Therapy of Primary Liver Cancer

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Primary liver cancer (PLC) is a fatal disease that affects millions of lives worldwide. PLC is the leading cause of cancer-related deaths and the incidence rate is predicted to rise in the coming decades. PLC can be categorized into three major histological subtypes: hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), and combined HCC-ICC. These subtypes are distinct with respect to epidemiology, clinicopathological features, genetic alterations, and clinical managements, which are thoroughly summarized in this review. The state of treatment strategies for each subtype, including the currently approved drugs and the potential novel therapies, are also discussed.

KEYWORDS: PRIMARY LIVER CANCER; HEPATOCELLULAR CARCINOMA; INTRAHEPATIC CHOLANGIOCARCINOMA; COMBINED HCC-ICC; PLC THERAPY

Introduction

Primary liver cancer (PLC) is a deadly malignancy with significant histological and biological heterogeneity, and ranks as the fourth leading cause of cancer-related death worldwide.[1,2] Therefore, it has become a major public healthy challenge. Over the past decades, the morbidity and mortality associated with PLC have steadily risen. According to Globocan's latest Global Cancer Statistics Report, 841,080 cases of liver cancer were reported worldwide in 2018, accounting for 4.7% of the total cancer cases in the same period, while deaths totaled 781,631, accounting for 8.2% of total cancer deaths.[3] On the basis of annual projections, the World Health Organization estimates that 1,276,679 patients will die from liver cancer in 2040. Incidence and mortality of PLC differ widely between regions. The highest incidence of PLC was observed in East Asia and in sub-Saharan Africa.[4] In particular, China experiences the highest number of cases of PLC, with a high incidence rate (18.3 cases/100,000 inhabitants).[5]

PLC manifests as three subtypes: hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), and combined HCC-ICC (cHCC-ICC), which differ notably in epidemiology, clinicopathological morphology, genetic alteration, and appropriate therapeutic responses. HCCs are primarily related to viral infection, alcohol abuse, and metabolic syndrome,[6] whereas ICCs are mainly associated with chronic liver inflammation and biliary tract diseases.[7,8] Risk factors for development of cHCC-ICC include overweight, obsess, nonalcoholic steatohepatitis, and liver cirrhosis.[9,10] HCCs show a solid and trabecular pattern with local invasion restricted to the liver,[11–13] whereas ICCs are ductular, papillary, or solid tumor structures with high metastasis to distal organs.[14–16] cHCC-ICCs are the combination of the HCC and ICC phenotypes present in liver tissue, and are classified into separate, combined, and mixed cHCC-ICC subclasses, which are more aggressive and have a poorer prognosis.[2,17–21]

The three PLC subtypes have distinct genetic alterations and molecular patterns. HCCs are associated with genetic alterations in specific chromosomal regions and genes, including TERT promoter mutation, TP53 deletion, and WNT signaling (CTNNB1 and AXIN1) activation.[22–29] ICCs show a unique mutational landscape with recurrent mutations, with the genetic alterations in TP53, KRAS, isocitrate dehydrogenase (IDH) 1/2, and fibroblast growth factor receptor (FGFR) gene fusions.[30–35] Combined cHCC-ICCs

show strong ICC-like features, whereas mixed cHCC-ICCs show HCC-like features.[36,37] Understanding the molecular alterations that initiate various PLC subtypes is of great importance for us to decipher the mechanisms of tumorigenesis. Genetic alterations can be transformed into biomarkers that may represent new therapeutic targets, affect the treatment decisions, and ultimately improve the treatment of liver cancer patients. HCCs mainly respond to targeted therapy, immunotherapy, and antiviral agents, while ICC patients benefit from classical chemotherapy, targeted therapy, and immunotherapy. Based on the pathological classification and the molecular features of cHCC-ICCs, combined cHCC-ICCs should be treated with similar therapies to ICCs, whereas mixed cHCC-ICCs are treated more like HCCs. In this review, we systematically summarize the epidemiology, pathogenesis, genetic alteration, and treatment for each subtype and comprehensively describe current therapy drugs and the potential novel therapies for PLC.

Epidemiology and Risk Factors. HCC. HCC represents the major histologic subtype, accounting for approximately 80% of all cases of PLC. The risk factors for HCC includes hepatitis B virus (HBV)/hepatitis C virus (HCV) infection; aflatoxin B1; alcoholic abuse; and nonalcoholic, metabolic symptoms, such as diabetes and obesity.[6] According to the Global Burden of Disease from 1990 to 2015, HBV and HCV accounted for 432,000 liver cancer deaths (54%), alcohol for 245,000 (30%), and other causes for 133,000 (16%) deaths. In particular, 55% of all HCC cases worldwide are reported from China[38] due to the locally high prevalence of HBV infection.

ICC. As the second most common liver carcinoma following HCC, ICC accounts for around 15% of PLC cases, with a high incidence of 2 per 100,000 population worldwide annually.[39] The most common risk factors for ICC are biliary tract diseases, including choledochal cysts; cholelithiasis; choledocholithiasis; liver flukes; viral hepatitis; metabolic syndrome; and other risk factors, including tobacco and alcohol use, and cirrhosis.[7] Recently, the incidence of ICC has been increasing more rapidly owing to risk factors[8] including increasing chronic liver disease and environmental toxins, and is found more often due to improved diagnostic tools and imaging.

cHCC-ICC. cHCC-ICC presents as a heterogeneous tumor showing both hepatocyte and cholangiocyte differentiation, and has a poor prognosis.[40] cHCC-ICC is a rather rare tumor with an incidence rate less than 5%.[1] The poor prognosis associated with cHCC-ICC is due to the limited treatment options and difficulty of diagnosis. To date, the largest cohort analysis, which included 529 patients diagnosed with cHCC-ICC between 2004 and 2014 across 18 registries,[41] reported that the incidence of cHCC-ICC in men and women was 0.08 and 0.03 per 100,000 per year respectively, with the average age of 63 years at diagnosis. One- and 5-year cause-specific survival rates for cHCC-ICC were 41.9% and 17.7%, respectively, with the median survival of 8 months. Among racial groups, cHCC-ICCs are most common in Asian races and Pacific Islanders. Obesity, nonalcoholic steatohepatitis, and liver cirrhosis were observed in some cHCC-ICC cohorts[9,10] and are potential risk factors for cHCC-ICC.

Clinicopathological Features. HCC. HCC shows a solid, trabecular, and pseudoglandular pattern with a high density of tumor cells. It has three subtypes: well-differentiated HCC, moderately differentiated HCC, and poorly

differentiated HCC.[11–13] Well-differentiated HCCs are often small (less than 2 cm in diameter) and are composed of cells with a higher nuclear to cytoplasmic ratio, arranged in a thin trabecular pattern with rare pseudoglandular structures. Moderately differentiated HCCs are usually larger tumors (larger than 3 cm) showing polygonal tumor cells in a thick trabecular arrangement with a frequent pseudoglandular pattern. Poorly differentiated HCCs are composed of pleomorphic tumor cells in a solid or compact growth pattern.

