern of cHCC-iCCA in this regard [60].

Experimental models

By establishing representative pre-clinical and translational models of cHCC-iCCA, one can fulfill the fundamental need of establishing a comprehensive understanding of the neoplastic development, progression, and potential therapies for this rare pathological entity **(Table 2)**.

1. Cell lines

The established cell lines of human cHCC-iCCA include KMCH-1 [106] and KMCH-2 [107]. KMCH-1 was derived from a surgical specimen of a 52-year-old male with cHCC-iCCA and possesses a chromosome number ranging from 60 to 98, with a modal number of 74. KMCH-1 cells can produce tumors after 1 month following subcutaneous or intraperitoneal transplantation in nude mice and demonstrate features of CCA *in vitro* and *in vivo* [106]. Furthermore, the top five preferentially expressed genes in KMCH-1 include *UROD*, *EGFR*, *HDAC1*, *SPRR2G*, and *GGPS1* (CRISPR, DepMap 22Q2 Public+Score, Chronos). KMCH-2 was established from a surgically resected cHCC-iCCA collected from a 40-year-old male, with chromosomes distributed in a range of 75 to 82 and a modal number of 79. KMCH-2 exhibits an albumin-producing characteristic of HCC *in vitro* without mucin production; however, subcutaneous tumors that develop in nude mice following a KMCH-2 injection exhibit features of adenocarcinoma with mucin production [107].

The CC-62 cell line was reported by Gil-Benso *et al.* as a murine cHCC-iCCA cell line [108]. CC-62 cells were derived from male Wistar rats after the administration of 2-acetylaminofluorene and their chromosome numbers ranged from 74 to 82, with a modal number of 79. They do not express *KRAS* or *p53* and a molecular analysis failed to detect any mutations; however, RT-PCR revealed transcripts for *c-met* and the absence of hepatocyte growth factor (*Hgf)* expression. In addition, CC-62 cells can form tumors within 1 month following subcutaneous transplantation into nude mice with morphological patterns of an epithelial, mucosecretory, and spindle-shaped carcinoma [108].

2. Animal models

Piscaglia *et al.* established a rat liver carcinogenesis protocol derived from the activation of oval cells (using 2-acetylaminofluorene/partial hepatectomy or a 2AAF/PH regimen) in combination with the administration of aflatoxin-B1 (also known as the "APA regimen") for the development of tumors with cHCC-iCCA features [109]. Interestingly, the model was highly efficient in producing mixed tumors; however, one limitation was that the tumors could only develop in cirrhotic livers, which is not the case for human cHCC-iCCA tumors, as the latter often develops in noncirrhotic livers. Furthermore, the mechanism of this model was based on oval cell proliferation and it appeared that these cells are the source of the observed mixed tumors in this model. This limited its applicability because it has not been demonstrated that oval cells are the sole cells of origin for cHCC-iCCA development. He *et al.* described a cHCC-iCCA model derived from a *MYC*-driven HCC model through the hepatocyte-specific deletion of the *NF-κB* essential modulator (*NEMO*) gene [98]. The deletion of *NEMO* resulted in a shift from *MYC*-driven HCC development to the development of mixed tumors. However, this highly specific genetic alteration may not explain the entire spectrum of cHCC-iCCA cases observed in the clinical setting. Moreover, *in vivo* models have been developed to examine the *Hippo-YAP* pathway for the purpose of triggering cHCC-iCCA formation [82, 85, 110, 111]. In these cases, the combined overexpression of *YAP* and *PI3KCA* resulted in cHCC-iCCA tumor formation in up to 50% of the animals [110]. Nishio *et al* examined the role of *Hippo-YAP* signaling in the development of mixed tumors. This group demonstrated that *Mob1a/1b*-deficient mice developed mixed tumors [111]. Further, cHCC-iCCA may be generated *via AKT/β-catenin*-initiated or aristolochic acid-induced tumorigenesis [89, 112]. In addition, murine HPCs/hepatoblasts transfected with oncogenic *H-Ras* and the *SV40* T antigen or with *Bmi1* and mutated *β-catenin* resulted in the formation of liver cancers with iCCA and/or HCC features [82, 83, 113, 114]. However, most of these specific genetically engineered models can mimic a subgroup of mixed tumors but cannot cover the spectrum of cHCC-iCCA tumors observed in the clinical setting.

3. Patient-derived organoids (PDOs)

Broutier *et al.* generated liver cancer PDOs and was able to establish two cHCC-iCCA PDO lines [115], in which drug screening was performed [115]. They demonstrated differential sensitivity of these cHCC-iCCA-derived PDO lines to various compounds, such as taselisib (*PI3K* inhibitor), LGK974 (*PORCN* inhibitor), dasatinib (tyrosine kinase inhibitor, TKI), gemcitabine (Gem), and SCH772984 (*ERK1/2* inhibitor). Interestingly, both organoid lines were resistant to most screened compounds. Only sorafenib (TKI), taselisib, and vorinostat (histone deacetylase inhibitor) exhibited cytotoxicity against both cHCC-iCCA-derived tumoroids causing a growth inhibitory effect [115]. The establishment of a larger cHCC-iCCA organoid biobank and its pharmacogenomic phenotyping may lead to the development of therapeutic strategies in an *ex vivo* setting that could be of value toward narrowing potential drug candidates for further development.

Clinical management

There is no established consensus for the treatment of cHCC-iCCA because of its low incidence; therefore, current interventions are often extrapolated from established therapies for either HCC or iCCA. In the current (8^{th}) edition of the AJCC TNM staging system, cHCC-iCCA is staged according to the iCCA classification [116]; however, this classification may not be suitable for the management and prognosis of cHCC-iCCA, because it is biologically different from both HCC and iCCA. Therefore, a specific staging system should be established in the future.

Surgical resection is currently the best treatment for cHCC-iCCA and may offer the longest OS (median OS of 25.7 months); however, it can only be performed in a minority of patients (34.2%) [15]. Unfortunately, the recurrence risk is high even after radical resection, with a median time to recurrence of 6–9 months [117]. The role of LT in the treatment of cHCC-iCCA remains controversial [5]. A median disease-free survival (DFS) of 14.2 months and a median OS of 37.1 months have been reported based on a systematic review of retrospective studies [8]. Meanwhile, a retrospective matched cohort study reported a more positive outcome with 5-year survival rates that were similar between cHCC-iCCA and the HCC controls (78% *vs*. 86%) undergoing LT [25]. A different retrospective study showed that the 5-year OS (67%) and the DFS (75%) were higher in patients treated with LT compared with resection for cHCC-iCCA in the setting involving cirrhosis, with survival rates comparable to those of patients with iCCA [118]. These studies may indicate that LT can improve patient survival compared with resection in cirrhotic patients with cHCC-iCCA tumors that are smaller than 5 cm [5, 25, 118]; however, there is insufficient evidence to recommend LT for the treatment of cHCC-iCCA [119].