ICC. ICC can be divided into two subtypes: a small duct type that originates from small intrahepatic ductules with no or minimal mucin production, and a large bile duct type that arises from large intrahepatic ducts proximal to the bifurcation of the right and left hepatic ducts, with high mucin production ability.[14–16] Further, ICC shows three different growth patterns: mass forming (MF), periductal infiltrating (PI), and intraductal growth (IG).[42] MF ICC is a firm, multilobulated, unencapsulated, white-gray tumor, owing to its extensive desmoplastic stroma. The PI subtype shows extensive infiltration along the intrahepatic hilum structure, and the IG subtype is usually restricted to tubes with papillary structures. MF ICC is the most common type associated with a poor prognosis, while IG type is rare but has a favorable prognosis.[17]

cHCC-ICC. Though the phenomenon of HCC and ICC being present in the same liver was first described in 1903,[17] cHCC-ICC was not systematically described until 1949, when it was classified into three subtypes depending on the location of HCC and ICC: type A (separate type) has separate nodules of hepatocellular and bile duct carcinoma; type B (combined type) shows contiguity with intermingling but with clearly defined areas; type C (mixed type) presents as intimate association without clear boundaries.[18] In 1985, another classification system with three subtypes was established: type I (collision tumors) is the simultaneous occurrence of both HCC and ICC in the same patient; type II (transitional tumors) is an identifiable intermediate transition between HCC and ICC; type III (fibrolamellar tumors) resembles the fibrolamellar variant of HCC but also contains mucin-producing pseudoglands.[19] Presently, the World Health Organization (WHO) 2010 classification is commonly used, in which cHCC-ICC is classified into two main types, the classic type and the stem cell (SC) type (subtype with SC features), with the SC type subdivided into three subtypes, including the typical subtype, intermediate subtype (INT), and cholangiocellular type[43].

The lack of a unified classification system greatly adds to the difficulty for cHCC-ICC research, and the clinicopathological characteristics of cHCC-ICC remain ill-defined. cHCC-ICC can exhibit stem/progenitor cell phenotypes consisting of small cells with scant cytoplasm; hyperchromatic nuclei embedded within a thick, desmoplastic stroma; a high nuclear/cytoplasmic ratio; and increased mitotic activity.[1] In addition, the immunohistochemistry has identified stemness-related markers (KRT19, CD56, EpCAM, CD117, CD113, OV6).[1,20] cHCC-ICC clinicopathologic characteristics include more frequent multifocal lesions, more microvascular emboli, and portal vein and lymph node invasion, all of which indicate a poor prognosis.[21]

Genetic Alterations. HCC. Wide-scale genomic studies have revealed that hundreds of somatic DNA alterations accrue in HCC, including chromosome aberrations and mutations. High-level DNA amplifications are enriched in chromosome locations 6p21 and 11q13 in HCC,[44] which occur in 5%–10% of cases. Recently, some oncogenic genes were identified in the regions of frequent DNA gain. For example, LINC01138 is an oncogenic long intergenic non-coding RNA located in this region, which has been identified as a driver of HCC.[45] VEGFA and CCND1/FGF19 have also identified in these regions and are potential therapeutic targets.[46] Loss of heterozygosity on chromosome 8p is a frequent event in HCC.[47] These DNA alterations are often associated with cancer progression due to the deletion of tumor suppressor genes. Intriguingly, in these regions, a variety of vulnerability genes have recently been identified. For example, TSLNC8 was characterized as a tumor suppressor gene on chromosome 8p12, the region that shows allelic loss in HCC, and was shown to inhibit the proliferation and metastasis of HCC.[48] The genetic mutations of HCC have been well studied. Mutations in the TERT promoter occur in approximately 60% of cases and cause recurrent viral insertion of HBV.[49] Deletion mutations in TP53 are the most frequent genetic alterations, accounting for about 30% of cases,[22–29] and are thought to be the initiating event driving the formation of precursor lesions. Mutated genes in WNT

signaling (CTNNB1 and AXIN1) and chromatin remodeling (ARID1A) account for approximately 27%–40% of cases.[22–29] Accumulation of activating mutations in oncogenes, including activation of AKT or mTOR and of the oxidative stress pathway activation, occurs throughout tumor progression, and could be potentially targeted with molecular therapies in the future.

ICC. ICC shows a unique mutational landscape with recurrent mutations, compared with HCC. It harbors the genetic alterations in TP53, KRAS, ARID1A, BAP1, IDH1, IDH2, PIK3CA, SMARCB1, EPHA2, SMAD4, GNAS, and PBRM1, as well as FGFR gene fusions.[30–35] Gain of function of IDH1 and IDH2 mutation on R132 and R172 two hotpot codons was observed in 10%–28% of ICC cases.[32] Fusions, amplifications, translocations, and rearrangements of FGFR genes are found in ICC and are closely related to the initiation and progression of ICC.[50] The activating mutation of KRAS (15%–20%) is another frequent genomic alteration in ICC.[31,51,52] The KRAS mutation often exists concurrently with FGFR2 fusions and IDH mutations, suggesting a possible cooperative role in ICC pathogenesis.[53,54] In addition, recent studies have shown that BRAF and Notch are considerably more prevalent in ICC and function in ICC pathogenesis.[55]

cHCC-ICC. cHCC-ICCs are genetically complex tumors. The combined subtype of cHCC-ICC shows strong ICC-like features, with the high expression of EPCAM, KRT19, PRDM5, and KRAS. The mixed subtype of cHCC-ICC shows HCC-like features with the high expression levels of AFP, GPC3, APOE, SALL4, and AFP81.[36]

The most frequent mutation observed in cHCC-ICCs is TP53, with a strikingly high 49.2% mutation frequency, much higher than that in HCC (20%–35%) and ICC (18%–38%).[36,56] Interestingly, several studies have found that the disruption of Trp53 alone in livers of mice can induce the formation of cHCC-ICC,[37,57] which further implies that TP53 may be the driver gene in cHCC-ICC. It is notable that Nestin, a type VI intermediate filament (IF) protein that is commonly used as a neuroectodermal SC marker, is highly expressed in cHCC-ICC and is strongly associated with poorer prognosis.[36] Hence, Nestin may be a promising biomarker for cHCC-ICC.

Challenges and Limitations of Current Treatment Strategies. Resection, Transplantation, Local and Regional Therapies. HCC. The commonly used staging system for HCC is the Barcelona Clinic Liver Cancer staging system (Figure 1). HCCs in the very early stage or intermediate stage can be treated with local regional therapies, which include radiofrequency ablation (RFA), resection (da Vinci surgery, laparoscopic surgery, or traditional surgery), transplantation (orthotopic liver transplantation, piggyback transplantation, split liver transplantation, auxiliary liver transplantation), percutaneous ethanol injections (PEI), or transcatheter arterial chemoembolization (TACE).[58]

ICC. Surgery is currently the only curative treatment for ICCs but only a minority of patients in early stages are considered candidates for resection. In surgery, ICC is usually treated with hepatic resection to achieve negative resection margins.[59] For patients with locally unresectable ICC, tumor ablation such as RFA, or hepatic artery-based therapies like yttrium-90 radioembolization, appear promising.[59–64]

cHCC-ICC. An accurate diagnosis is of paramount importance for the treatment of cHCC-ICC. Currently, major hepatectomy is the optimal management for cHCC-ICC.[65] The rarity of this cancer as well as the lack of biomarkers have made this cancer difficult to diagnosis and manage. Surgical resection remains the only curative option for patients with cHCC-ICC.

The treatment options for cHCC-ICC are similar to those for HCC and ICC and include surgery, radiation, yttrium-90 radioembolization, chemotherapy, combined radiation and chemotherapy, combined surgery and chemotherapy, and triple therapy (surgery, radiation, and chemotherapy).[41,66–69] A recently retrospective analysis from 2001 to 2015 of 623 PLC patients, including 47 cHCC-ICC, 468 HCC, and 108 ICC patients, who underwent resection found that although cHCC-ICC is more poorly differentiated than HCC and ICC, it had a similar 5-year survival rate (49.7%, 54.8%, and 68.7%, respectively) and 3-year recurrence rate (57.9%, 61.5%, 56%, respectively).[70]

Systemic Chemotherapy. HCC. Systemic chemotherapy has limited efficacy on HCC: several clinical trials of chemotherapy have shown low response rates and worse toxicity without a significant improvement in
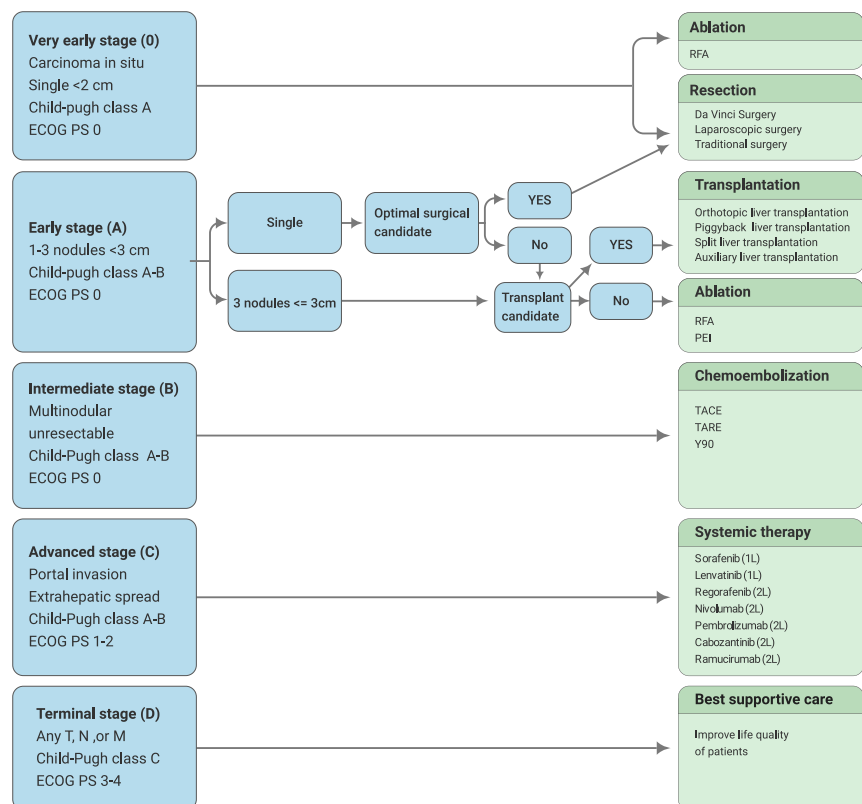
Figure 1. Barcelona Clinic Liver Cancer Staging System and Corresponding Treatment Options. The schematic diagram illustrates therapeutic choice by which a treatment theoretically recommended for a different stage is the best treatment option. 1L, first-line; 2L, second-line; ECOG, Eastern Cooperative Oncology Group; M, metastasis stage; N, nodal stage; PEI, percutaneous ethanol injection; PS, performance status; T, tumor stage; TACE, transarterial chemoembolization; TARE, transarterialradioembolization; Y-90, Y-90 radioembolization

the overall survival (OS), including gemcitabine- and doxorubicin-based treatment, FOLFOX (5-fluorouracil [5-FU], leucovorin, oxaliplatin) and PIAF (cisplatin/interferon alpha-2b/doxorubicin/5-FU).[71–74] This suggests a limited role for traditional chemotherapy in the treatment of advanced HCC.

ICC. Current first-line standard of treatment for ICC is the combination of gemcitabine and platinum-derived chemotherapy (Figure 2B). With the poor prognosis, the median survival of advanced ICC patients is less than one year. Very limited effective treatments are available for patients who progress on first-line chemotherapy, so there is a high medical demand.

First-Line Treatment. Effective molecular targeted therapy and immunotherapy is lacking, so chemotherapy, with gemcitabine, platinum compounds, and fluoropyrimidines, is still the mainstream of standard treatment for unresectable ICC.

The primary chemotherapy for ICC is gemcitabine, which was established as the first-line therapy for advanced biliary tract cancer (BTC) in 1999. In 2010, the randomized, controlled, ABC-02 phase III clinical trial compared the benefit of gemcitabine plus cisplatin (CisGem) chemotherapy with the single agent gemcitabine.[75] This study showed an advantage for CisGem in OS (11.7 months versus 8.1 months; hazard ratio [HR], 0.64; 95% confidence interval [CI], 0.52–0.80) and progression-free survival (PFS) (8.0 months versus 5.0 months, p < 0.001). This effectiveness was confirmed in a Japanese randomized phase II study, BT22 (median OS, 11.2 months versus 7.7 months; HR, 0.69).[76] Based on these promising results, CisGem is currently regarded as the standard of care in the first-line treatment for advanced cholangiocarcinoma.

Other than cisplatin, gemcitabine plus other agents such as oxaliplatin, S-1, capecitabine, bevacizumab, and Nab-paclitaxel have also been considered as the first-line choices for advanced cholangiocarcinoma based on the promising outcomes from several phase II or III trials.[77–91]

A recent multicenter, randomized, phase III clinical trial (NCT01470443) showed that XELOX has the comparable efficacious effect to GEMOX in terms of tumor response, survival rate (OS and PFS), and safety. Also, XELOX has an advantage of low hospital visits, compared with GEMOX. Thus, XELOX could be an alternative for cholangiocarcinoma therapies.