Currently, evidence from large randomized prospective trials for the treatment of recurrent, metastatic, or unresectable cHCC-iCCA is lacking. Therefore, a tumor-agnostic and therapeutic approach should be applied, and tumor specimens should ideally be sent for genomic analysis for identifying targetable genetic alterations. Furthermore, immunotherapy may be applied to patients with a microsatellite instability-high (MSI-H) status [23] or with a high mutational burden [120], which may be considered in polymerase epsilon-mutated tumors [121] because of the tumor-agnostic efficacy of immunotherapy in such cases. The administration of immune checkpoint inhibitors (ICIs) has become a standard treatment for advanced HCC, although their efficacy for cHCC-iCCA must be evaluated in more detail when administered alone or in combination with anti-vascular endothelial growth factor compounds, TKIs, or other chemotherapy-based regimens. This is particularly relevant in light of a recent study that demonstrated the efficacy of immunotherapy in biliary tract cancer [122].

Local therapy

TACE, percutaneous ethanol injection, radiofrequency ablation, and cryoablation may be beneficial for the treatment of cHCC-iCCA recurrences in selected patients [123, 124]. The pattern of cHCC-iCCA local recurrence often appears to be a configuration observed in iCCA rather than in HCC, thus potentially indicating a dominant prognostic role for iCCA-associated elements in cHCC-iCCA following local therapy [125]. However, patients in whom the tumors are not resectable after local recurrence and distant metastasis is absent may be candidates for local palliative therapy.

Surgery is associated with superior survival for patients with cHCC-iCCA; however, it is only feasible in a minority of these patients (34.2%) [15]. In a retrospective cohort of 79 patients, 18 with unresectable cHCC-iCCA received liver-directed therapy (including TACE, radioembolization, or hepatic arterial infusion chemotherapy) [15]. These locoregional therapies resulted in an overall response rate of 47% (20% to TACE, 50% to radioembolization, and 66% to hepatic arterial infusion chemotherapy) with a median progression-free survival (PFS) and OS of 8.3 and 16.0 months, respectively. The retrospective study concluded that liver-directed therapies have a superior objective response over systemic chemotherapy and may offer a survival advantage and potentially achieve the downstaging of patients, which may permit surgical resection [15].

Connell *et al.* expressed concern that the majority of cHCC-iCCA cases are less vascularized but more fibrotic than HCC cases, thereby suggesting that these cases are less likely to respond to TACE [21]. The efficacy of TACE for primary unresectable and recurrent cHCC-iCCA has been associated with tumor vascularity (median OS of 16 months for hypervascular tumors and 4 months for hypovascular tumors), thereby demonstrating a poorer survival compared with that observed in patients with HCC [13]. cHCC-iCCA cases with a non-rim arterial phase hyper-enhancement (APHE) pattern of imaging exhibit a better radiological response rate (36% *vs*. 0%) and survival (52.8 *vs*. 12.4 months) compared with cHCC-iCCA cases with a rim APHE pattern of imaging treated with resection and TACE [10, 14].

Occurrence of cHCC-iCCA after locoregional treatment?

Cases of cHCC-iCCA occurring in patients with HCC after TACE have recently been reported. Nishihara *et al.* have reported that preoperative TACE may increase *CK19* expression in patients with HCC, thereby possibly influencing cell differentiation toward a more aggressive biliary phenotype [126]. A combined hepato-cholangiocellular phenotype also appears to be more frequent in patients with HCC who have undergone TACE [12]; however, this occurs episodically and the idea of a possible role of TACE in determining the phenotype of HCC remains speculative at best, because its verification would require histological specimens before and after the delivery of TACE, which are rarely ever obtained in clinical routine.

Systemic treatment

Because no specific evidence-based treatment for cHCC-iCCA has been established [127, 128], advanced cHCC-iCCA is often treated similar to HCC or, more often, as iCCA [5, 129-132]. Rogers *et al.* have reported a clinical benefit in three cases of patients with unresectable cHCC-iCCA treated with Gem-platinum with or without bevacizumab (Bev) [133] **(Table 3)**. Kobayashi *et al.* reported that the median OS in patients treated with Gem/cisplatin (Cis), fluorouracil (5-FU)/Cis, and sorafenib was 11.9, 10.2, and 3.5 months, respectively **(Table 3)** [134]. A multicenter study of 30 patients treated with Gem and oxaliplatin (Oxp) or Cis/Bev [135] indicated that 8 patients (28.6%) showed a partial response and 14 (50%) had stable disease with a median PFS of 9.0 months and an OS of 16.2 months **(Table 3)** [135]. In a single center cohort of 68 patients with unresectable cHCC-iCCA who have received systemic treatment, 57 received Gem-based regimens; from this subgroup, 16 received Gem/5-FU and 41 were treated with Gem-platinum **(Table 3)** [23]. OS was 11.7 months for Gem/5-FU, 11.5 months for Gem-platinum, and 9.6 months (7 patients) for sorafenib monotherapy [23]. Because no confirmed objective responses were observed in patients with cHCC-iCCA treated with sorafenib, it has widely been considered that cHCC-iCCA should be initially treated similar to iCCA rather than HCC. However, a large multicenter analysis conducted by Gignate *et al.* evaluated the outcomes of patients with cHCC-iCCA following treatment with either TKI or platinum-based chemotherapy [136]. The median OS with TKI or platinum-based chemotherapy was 8.3 or 11.9 months, respectively. Despite the numerical difference, these results were not statistically significant; the authors concluded that TKI therapy and platinum-based chemotherapy exhibited a similar efficacy [136]. Nevertheless, it should be noted that frequently encountered circumstances, such as impaired liver function (due to cirrhosis), may restrict the feasibility of administering platinum-based regimens; therefore, TKI therapy represents a better alternative.

More recently, ICIs have been approved for the treatment of HCC. Although cHCC-iCCA was excluded from clinical trials with ICIs, encouraging reports have been published on the effects of ICIs on cHCC-iCCA. In fact, a complete remission of cHCC-iCCA with lung metastases following third-line treatment with pembrolizumab was reported **(Table 3)** [137]. Moreover, in another case of cHCC-iCCA, a significant radiological response and an improvement of patient quality of life were achieved with nivolumab monotherapy and its combination with ipilimumab, thereby suggesting the efficacy of ICIs in selected patients **(Table 3)** [138].

Recently, the phase III study TOPAZ-1 demonstrated that durvalumab (Durva; a PD-L1 inhibitor) and Gem-Cis have significantly improved median OS (12.8 *vs*. 11.5 months) and PFS (7.2 *vs*. 5.7 months) in patients with advanced biliary tract cancer compared with Gem-Cis alone [139]. TOPAZ-1 revealed that by adding immunotherapy to standard chemotherapy, the survival of patients with biliary tract cancer was improved with manageable safety [139]. Durva+Gem-Cis has recently been approved by the FDA and EMA and is a new first-line standard of care regimen for iCCA. Moreover, the phase III HIMALAYA trial showed that Durva combined with tremelimumab (Treme; a CTLA-4 inhibitor) can reduce the risk of death by 22% in patients with advanced HCC compared with those receiving sorafenib alone (median OS: 16.4 *vs*. 13.8 months) [140]. The overall response rate for Durva+Treme was 20.1% (*vs*. 17% for Durva and 5.1% for sorafenib), thereby supporting that a combination immunotherapy of Durva+Treme is a new first-line systemic therapy for advanced HCC after its recent FDA approval [140]. However, Durva alone exhibited substantial efficacy in the HIMALAYA trial [140]. Based on the evidence provided by the TOPAZ-1 and HIMALAYA trials, Durva+Gem-Cis may

reflect a promising regimen for the treatment of cHCC-iCCA. This combination offers efficacy for both tumor subtypes (HCC and iCCA), although future studies should evaluate this regimen prospectively.