Second-Line Treatment. There is no established standard second-line chemotherapy for advanced cholangiocarcinoma, and all regimens have shown limited efficacy, with a median PFS of around 3 months and median OS of about 7 months.[92]

FOLFOX (L-folinic acid, 5-FU, and oxaliplatin) is an optional second-line treatment option based on the randomized, phase III, multicenter, open-label ABC-06 study (NCT01926236). FOLFOX showed increased benefit for median OS, 6 months and 12 months, and OS rate (%): 6.2 months, 50.6% and 25.9% compared with 5.3 months, 35.5% and 11.4% for the control group (active symptom control [ASC] arm).[92]

Currently several phase II and III chemotherapy clinical trials are under way (Table 3). Combined therapy with chemotherapy shows promise in the treatment of cholangiocarcinoma: selective internal radiotherapy (SIRT) plus chemotherapy or hepatic arterial infusion plus systemic chemotherapy both had antitumor activity and are promising for the treatment of ICC.[93,94]

cHCC-ICC. In contrast to surgery-based treatments for resectable cHCC-ICC, systemic therapy is the nonstandard option for advanced and unresectable cHCC-ICC, based on the standard treatment strategy for the unresectable HCC or ICC. Chemotherapy for advanced or unresectable cHCC-ICC is largely understudied, with only a few case reports and some retrospective studies having been published.[9,10,95–101] Recently, a multicenter retrospective analysis has been conducted by Kobayashi and colleagues.[10]

According to divided-group treatment with (1) gemcitabine plus cisplatin (n = 12), (2) 5-FU plus cisplatin (n = 11), (3) sorafenib monotherapy (n = 5), (4) others (n = 8), they found that 36 patients with platinum-containing treatment had longer OS time than those treated by sorafenib monotherapy, showing OS of 11.9 months (95% CI, 4.9–18.8), 10.2 months (95% CI, 3.9–16.6), 3.5 months (95% CI, 0.0–7.6), and 8.1 months (95% CI, 0.9–15.4), respectively.

A similar conclusion was drawn in another retrospective study of 123 cHCC-ICC patients, with 68 receiving gemcitabine-based therapy (gemcitabine + platinum or gemcitabine + 5-FU) or targeted agents (sorafenib).[9] Median PFS favored gemcitabine/platinum and gemcitabine/5-FU (8.0 and 6.6 months, respectively) over sorafenib monotherapy (4.8 months).

A

Advanced HCC								
1st line	Sorafenib*	Lenvatinib*		Atezolizumab plus Bevacizumab		Tremelimumab plus Durvalumab		Donafenib

2nd line	VEGF/R inhibitors	TGF-β inhibitors	c-MET inhibitors	CDKs inhibitors	FGFR4 inhibitors	PD-1 inhibitors	PD-L1 inhibitors	CLAT-4 inhibitors	Other novel therapies
	Regorafenib* Cabozantinib* Ramucirumab* Bevacizumab Apatinib	Galunisertib	Tepotinib Tivantinib	Palbociclib Ribociclib Milciclib	Fisogatinib	Nivolumab* Pembrolizumab Tislelizumab Toripalimab Sintilimab Camrelizumab Penpulimab CS1003	Atezolizumab Durvalumab Avelumab CS1001	Ipilimumab* Tremelimumab	ALT-803 (IL-15 superagonist) KY1044 (T cell CO-stimulator) Pexa-Vec(oncolyticvirus) BMS-986253 (IL-8 antibody) Entecavir /tenofovir DF /Vemlidy (antiviral) CAR-T DC vaccines

B

Advanced ICC								
1st line	Gemcitabine plus Cisplatin*		Gemcitabine plus Oxaliplatin			Capecitabine plus Oxaliplatin		

2nd line	Chemotherapy	FGFR inhibitors	IDH1/2 inhibitors	VEGFR inhibitors	PARP inhibitors	PD-1 inhibitors	PD-L1 inhibitors	CLAT-4 inhibitors	Other novel therapies
	FOLFOX	Pemigatinib* Infigratinib Futibatinib Derazantinib Erdafitinib Debio 1347	Ivosidenib FT-2102 BAY 1436032	Ramucirumab Apatinib Surufatinib	Niraparib Olaparib Rucaparib	Pembrolizumab Camrelizumab Nivolumab	Durvalumab	Tremelimumab Ipilimumab	CB-103(Notch) CX-2009(CD166) Entinostat(HDAC1/3) Trastuzumab(HER2) Bortezomib (Proteasome) Bintrafusp alfa (PD-L1xTGF-β) XmAb22841 (CTLA-4 x LAG-3) M7824(PD-L1XTGFβ)

Figure 2. Treatment Strategy for Advanced HCC and ICC. The schematic illustration represents FDA-approved drugs for treatment of advanced HCC and ICC. First-line drugs for HCC include sorafenib, lenvatinib, atezolizumab plus bevacizumab, tremelimumab plus durvalumab and donafenib, whereas, for ICC, the combination of gemcitabine and cisplatin is currently proposed as first line. The bottom row represents corresponding second-line therapies that come in when patients are not suitable for their first-line therapy.

Molecular Targeted Therapy. HCC. *First-Line Drugs.* Sorafenib. Sorafenib was the first US Food and Drug Administration (FDA)-approved first-line systemic targeted drug for advanced HCC. It is an oral small-molecule multikinase inhibitor targeting VEGFR1, VEGFR2, VEGFR3, PDGFRβ, and Raf. Two large, international, multicenter clinical trials, SHARP and Asian-Pacific, have proved that sorafenib can suppress tumor progression and prolong OS in patients with advanced HCC.[102,103] These trials showed that sorafenib can increase PFS and OS by ~3 months in patients with advanced HCC in Western countries. As the first generation of targeted drugs for HCC, sorafenib has been used for over a decade. During this time, many patients have benefited, though others quickly developed resistance to sorafenib.[104]

Lenvatinib. Lenvatinib is becoming available for HCC patients who develop sorafenib resistance. Lenvatinib is an oral tyrosine kinase inhibitor, inhibiting VEGFR1−3, FGFR1−4, PDGFR, RET, and KIT. In August 2018, the FDA approved lenvatinib for first-line treatment of patients with unresectable HCC after lenvatinib was proved to be noninferior to sorafenib in the phase 3 REFLECT trial.[105]

Median OS in the lenvatinib arm and sorafenib arm was 13.6 months and 12.3 months (HR, 0.92; 95% CI, 0.79, 1.06), respectively. The adverse effects were hypertension (42%), diarrhea (39%), and decreased appetite (34%) with lenvatinib, and palmar-plantar erythrodysesthesia (52%), diarrhea (46%), decreased weight (31%), hypertension (30%), and decreased appetite (27%) with sorafenib.