Current and future clinical trials

As of March 2022, there were only four registered clinical trials for the treatment of cHCC-iCCA (ClinicalTrials.gov), two of which were observational studies (NCT03178409 and NCT04848805; **Table 4**) comparing the prognosis of patients undergoing liver surgery or LT. In 2017, a phase II trial was established to assess the efficacy of derazantinib, a potent anti-*FGFR1-3* oral kinase inhibitor, for the treatment of patients harboring *FGFR2* gene fusions, mutations, or amplifications (NCT03230318). Recently, a new randomized phase II trial was launched to evaluate whether atezolizumab (Atezo)/Bev *plus* Gem-Cis is superior to Gem-Cis alone in advanced tumors (NCT05211323). Because no specific systemic treatment has been established and as the peculiar biology of this tumor remains unclear, cHCC-iCCA cases are typically treated either as HCC or iCCA cases. The current development of iCCA-targeted therapies may prompt new cHCC-iCCA treatment research based on biomarker trials, which could involve molecular alterations of *FGFR2*, *IDH1/2*, *ERBB-2/HER2*, $BRAF^{V600E}$, and *TRK* as well as the evaluation of immunotherapy in patients with MSI-H status [141]. Because of the low incidence of this tumor, cooperative initiatives within international consortia and the creation of international registries are required to facilitate trials exploring systemic treatments and meaningful data collection.

Conclusions

Nearly one third of the cHCC-iCCA cases are diagnosed at a metastatic stage. No particular etiology has been associated with the occurrence of cHCC-iCCA. Instead, this tumor appears to share the same risk factors as HCC and iCCA, including liver cirrhosis, HBV, HCV, alcohol abuse, and chronic biliary disease. A deeper understanding of its etiology will enable the development of surveillance methods that do not exist for this tumor type.

A potential consensus lies in the concept that cHCC-iCCA emerges from a monoclonal cell of origin that includes HPCs and/or hepatocytes. The role of diverse tumor microenvironments (inflammatory, necroptotic, and apoptotic) in determining the differentiation of liver cells into the different lineages and phenotypes of PLCs remain uncertain and should be evaluated in future studies. The genetic landscape of cHCC-iCCA appears to be at a crossroad for the HCC and iCCA; however, whether cHCC-iCCA is genetically closer to HCC or iCCA remains inconclusive. Exploring new targets and signaling pathways in this tumor should be prioritized to identify future therapeutic approaches.

"Stem cell features" can be observed in all forms of cHCC-iCCA and are used to define the different subcategories of this tumor type. Currently, the diagnosis of cHCC-iCCA is primarily based on the histological (through H&E staining) recording of unequivocal hepatocytic and cholangiocytic differentiation, with a definable intimate intermingle **(Figure 6 A–B)**. Immunohistochemistry for hepatic and biliary markers assist in the confirmation of the diagnosis **(Figure 6 A–B)**, and the differential diagnoses of $CK19^{+}$ HCC and CLCs must be considered. To the best of our knowledge, the proportion of large and small duct morphologies in cHCC-iCCA is unclear. We believe that it would be of interest to examine the expression of mucin in future studies. Moreover, *nestin* (a new promising marker of prognostic relevance for cHCC-iCCA) may prove useful in differentiating cHCC-iCCA from HCC. The radiological features of cHCC-iCCA are heterogeneous and may exhibit the following: (i) typical features of both HCC and iCCA, (ii) an HCC-dominant pattern, or (iii) an iCCA-dominant pattern. Sometimes, a non-matching biomarker profile may lead the way to the diagnosis of cHCC-iCCA. For the atypical radiologic characteristics or the discordance between imaging and serum markers, liver biopsy should be considered for performing H&E and immunohistochemical staining to confirm the biphenotypic pattern. However, biopsy has limited sensitivity and specificity that may lead to misdiagnosis; therefore, the diagnosis of cHCC-iCCA should be reconsidered in the case of treatment failure.

The application of the TNM system for iCCA in cHCC-iCCA is evidently not optimal; therefore, cHCC-iCCA–exclusive independent TNM staging systems should be established in the future. Whether such

a staging system should include liver function (such as in the case of the BCLC system for HCC) also remains a matter of debate, because up to 50% of the cHCC-iCCA cases develop in a background of liver cirrhosis. Typically, the prognosis of cHCC-iCCA appears to be worse than that of HCC. Treatment of cHCC-iCCA currently mirrors the standard treatment for HCC or iCCA and mostly depends on the physician's discretion for each case made after considering the patient's performance status and comorbidities. Surgery is the best option for patients with localized disease. However, this option is only suitable for a minority of patients and is associated with high postoperative recurrence. TACE may represent a feasible option in hypervascular cHCC-iCCA. Gem- and platinum-based chemotherapy as well as TKI are widely used for the treatment of cHCC-iCCA; however, the efficacy of these regimens is limited. The results of a recruiting phase II trial (NCT05211323) evaluating the combination of Gem, Cis, Atezo, and Bev are eagerly awaited. The recent TOPAZ-1 trial of combined chemotherapy and immunotherapy has changed the standard of care for patients with CCA [139]. As PD-1/PD-L1-blocking agents proved to be effective for the treatment of HCC, their combination could be considered as a potential therapeutic regimen for cHCC-iCCA in future studies [140, 142].
We strongly encourage the international community to build up registries that will enable a larger prospective evaluation of patients with cHCC-iCCA and accumulate evidence from a larger number of cases, because this tumor cannot be effectively studied locally owing to its low incidence. Based on the high prevalence of druggable alterations in iCCA, we bring to the discussion whether all cHCC-iCCA cases should be submitted to molecular profiling to identify druggable targets. This approach is befitted for this rare tumor entity, particularly because no evidence-based therapeutic recommendations exist.

Statement of Ethics
An ethics statement is not applicable because this study is exclusively based on the published literature.

Conflict of Interest Statement
Florian Reiter received honoraria for lectures and travel support from the Falk Foundation, Novartis, Ipsen, and Gilead. Najib Ben Khaled has received reimbursement for meeting attendance fees and travel expenses from EISAI and lecture honorarium from Falk. Andreas Geier is advisory board or steering committee member to AbbVie, Alexion, Bayer, BMS, EISAI, Gilead, Intercept, Ipsen, MSD, Novartis, Pfizer, Roche, Sanofi-Aventis, Sequana. Enrico De Toni has served as a paid consultant for AstraZeneca, Bayer, BMS, EISAI, Eli Lilly & Co, Pfizer, IPSEN, and Roche; received reimbursement of meeting attendance fees and travel expenses from Arqule, AstraZeneca, BMS, Bayer, Celsion, and Roche; received lecture honoraria from BMS and Falk; and received third-party funding for scientific research from Arqule, AstraZeneca, BMS, Bayer, Eli Lilly, and Roche. The funding agencies were not involved in the study design and the data collection, analysis, and interpretation. The other authors have no conflicts of interest to declare.

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Author Contributions
Liangtao Ye, Julia Schneider, Najib Ben Khaled, Enrico De Toni, Changhua Zhang, and Florian Reiter contributed to the literature review and search, writing, formatting, and editing of the manuscript. Peter Schirmacher, Yulong He, and Andreas Geier contributed to critical revision of the manuscript

for important intellectual content. Liangtao Ye, Andreas Geier, Enrico De Toni, Changhua Zhang, and Florian Reiter designed the figures, answered the questions from the peer reviewers, and replied to their comments. Peter Schirmacher, Carolin Seifert, and Lea Frey contributed by improving the revision and provided images from the pathological sections of cHCC-iCCA. Liangtao Ye, Julia Schneider, Najib Ben Khaled, Peter Schirmacher, Lea Frey, Carolin Seifert, Yulong He, Andreas Geier, Enrico De Toni, Changhua Zhang, and Florian Reiter approved the submitted manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding authors.

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Figure Legends

Figure 1. Evolution of cHCC-iCCA classification. cHCC-iCCA, combined hepatocellular carcinoma-intrahepatic cholangiocarcinoma; ed., edition; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; WHO, World Health Organization. References, Sempoux 2019 [7], Wells 1903 [29], Allen 1949 [30], Goodman 1985 [34], Fritz 2000 [36], Bosman 2010 [50].

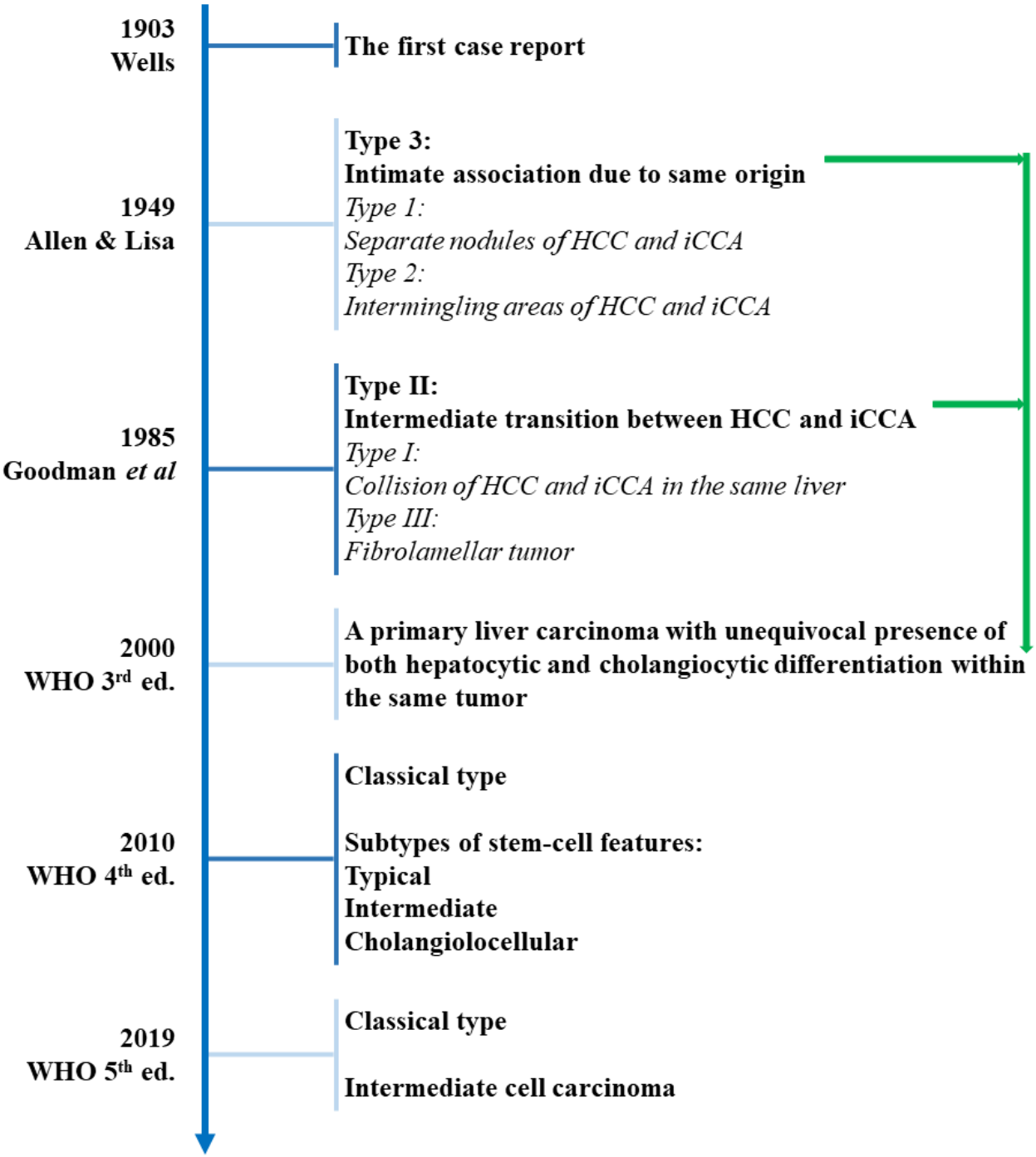
Figure 2. cHCC-iCCA classification. HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; WHO, World Health Organization (figure modified from "*Treatment of Combined Hepatocellular and Cholangiocarcinoma*" by Leoni 2020 [143]).