Donafenib. Similar to sorafenib, donafenib is a novel multikinase inhibitor targeting RAF kinase and various receptor tyrosine kinases (RTKs), including VEGF receptor (VEGFR) and BRAF.[106] According to the report from 2020 International Conference of the American Society of Clinical Oncology (CSCO), donafenib significantly improves OS over sorafenib (12.1 versus 10.3 months) with fewer side effects and higher patient tolerance for advanced HCC patients in its phase II/III open-label trial.[107] The grade 3 and above adverse reaction rates for donafenib and sorafenib were 57.4% and 67.5%, respectively.

Thus, donafenib was recommended as the first-line therapy in the CSCO guidelines for HCC.

Second-Line Drugs. Regorafenib. Regorafenib, an oral multikinase inhibitor, inhibits the activity of protein kinases involved in multiple biological processes, such as tumorigenesis, tumor angiogenesis, distant metastasis, and tumor immune escape. These kinases include VEGFR 1−3, TIE2, RAF1, KIT, RET, RAF, BRAF, PDGFR, FGFR, and CSF1R. The randomized, double-blind, multicenter, phase III clinical trial RESORCE showed that regorafenib significantly improves the OS of patients, as compared with the placebo, from 7.8 to 10.6 months (HR, 0.63, p < 0.0001).[108] Grade 3−4 adverse events were reported in 40% of patients receiving regorafenib and 11% of patients receiving the placebo. In 2017, regorafenib received FDA approval as the second-line drug for the treatment of patients with advanced HCC who fail to respond to the sorafenib treatment.

Cabozantinib. Cabozantinib is an oral inhibitor and targets multiple kinases, including VEGFR2, c-MET, RET, ROS1, TYRO3, MER, KIT, TRKB, FLT3, TIE-2, as well as the GAS6 receptor (AXL)[109,110]. It was originally approved for medullary thyroid cancer in 2012 and advanced renal carcinoma in 2016. According to the randomized, double-blind, multicenter, phase 2 clinical trial conducted across 95 centers in 19 countries, median OS was 10.2 months for patients receiving cabozantinib, and 8 months for patients treated with placebo (HR, 0.76; p = 0.005).[111] Median PFS was 5.2 months and 1.9 months, respectively. Grade 3 or 4 adverse events occurred in 68% of patients in the cabozantinib arm and 36% in the placebo arm. The observed hepatotoxicity can be mostly controlled through dose modifications. Based on the encouraging results of prolonged OS and PFS, cabozantinib received its FDA approval for HCC in 2018.

Ramucirumab. Ramucirumab is a completely human monoclonal antibody that can specifically inhibit VEGFR-2.[112] For patients with alpha-fetoprotein ≥400 ng/mL and who have been previously treated with sorafenib, ramucirumab was approved as a monotherapy by the FDA on May 10, 2019.

Table 1. Systemic Therapies Currently or Promising Approved for Advanced HCC and ICC

Drugs	Target	Therapy Line	Approved Year	Trial
HCC				
Sorafenib (Nexavar)	VEGFR-2, VEGFR-3, PDGFR-β, RAF kinases	1	2007	SHARP Asian-Pacific
Lenvatinib (Lenvima)	FGFR, VEGFR, PDGFR-α, RET, KIT	1	2018	REFLECT
Regorafenib (Stivarga)	Tie2, VEGFR, PDGFR, FGFR	2	2017	RESORCE
Nivolumab (Opdivo)	PD-1	2	2017	CHECKMATE-040
Cabozantinib (Cabometyx)	c-Met, VEGFR-2, AXL, RET	2	2018	CELESTIAL
Pembrolizumab (Keytruda)	PD-1	2	2018	KEYNOTE-224
Ramucirumab (CYRAMZA)	VEGFR-2	2	2019	REACH-2
Nivolumab + ipilimumab (Opdivo + Yervoy)	PD-1, CTLA-4	2	2020	Cohort 4 of CHECKMATE-040
Atezolizumab + bevacizumab	PD-L1/VEGF	1	promising	IMbrave150
Tremelimumab + durvalumab	PD-1, CTLA-4	1	promising	NCT02519348
Donafenib	VEGFR, BRAF	1	promising	NCT02645981
Apatinib	VEGFR-2	2	promising	NCT02329860
ICC				
Gemcitabine + cisplatin	chemotherapy	1	2010	ABC-02
Pemigatinib (Pemazyre)	FGFR1−3	2	2020	FIGHT-202
Ivosidenib	IDH-1/2	2	promising	ClarIDHy

Approval was based on REACH 2 (NCT02435433), a randomized, double-blind, multicenter phase III study of 292 patients with AFP ≥ 400 ng/mL who had disease progression after sorafenib or were intolerant to sorafenib.[113] More recently, a study further confirmed the efficacy of ramucirumab in elderly patients with HCC and elevated AFP after sorafenib in REACH and REACH-2, with a survival benefit observed across all age subgroups and a tolerable safety profile, supporting its value irrespective of age, including for patients ≥ 75 years.[114]

Apatinib. Apatinib, a tyrosine kinase inhibitor targeting VEGFR-2, significantly prolonged OS and PFS in Chinese patients with advanced HCC who had previously been treated with sorafenib and/or chemotherapy, according to the results of a randomized, placebo-controlled, phase III trial conducted in 31 sites in China.[115] Median OS was almost 2 months longer for patients who received apatinib compared with patients receiving the placebo (8.7 months versus 6.8 months), and median PFS was more than 2 months longer (4.5 months versus 1.9 months).[115] The most common grade 3 or worse adverse events occurred at a rate of 69.2% in the apatinib arm and 3.1% in the placebo arm. With the significantly prolonged OS and PFS and a manageable safety profile, apatinib has potential to become a new second-line therapy for liver cancer.

Novel Therapeutic Targets. Even with all these available treatments (Table 1), the median PFS for HCC patients remains less than a year. Thus, novel treatment is still a critical unmet need for treatment of HCC. Based on the genomic profile and biomarkers reported in HCC, several clinical trials targeting various pathways are currently ongoing (Table 2). Recently, a first-in-human phase I study (NCT02508467) of fisogatinib (BLU-554), an orally bioavailable inhibitor of human FGFR4, demonstrated its antitumor activity in HCC, and further validated that the aberrant FGF19-FGFR4 signaling pathway may be a driver event.[116] In addition, the TGF-β1 receptor type I inhibitor galunisertib also showed an acceptable safety and prolonged OS outcome in combination with sorafenib in a phase II trial (NCT01246986).[117,118] Other potential candidates, including the cyclin-dependent kinase (CDK) inhibitors regulating the cell cycle pathways (ribociclib, palbociclib,[119,120] abemaciclib, and milciclib) as well as the c-MET inhibitors tepotinib[121] and tivantinib[122] are being evaluated in HCC clinical trials.