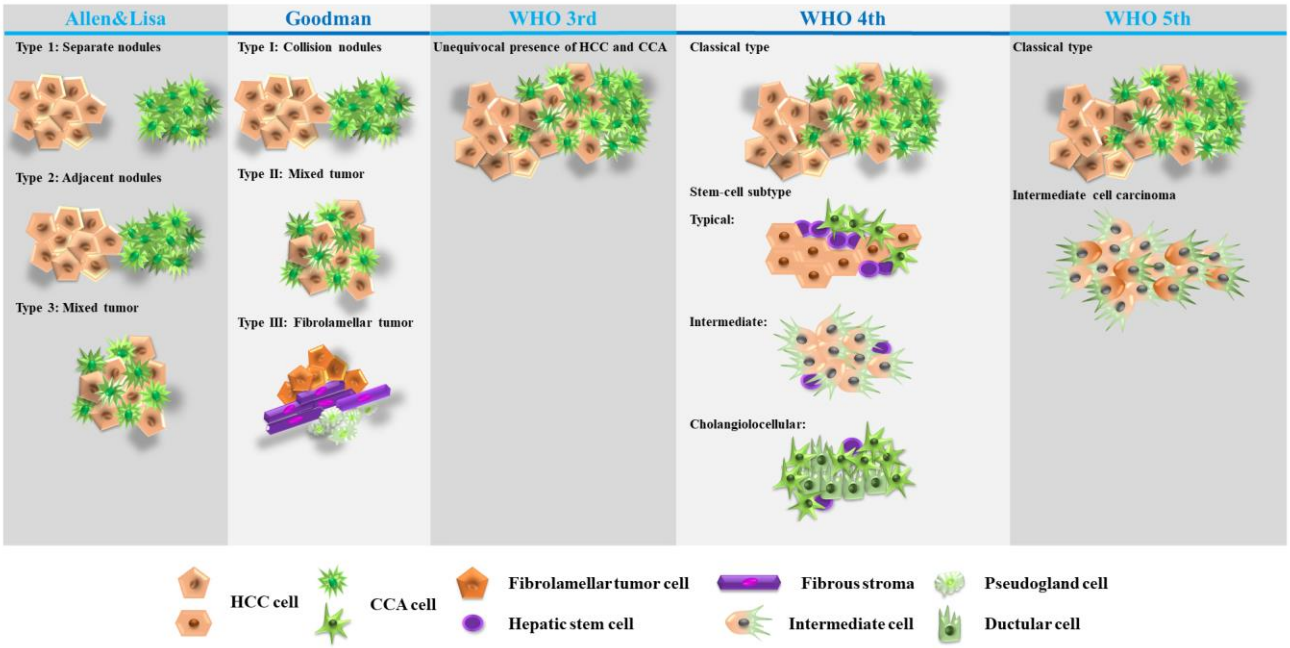
Figure 3. Representative images of intermediate cell carcinoma. H&E **(A)** and immunohistochemistry staining of intermediate cell carcinoma in a patient with CK19 **(B)**, Arginase **(C)**, HepPar-1 **(D)**, Glypican-3 **(E)**, EpCAM **(F)**, CD34 **(G)**, and Ladewig staining **(H)**. Magnification, 40×, scale bar=20 μm. H&E, hematoxylin and eosin; CK19, cytokeratin 19; HepPar-1, hepatocyte paraffin 1; EpCAM, epithelial cellular adhesion molecule.

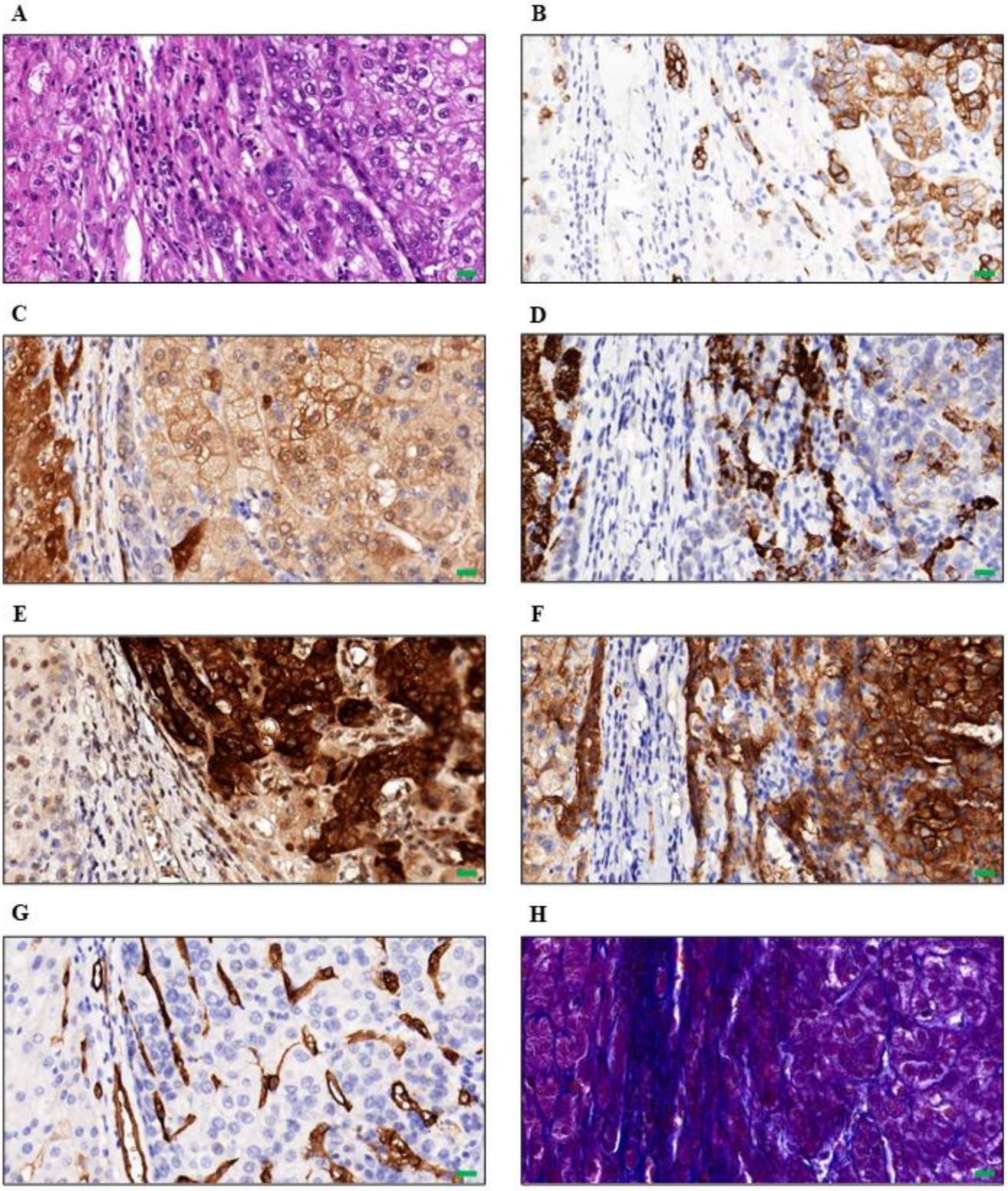
Figure 4. Potential cells of origin of cHCC-iCCA under the background of the tumor microenvironment. cHCC-iCCA, combined hepatocellular carcinoma-intrahepatic cholangiocarcinoma; CLC, cholangiolocellular carcinoma; HCC, hepatocellular carcinoma; HPC, hepatic progenitor cell; iCCA, intrahepatic cholangiocarcinoma (the figure is modified based on "*Cell of origin in biliary tract cancers and clinical implications*" by Moeini 2021 [82]). References, Wang 2020 [89], Chen 2012 [92], Tarlow 2014 [93], Schaub 2018 [97], Hill 2018 [144].