ICC. Molecular targeted therapy controls tumor cell proliferation, apoptosis, adhesion, and movement by inhibiting the surface molecules of tumor cell membranes and thereby inhibiting intracellular signaling pathways. ICC genetic alterations primarily include FGFR, IDH, epidermal growth factor (EGFR), and breast cancer type 1 susceptible protein associated protein-1 (BAP1).[123−125] Genetic alterations of these genes all have implications for therapy. At present, a variety of molecular targeted drugs are in the clinical research stage (Table 3), some of which have made progress in the treatment of ICC (Table 1).

FGFR Inhibitors. The most promising target therapy for cholangiocarcinoma identified in recent years is the inhibitor of the fibroblast growth factor (FGF) signaling pathway, which consists of 22 members labeled FGF1−23 (FGF15 = FGF19, called FGF15/19) and four interacting transmembrane receptors (FGFR1−4).[126] FGF signals regulate cell proliferation, in which FGFR2 fusions occurred in 10%−20% of ICC patients and are considered as a promising therapeutic target.[33,51,127,128] Currently, several FGFR inhibitors are being evaluated in clinical trials for cholangiocarcinomas with FGFR genetic aberrations.

Pemigatinib (INCB054828). Pemigatinib is the first and only targeted therapy so far approved (in 2020) by the FDA for the treatment of this rare cancer. It is a selective, potent oral inhibitor of FGFR 1, 2, and 3.[129] Approval was based on findings from the phase II FIGHT-202 trial (NCT02924376), which enrolled 107 patients with locally advanced or metastatic cholangiocarcinoma with FGFR2 fusions or rearrangements (cohort A), other FGF/FGFR genetic alterations (cohort B), or no FGF/FGFR genetic alterations (cohort C). For those in cohort A, treatment with pemigatinib resulted in a median OS of 21.1 months and median PFS of 6.9 months. The FIGHT-202 study suggests that locally advanced or metastatic cholangiocarcinoma patients with FGFR2 fusions or rearrangements may benefit from potent oral FGFR1, 2, and 3 inhibitor treatment. Median PFS was 6.9 months for patients with FGFR2 alterations, 2.1 months for patients with other FGF/FGFR alterations, and 1.7 months for those with no alterations in these genes. Median OS was 21.1 months, 6.7 months, and 4.0 months for the respective cohorts.[130] With the promising results of phase II, the phase III clinical trial of pemigatinib is currently underway (NCT03656536).

Table 2. Selected Ongoing Systemic Therapy Clinical Trials for Advanced HCC

Drug	Target	Sponsor	Status	Phase	Enrollment	Trial Identifier
Targeted Therapy						
Cabozantinib	VEGFR	Hospices Civils de Lyon	recruiting	phase 4	170	NCT03963206
Lenvatinib	VEGFR	Eisai Pharmaceuticals India Pvt Ltd	not yet recruiting	phase 4	50	NCT04297254
Donafenib	VEGFR	Suzhou Zelgen Biopharmaceuticals	completed	phase 2 phase 3	668	NCT02645981
Milciclib	CDK2	Tiziana LifeSciences	active, not recruiting	phase 2	31	NCT03109886
Palbociclib	CDK46	Pfizer	active, not recruiting	phase 2	23	NCT01356628
Ribociclib	CDK46	Texas University	recruiting	phase 2	40	NCT02524119
Galunisertib versus LY2157299 + sorafenib versus placebo + sorafenib	TGF-β	Eli Lilly	active, not recruiting	phase 2	120	NCT02178358
Immunotherapy						
Tislelizumab versus sorafenib	PD-1	BeiGene	active, not recruiting	phase 3	674	NCT03412773
Toripalimab versus placebo	PD-1	Shanghai Junshi Bioscience	recruiting	phase 2 phase 3	402	NCT03859128
Nivolumab versus placebo	PD-1	Bristol-Myers Squibb	recruiting	phase 3	530	NCT03383458
Nivolumab versus sorafenib	PD-1	Bristol-Myers Squibb	active, not recruiting	phase 3	1,723	NCT02576509
Pembrolizumab versus placebo	PD-1	Merck Sharp & Dohme	recruiting	phase 3	950	NCT03867084
Avelumab	PD-L1	Seoul National University Hospital	active, not recruiting	phase 2	30	NCT03389126
Combined Therapy						
Lenvatinib + pembrolizumab versus lenvatinib + placebo	VGFR, PD-1	Merck Sharp & Dohme	active, not recruiting	phase 3	750	NCT03713593
CS1003 + lenvatinib versus placebo + lenvatinib	VGFR, PD-1	CStone Pharmaceuticals	recruiting	phase 3	525	NCT04194775
Tislelizumab + regorafenib versus placebo + regorafenib	VEGF, PD-1	National Taiwan University Hospital	not yet recruiting	phase 2	125	NCT04183088
Toripalimab + lenvatinib	VGFR, PD-1	Peking Union Medical College Hospital	not yet recruiting	phase 2	76	NCT04368078
Durvalumab + bevacizumab versus placebo	VEGF, PD-L1	AstraZeneca	recruiting	phase 3	888	NCT03847428
Atezolizumab + bevacizumab versus sorafenib	VEGF, PD-L1	Hoffmann-La Roche	recruiting	phase 3	480	NCT03434379
Atezolizumab + bevacizumab	VEGF, PD-L1	National Health Research Institutes, Taiwan	not yet recruiting	phase 2	48	NCT04180072
Cabozantinib + atezolizumab versus sorafenib	VEGF, PD-L1	Exelixis	recruiting	phase 3	740	NCT03755791
Atezolizumab + bevacizumab versus active surveillance	VEGF, PD-L1	Hoffmann-La Roche	recruiting	phase 3	662	NCT04102098
Regorafenib + nivolumab	VEGF, PD-1	Fundación Clinic per a la Recerca Biomédica	not yet recruiting	phase 1 phase 2	60	NCT04170556
Lenvatinib + pembrolizumab versus lenvatinib + placebo	VEGFR, PD-1	Merck Sharp & Dohme	recruiting	phase 3	750	NCT03713593
Camrelizumab + apatinib	VEGFR, PD-1	Zhejiang University	recruiting	phase 1 phase 2	120	NCT04035876
Camrelizumab + apatinib versus sorafenib	VEGFR, PD-1	Jiangsu HengRui	recruiting	phase 3	510	NCT03764293

(Continued on next page)