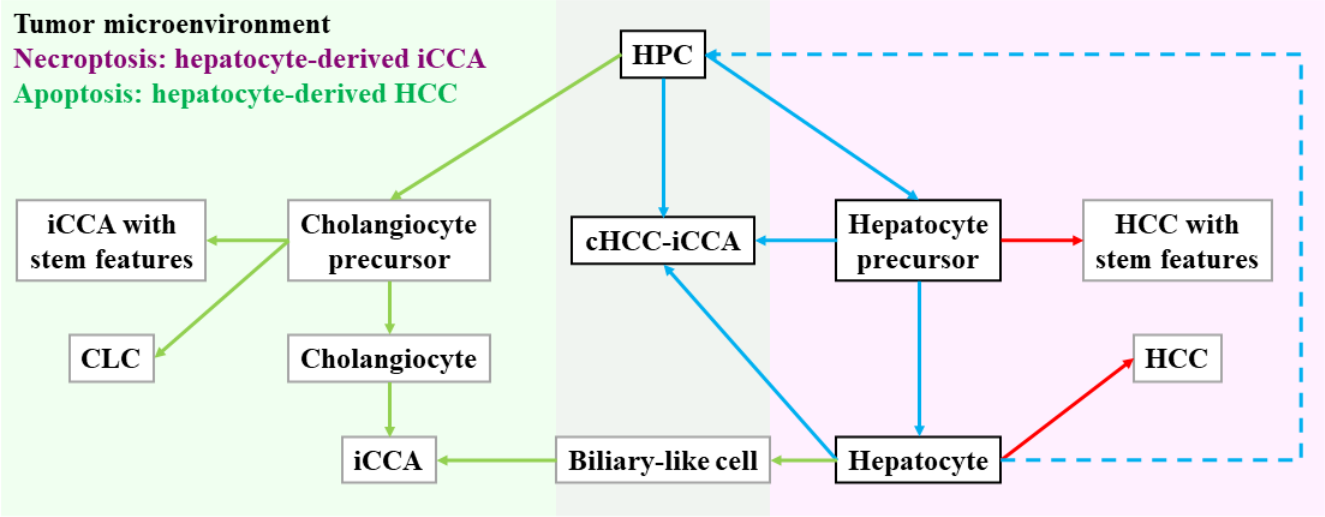
Figure 5. The genetic landscape of cHCC-iCCA. Boxes show common genetic alterations in cHCC-iCCA. Some of these alterations are found in all primary liver tumors (purple box), whereas some are more common in cHCC-iCCA and HCC (blue box) or cHCC-iCCA and iCCA (orange box). Therapeutic agents approved by the FDA or EMA for a specific genetic alteration independent of tumor type are listed. cHCC-iCCA, combined hepatocellular carcinoma-intrahepatic cholangiocarcinoma; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; n.a., not available. References, Cazals-Hatem 2004 [28], Moeini 2017 [51], Xue 2019 [60], Wang 2018 [81], Moeini 2021 [82], Coulouarn 2012 [87], Sasaki 2017 [95], Liu 2018 [100], Joseph 2019 [103], Fujimoto 2015 [104], Müller 2020 [105], Sasaki 2019 [145].

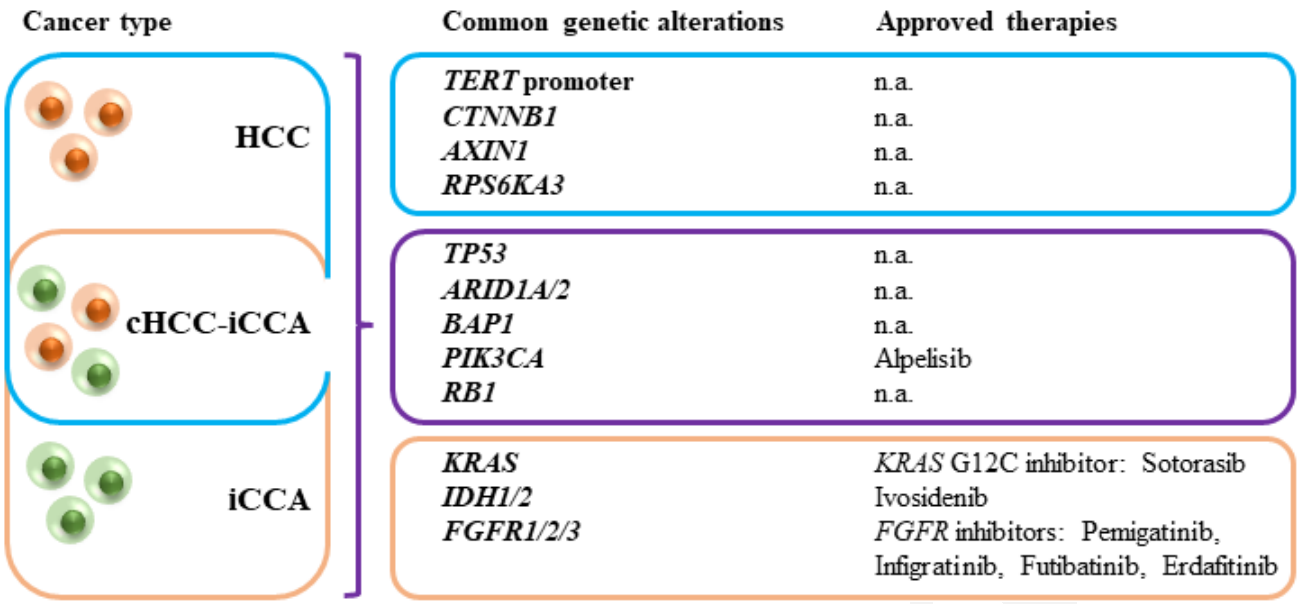
Figure 6. Representative images of classical type of cHCC-iCCA. A. Immunohistochemistry staining of cHCC-iCCA in patient 1 by CK7 (CCA marker) and HepPar-1 (HCC marker). Magnification, 60×, scale bar=20 μm. **B.** H&E and immunohistochemistry staining of cHCC-iCCA in patient 2 by CK7, HepPar-1, and Arginase (HCC marker). Magnification, 2×, scale bar=500 μm; 5×, scale bar=200 μm; 63×, scale bar=20 μm. CCA, cholangiocarcinoma; cHCC-iCCA, combined hepatocellular carcinoma-intrahepatic cholangiocarcinoma; CK7, cytokeratin 7; HCC, hepatocellular carcinoma; HepPar-1, hepatocyte paraffin 1; H&E, hematoxylin and eosin.