Table 2. *Continued*

Drug	Target	Sponsor	Status	Phase	Enrollment	Trial Identifier
Sintilimab + lenvatinib	VEGFR, PD-1	Beijing Cancer Hospital	not yet recruiting	phase 2	56	NCT04042805
Sintilimab + IBI305 versus sorafenib	VEGF, PD-1	Innovent Biologics	recruiting	phase 2 phase 3	566	NCT03794440
Regorafenib + avelumab	VEGF, PD-L1	Institut Bergonié	recruiting	phase 1 phase 2	362	NCT03475953
Sorafenib + toripalimab	VEGF, PD-1	Sichuan University	not yet recruiting	phase 1 phase 2	39	NCT04069949
Galunisertib + nivolumab	TGF-β, PD-1	Eli Lilly	active, not recruiting	phase 2	75	NCT02423343
Fisogatinib + CS1001	FGFR4, PD-L1	CStone Pharmaceuticals	recruiting	phase 1 phase 2	52	NCT04194801
AK105 + anlotinib versus sorafenib	RTK, PD-1	Chia Tai Tianqing	not yet recruiting	phase 3	648	NCT04344158
Anlotinib + sintilimab	RTK, PD-1	Nanjing Medical University First Hospital	recruiting	phase 2	20	NCT04052152
Abemaciclib + nivolumab	CDK4/6, PD-1	Abramson Cancer Center, Pennsylvania University	Suspended (COVID-19)	phase 2	27	NCT03781960
Durvalumab + tremelimumab versus durvalumab versus sorafenib	PD-L1, CTLA-4	AstraZeneca	active, not recruiting	phase 3	1,310	NCT03298451
Nivolumab + ipilimumab versus sorafenib/lenvatinib	PD-1, CTLA-4	Bristol-Myers Squibb	recruiting	phase 3	1,084	NCT04039607
Durvalumab + tremelimumab versus durvalumab monotherapy versus tremelimumab monotherapy versus durvalumab + bevacizumab	VEGF, PD-L1, CTLA-4	Med Immune LLC	active, not recruiting	phase 2	433	NCT02519348
Galunisertib versus galunisertib + sorafenib ramucirumab	TGF-β,VEGF, VEGFR	Eli Lilly	active, not recruiting	phase 2	193	NCT01246986
Lenvatinib + pembrolizumab + TACE versus placebo + TACE	VEGFR, PD-1, TACE	Merck Sharp & Dohme	not yet recruiting	phase 3	950	NCT04246177
TAI + lenvatinib versus lenvatinib	VEGFR, chemoinfusion	Sim Yat-sen University	recruiting	phase 3	206	NCT04053985
SBRT + sintilimab versus SBRT	PD-1, radiation	Mian XI, Sim Yat-sen University	recruiting	phase 2 phase 3	116	NCT04167293
Donafenib + anti-PD-1 antibody	VEGFR, PD-L1	Zhejiang University	recruiting	phase 1	30	NCT04418401
Others						
ALT-803 + avelumab	PD-L1, IL-15 superagonist	Altor Bioscience	recruiting	phase 2	611	NCT03228667
KYI044 monotherapy versus KY1044 + atezolizumab	PD-LLT cell CO-stimulator	Kymab Limited	recruiting	phase 1 phase 2	412	NCT03829501
Pexa-Vec + nivolumab	PD-1, oncolytic virus	Transgene	active, not recruiting	phase 1 phase 2	30	NCT03071094
Nivolumab + BMS-986253 versus nivolumab + cabiralizumab versus nivolumab monotherapy	VEGFR2, PD-1, interleukin-8	NYU Langone Health	not yet recruiting	phase 2	74	NCT04050462
Entecavir/tenofovir disoproxil monotherapy	Antiviral therapy (HBV)	West China Hospital	recruiting	phase 4	450	NCT04032860
Vemlidy versus placebo	Antiviral therapy (HBV)	Taipei Veterans General Hospital, Taiwan	not yet recruiting	phase 4	402	NCT04290936
Pexastimogene devacirepvec versus sorafenib	Vaccinia virus-based oncolytic immunotherapy	SillaJen, Inc	active, not recruiting	phase 3	600	NCT02562755

Table 2. *Continued*

Drug	Target	Sponsor	Status	Phase	Enrollment	Trial Identifier
Immune Cell						
CD147-CAR-T	CAR-T therapy	Xijing Hospital	recruiting	phase 1	34	NCT03993743
Anti-DR5 CAR-T/TCR-T cells immunotherapy	CAR-T therapy	Shenzhen BinDeBio Ltd	recruiting	phase 1 phase 2	50	NCT03941626
CAR-GPC3 T cells	CAR-T therapy	Zhejiang University	recruiting	phase 1	36	NCT03980288
GPC-3 or TGF-β targeting CAR-T cell therapy	CAR-T therapy	Guangzhou Medical University Second Hospital	recruiting	phase 1	30	NCT03198546
GPC3-CAR (GLYCAR T cells) + fludarabine and cytoxan	CAR-T therapy	Baylor College of Medicine	recruiting	phase 1	14	NCT02905188
c-Met/PD-L1 CAR-T cell injection	CAR-T therapy	Second Hospital Nanjing Medical University	not yet recruiting	early phase 1	50	NCT03672305
IMA202 product	TCR-T therapy	Immatics US, Inc	recruiting	phase 1	16	NCT03441100
Microwave ablation + neoantigen vaccines	Neoantigen DC Vaccines	Chinese PLA General Hospital	recruiting	phase 1	24	NCT03674073
DC vaccines	Neoantigen DC Vaccines	Sichuan University	recruiting	phase 1	80	NCT04147078
Autologous DC + conjugate vaccine	Vaccine	Mayo Clinic	recruiting	early phase 1	26	NCT03942328

CAR-T, chimeric antigen receptor T cell; SBRT, stereotactic body radiotherapy; TAI, transarteria1 chemoinfusion.

Infigratinib (BGJ-398). Infigratinib (BGJ-398) was the first FGFR inhibitor investigated for treatment of cholangiocarcinoma. It is an oral drug that selectively binds to FGFR2 and shows impressive antitumor efficiency and a manageable safety profile in participants with advanced FGFR-altered cholangiocarcinoma (NCT02150967).[131] The FDA granted fast-track designation to infigratinib early in 2020 for first-line treatment of patients with unresectable advanced or metastatic cholangiocarcinoma who harbor FGFR2 gene fusions or translocations. It is currently undergoing a phase III trial (NCT03773302) to assess the efficacy and safety of infigratinib versus standard treatment chemotherapy with CisGem in first-line treatment of cholangiocarcinoma patients. Patients will be randomized 2:1 to receive infigratinib or CisGem.

Futibatinib (TAS-120). Futibatinib (TAS-120) is a highly potent and selective irreversible pan-FGFR inhibitor for all four FGFR subtypes (FGFR1–4).[132] Futibatinib demonstrated a clinically promising benefit with a manageable toxicity profile in patients with cholangiocarcinoma harboring FGFR2 gene fusions in phase I/II (NCT02052778).[133,134] Furthermore, futibatinib can overcome acquired resistance to the ATP-competitive FGFR inhibitors BGJ398 and Debio 1347 and still show promise to patients who had previously progressed on FGFR inhibitors.[135] A phase III, open-label, randomized study of futibatinib versus CisGem chemotherapy as first-line therapy of patients with advanced cholangiocarcinoma harboring the FGFR2 gene rearrangement (FOENIX-CCA3) has been initiated (NCT04093362).