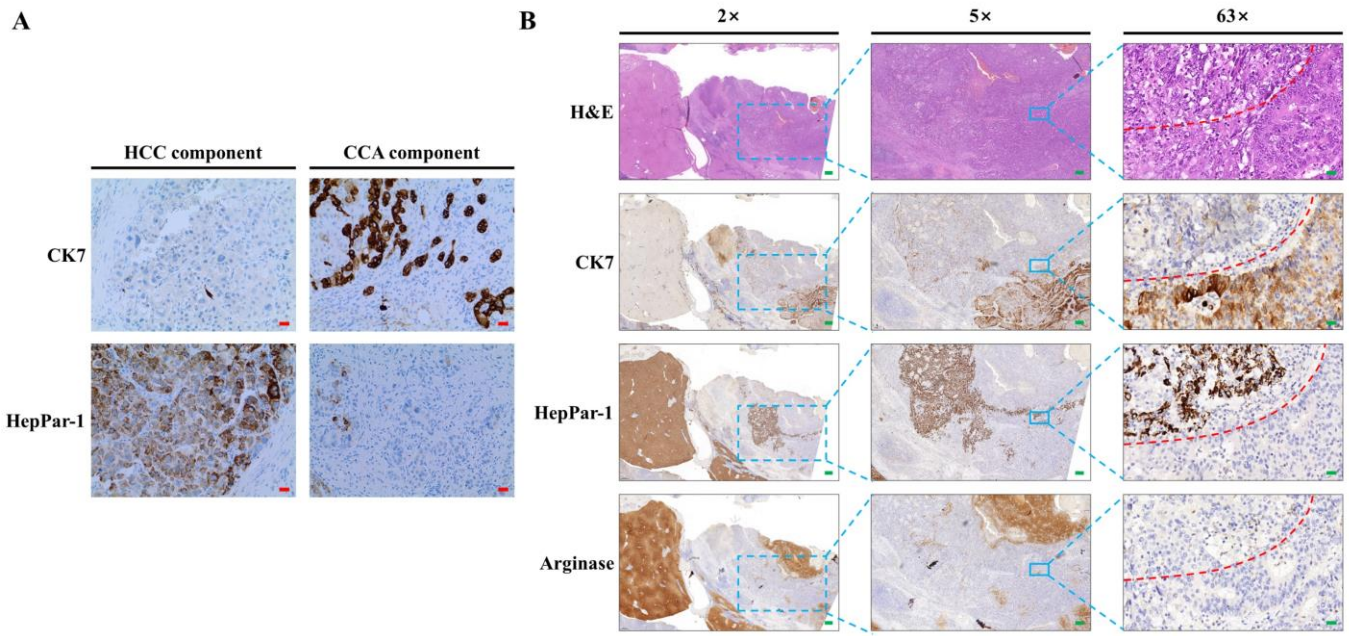


Table 1 Summary of genetic landscapes in cHCC-iCCA in comparison with independent HCC or iCCA.

Reference	Year	Genetic alterations in cHCC-iCCA	In HCC[Müller 2020 [105]]*	In iCCA[Moeini 2021 [82]]*
Cazals-Hatem[28]	2004	*TP53*; LoH at chromosomes *3p* and *14q*	*TP53* (15-45%); distinct: LoH at chromosomes *8p*, *17q*, *4q*, *16q*, *13q*, *6q*, and *7p*[*Stavraka 2018* [99]]	*TP53* (30%), MSI-H
Coulouarn[87]	2012	Dysregulation in *TGF-ß* and *Wnt/CTNNB1* signalings; increased *LEF1* and *SOX9* toward biliary phenotype	*CTNNB1* (15-35%)	-
Fujimoto[104]	2015	*TERT* promoter (53%), *ARID2* (27%), *PBMR1* (20%)	*TERT* promoter (40-60%)	-
Sasaki[95]	2017	*TP53* (45.3%), *TERT* promoter(31.3%), *ARID1A* (13.2%), *IDH1/2* (11.8%), *KRAS* (7.5%)	*TP53*, *TERT* promoter, *ARID1A* (5-15%)	*TP53*, *IDH1/2* (20%), *ARID1A* (15%), *KRAS* (15%)
Moeini[51]	2017	*TP53* (22%), *TERT* promoter (11%), *BRAF* (5%), *FGFR2-BICC1* fusion (5%), *IDH1* (5%)	*TP53*, *TERT* promoter	*TP53*, *IDH1*, *FGFR1-3* (20%), *BRAF* (3%)
Liu[100]	2018	*TP53*, *RYR3*, *FBN2*, *CTNNB1*, *ARID1A*, *KNCC3*, *MYC*	*TP53*, *ARID1A*, *CTNNB1*; distinct: *RYR3*, *FBN2*, *MYC* (less in HCC)	*ARID1A*
Wang[81]	2018	*TP53*, *mTOR*, *ARID2*	*TP53*	*TP53*
Xue[60]	2019	*TP53* (49%), *TERT* promoter(23%), *AXIN1* (10%), *ADGRV1* (10%), *HYDIN* (10%)	*TP53*, *TERT* promoter; distinct: *CTNNB1* (common in HCC)	Distinct: *KRAS* and *IDH1/2* (high in iCCA)
Joseph[103]	2019	*TP53* (80%), *TERT* promoter(80%), *MET/ERBB2/KRAS/PTEN* (55%), *CCND1/CCNE1/CDKN2A* (40%), *ARID1A/ARID2* (20%), *CTNNB1/AXIN/APC* (20%)	*TP53*, *TERT* promoter, *CTNNB1*, *ARID1A*	*TP53*, *KRAS*, *ARID1A*, *CNKN2A/B* (15%), *ERBB2/3* (7%), *MET* (5%)
Sasaki[145]	2019	*TP53* (46%), *TERT* promoter (25%), *ARID1A* (21%), *PBRM1* (20%), *IDH1/2* (8%), *KRAS* (5%), *ARID2* (3%)	*TP53*, *TERT* promoter, *ARID1A*	*TP53*, *KRAS*, *ARID1A*, *IDH1/2*

cHCC-iCCA, combined hepatocellular carcinoma-intrahepatic cholangiocarcinoma; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; LoH, loss of heterozygosity; MSI-H, microsatellite instability-high. *, genetic features of cHCC-iCCA in comparison with individual HCC or iCCA, but not refer to the HCC or iCCA portions inside cHCC-iCCA.

Table 2 Potential *in vitro*, *in vivo*, and *ex vivo* models of cHCC-iCCA.

Reference	Year	Origin	Type	Signaling	Method	Model
in vitro						
Murakami[106]	1987	Hepatectomy	cHCC-iCCA	n.a.	Primary human cell culture	KMCH-1
Yano[107]	1996	Hepatectomy	cHCC-iCCA	n.a.	Primary human cell culture	KMCH-2
Gil-Benso[108]	2001	2AAF-induced	cHCC-iCCA	n.a.	Primary cell culture of rats	CC-62
in vivo						
Carlson[114]	2005	Hepatocytes	cHCC-iCCA	*V12NRAS*	Transposon-based	C57BL/6J p19*Arf*-null
Piscaglia[109]	2009	HPCs	cHCC-iCCA	2AAF/partial-hepatectomy/aflatoxin-B1	Chemical and surgery	F344 rats; established cell lines named LCSCs
Lu[85]	2010	HPCs, hepatoblasts	HCC, iCCA	*Hippo-YAP*	GEMM	*Alb-Cre*; $sav1^{flox/flox}$; $mst1^{flox/flox}$; $mst2^{flox/flox}$
O'Dell[86]	2012	HPCs, hepatocytes and cholangiocytes	cHCC-iCCA, HCC, iCCA	*RAS* and *TP53*	GEMM	*Alb-Cre*; $KRAS^{LSL-G12D/+}$; $TP53^{flox/flox}$
Chen[84]	2014	HPCs	cHCC-iCCA	*PTEN* and *GRP94*	GEMM	*Alb-Cre*; $PTEN^{flox/flox}$; $GRP94^{flox/flox}$
Tschaharganeh[61]	2014	HPCs, hepatoblasts	HCC, iCCA	*TP53*	GEMM	*Alb-Cre*; $TP53^{flox/flox}$
Li[110]	2015	Hepatocytes	cHCC-iCCA, HCC, iCCA	*YAP* and *AKT*	Transposon-based	Overexpression of *YAP* and *PIK3CA*
Nishio[111]	2016	HPCs, hepatocytes and cholangiocytes	cHCC-iCCA, iCCA	*YAP/TAZ* and *TGF-ß*	GEMM	*Alb-Cre*; $Mob1a^{flox/flox}$; $Mob1b^{-/-}$
Hill[144]	2018	Hepatocytes	cHCC-iCCA, HCC, iCCA	*RAS* and *TP53*	GEMM with liver injury	DDC diet; *AAV8-TBG-Cre*; $KRAS^{LSL-G12D/+}$; $TP53^{flox/flox}$
He[98]	2019	Hepatocytes	cHCC-iCCA, HCC	*NF-κB* and *MYC*	GEMM	Overexpression of *MYC* and liver-specific deletion of *NEMO*; *Alb-Cre*; $MYC^{LAP\ tTA}$+doxycycline; $NEMO^{\Delta LPC}$
Cai[88]	2020	Hepatoblasts	cHCC-iCCA	*TP53*	GEMM	Isolation of hepatoblasts from B6.129S2-$TP53^{tm1Tyj}$/*J* ($p53^{-/-}$) mice; intra-splenic injection in C57BL/6 mice
Lu[112]	2020	n.a.	cHCC-iCCA, HCC	Aristolochic acid	Chemical-induced	C57BL/6J
Wang[89]	2020	Hepatocytes	cHCC-iCCA, HCC, iCCA	*AKT* and *CAT*	Hydrodynamic transfection	BALB/c; *AKT/CAT* plasmid injection
ex vivo						
Broutier[115]	2017	Hepatectomy	cHCC-iCCA	n.a.	Patient-derived organoids	Drug screening assays

2AAF, 2-acetylaminofluorene; cHCC-iCCA, combined hepatocellular carcinoma-intrahepatic cholangiocarcinoma; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; GEMM, genetically engineered mouse model; HCC, hepatocellular carcinoma; HPCs, hepatic progenitor cells; iCCA, intrahepatic cholangiocarcinoma; Mob1a/1b, mps one binder kinase activator; NEMO, NF-κB essential modulator; n.a., not applicable.

Table 3 Systematic chemotherapy and immunotherapy of cHCC-iCCA: chronological overview.

Reference	Year	Background	Treatment (n)	Primary endpoint	Study type	Results	Enrollment, n
Hayashi[123]	2006	With lymph node metastases; received TACE	Cisplatin+5-FU+radiation	-	A case	SD over 42 mo	1
Kitamura[129]	2008	With lymph node metastases; HCV positive	5-FU+cisplatin; gemcitabine	-	A case	SD of 6 mo	1
Hatano[124]	2009	Hepatectomy+interferon/5-FU arterial chemotherapy; lymph node metastases after 9 mo	S-1	-	A case	PR	1
Shimizu[130]	2009	Hepatectomy with lymph node recurrence	UFT (tegafur/uracil)	-	A case	SD of 7 mo	1
Kim[131]	2010	Recurrence with lung and bone metastases; sorafenib failed	Doxorubicin+cisplatin (8 cycles); 5-FU (15 cycles till reported)	-	A case	SD of 18 mo	1
Tani[132]	2011	With lung metastases; HBV positive	Gemcitabine+carboplatin+5-FU (10 mo); hepatic arterial infusion+cisplatin (4×); extended left hepatectomy; continued chemotherapy	-	A case	SD till 18 mo	1
Chi[128]	2012	Recurrence with lung metastases	Gemcitabine+cisplatin	-	A case	SD of 12 mo; died of disease at 41 mo	1
Connell[127]	2015	Recurrent unresectable or metastatic	Gemcitabine or 5-FU (5); PCT (6); PCT+sorafenib (6); sorafenib (8); clinical trials (3)	PFS and OS	Retrospective	PFS (2.37 mo), OS (10 mo). Gemcitabine or 5-FU/sorafenib/PCT/PCT+sorafenib: PFS: 1.8/3.1/4.5/8.2 mo; OS: 1.8/7.6/8.4/14.7 mo	28
Rogers[133]	2017	Unresectable	Gemcitabine (1); gemcitabine+cisplatin+IMRT (1); gemcitabine+bevacizumab (2); sorafenib (3; 1 with stereotactic radiation)	PFS and OS	Retrospective	PFS (3.4 mo), OS (8.3 mo). SD (2): gemcitabine+cisplatin+IMRT/+bevacizumab progression (5)	7
Kobayashi[134]	2018	Unresectable	Gemcitabine+cisplatin (12); 5-FU+cisplatin (11); sorafenib (5); others* (8)	PFS and OS	Multicenter retrospective	ORR 5.6%. PFS (2.8 mo), OS (8.9 mo). Gemcitabine+cisplatin/5-FU+cisplatin/sorafenib/others: OS: 11.9/10.2/3.5/8.1 mo	36
Salimon[135]	2018	Unresectable	Gemcitabine+oxaliplatin (18); gemcitabine+oxaliplatin+bevacizumab (9); gemcitabine+cisplatin (3)	PFS and OS	Multicenter retrospective	PFS (9.0 mo), OS (16.2 mo). PR (8), SD (14), progression (6)	30
Trikalinos[23]	2018	Local treatments** (11); hepatectomy (11; 1 with TACE)	Gemcitabine+platinum (41); gemcitabine+5-FU (16); sorafenib (7); 5-FU (3); erlotinib+bevacizumab (1)	PFS and OS	Retrospective	OS (12.1 mo). Gemcitabine+platinum/others: OS: 11.7/11.5 mo. Gemcitabine+platinum/gemcitabine+5-FU/sorafenib: PFS: 8.0/6.6/4.8 mo	68
Tahover[138]	2019	No genomic alterations, stable microsatellite status; 7 of 9 immune checkpoint genes overexpressed (A variant in CDK12)	Ipilimumab+nivolumab; nivolumab monotherapy	-	A case	CR over 11 mo	1
Rizell[137]	2020	Hepatectomy with lung metastases; sorafenib failed; gemcitabine+cisplatin failed	Pembrolizumab	-	A case	CR over 33 mo	1

5-FU, 5-fluorourcil; CR, complete remission; cHCC-iCCA, combined hepatocellular carcinoma-intrahepatic cholangiocarcinoma; HBV, hepatitis B virius; HCV, hepatitis C virius; IMRT, intensity-modulated radiation therapy; mo, months; OS, overall survival (median); ORR, overall response rate; PR, partial response; PFS, progression-free survival (median); PCT, platinum-based combination

therapy; SD, stable disease; TACE, transhepatic arterial chemoenbolization; *, including: S‐1 (4); gemcitabine (2); 5-FU+interferon (1); S-1+gemcitabine (1); **, including TACE, yttrium-90, hepatic artery infusion, or in combination.

Table 4 Registered trials for the treatment of cHCC-iCCA.

Trial identifier	Design	Intervention	Primary endpoint	Study type	Planned enrollment, n	Status
NCT03178409	cHCC-iCCA compared with HCC and iCCA	Hepatectomy	OS and DFS	Observational case-control	100	completed
NCT04848805	cHCC-iCCA compared with HCC	Liver transplantation	Retrospective	Observational	279	Recruiting
NCT03230318	cHCC-iCCA with *FGFR2* Gene Fusions or *FGFR2* Gene Mutations or Amplifications	Derazantinib, 300 mg/day orally	ORR and PFS	Phase II	143	Recruiting
NCT05211323	Advanced cHCC-iCCA	Atez/Bev plus Gem/Cis vesus Gem/Cis alone	PFS	Phase II	88	Recruiting