Derazantinib (BAL087, Formerly ARQ 087). Derazantinib is an orally administered small-molecule pan-FGFR kinase inhibitor with potent activity against FGFR1–3. Derazantinib has demonstrated antitumor activity and a manageable safety profile in phase I study in ICC patients,[136–138] and has received US and EU orphan drug designation for ICC. Phase II clinical trials are currently ongoing. Basilea announced positive interim results of a phase II trial for derazantinib in ICC patients in 2019. The interim analysis was conducted after 42 patients were included in the study, in which 29 patients received at least one post-baseline imaging assessment. The objective response rate (ORR) was 21% and the disease control rate for patients with partial remission or stable disease was 83%. To date, the safety data obtained from all 42 patients are consistent with the results of previous clinical studies. So far, the results are encouraging.

Erdafitinib (JNJ-42756493). Erdafitinib (JNJ-42756493) is a potent pan-FGFR1–4 inhibitor with demonstrated antitumor activity in patients with metastatic urothelial cancer and cholangiocarcinoma with FGFR alterations.[139] Asian advanced cholangiocarcinoma patients with FGFR alterations treated with erdafitinib in a phase IIa study (NCT02699606) showed promising efficacy and manageable safety profiles similar to those with other tumor types.[140]

Debio 1347. Debio 1347 is a novel orally administered small molecule that is a highly selective FGFR1–3 ATP-competitive inhibitor. The preliminary phase I clinical trial result showed an encouraging clinical activity and manageable treatment-emergent adverse events in solid tumors harboring a fusion of FGFR1, FGFR2, or FGFR3 (FUZE clinical trial), including 9 cholangiocarcinomas (NCT1948297).[141] The phase II FUZE trial of Debio 1347 (NCT03834220) for patients with advanced solid tumors harboring FGFR fusions, including a cohort for patients with cholangiocarcinoma, is currently being assessed. Recent studies have reported that secondary single nucleotide variants, including p.E565A and p.L617M, appear in cells after FGFR inhibition, resulting in acquired resistance to these FGFR inhibitor therapies.[142] The study confirmed the upregulation of the PI3K/AKT/mTOR signaling pathway in drug-resistant cells, and proved that the combination of FGFR and mTOR inhibitor can desensitize cells to FGFR drug resistance.[142]

IDH-1/2 Inhibitors. IDH catalyzes the conversion of isocitrate to α-ketoglutarate. The mutant forms of IDH1 and IDH2 catalyze the non-reversible accumulation of 2-hydroxyglutarate (2HG), an oncometabolite of α-ketoglutarate that is related to DNA methylation and can promote tumor cell proliferation, invasion, and tumor angiogenesis.[124] Because IDH1 and IDH2 mutates in about 10%–28% of ICCs, small-molecule targeted inhibitors of mutant IDH1 and IDH2 have been developed and are undergoing pre-clinical and clinical trials.

Ivosidenib (AG120). Ivosidenib is an oral IDH1 inhibitor developed by Agios and is currently approved to treat relapsed or refractory acute myeloid leukemia with an IDH1 mutation. It is now being evaluated for treatment of ICC. In the phase I trial, AG120 showed good tolerance and clinical benefit, with 40% PFS rate at 6 months in patients with advanced cholangiocarcinoma (NCT02073994). The phase III clinical trial of AG120, ClarIDHy, is a global, multicenter, double-blind study randomizing 186 participants with IDH1 mutations in a 2:1 ratio to AG-120 or placebo (NCT02989857).[143]

Table 3. Selected Ongoing Systemic Therapy Clinical Trials for Advanced Cholangiocarcinoma

Drug	Target	Sponsor	Status	Condition or Disease	Phase	Enrollment	Trial Identifier
Chemotherapy							
FOLFIRINOX versus GEMOX	chemotherapy	Shi Ming, Sim Yat-sen University	recruiting	intrahepatic cholangiocarcinoma	phase 3	188	NCT03771846
Anlotinib versus anlotinib + levamisole	chemotherapy	Zhengzhou University First Hospital	recruiting	intrahepatic cholangiocarcinoma	phase 3	152	NCT03940378
Melphalan/PHP versus CisGem	chemotherapy	Delcath Systems Inc	recruiting	intrahepatic cholangiocarcinoma	phase 2 phase 3	295	NCT03086993
Gemcitabine + capecitabine versus capecitabine	chemotherapy	Tianjin Medical University	recruiting	cholangiocarcinoma	phase 3	460	NCT03779035
Nab-paclitaxel, cisplatin, gemcitabine versus CisGem	chemotherapy	Southwest Oncology Group	recruiting	intrahepatic cholangiocarcinoma	phase 3	268	NCT03768414
CisGem versus capecitabine	chemotherapy	Universitatsklinikum Hamburg-Eppendorf	recruiting	cholangiocarcinoma, gall bladder carcinoma	phase 3	781	NCT02170090
Targeted Therapy							
BGJ398 (infigratinib)	FGFR2	QED Therapeutics, Inc	recruiting	cholangiocarcinoma FGFR2 gene mutation	phase 2	160	NCT02150967
Infigratinib versus CisGem	FGFR2	QED Therapeutics, Inc	recruiting	cholangiocarcinoma FGFR2 gene mutation	phase 3	384	NCT03773302
Pemigatinib versus CisGem	FGFR2	Incyte Corporation	recruiting	unresectable cholangiocarcinoma	phase 3	432	NCT03656536
Derazantinib	FGFR2	Basilea Pharmaceutica	recruiting	intrahepatic cholangiocarcinoma	phase 2	143	NCT03230318
TAS-120 versus CisGem	FGFR2	Taiho Oncologa, Inc	not yet recruiting	cholangiocarcinoma	phase 3	216	NCT04093362
Ponatinib	FGFR, VEGFR	Sameek Roychowdhury	recruiting	solid tumor with FGFR mutations	phase 2	45	NCT02272998
AG-120 versus placebo	IDH1	Agios Pharmaceuticals	active, not recruiting	advanced cholangiocarcinoma	phase 3	186	NCT02989857
FT-2102	IDH1	Forma Therapeutics, Inc	recruiting	solid tumors including ICC	phase 1 phase 2	200	NCT03684811
BAY 1436032	IDH1	Bayer	active, not recruiting	solid tumors including ICC	phase 1	81	NCT02746081
Ramucinimab	VEGFR2	MD Anderson Cancer Center	recruiting	cholangiocarcinoma	phase 2	50	NCT02520141
Apatinib	VEGFR2	Zhengzhou University First Hospital	recruiting	intrahepatic cholangiocarcinoma	phase 2	30	NCT03521219
Surufatinib versus capecitabine	VEGFR	Hutchison Medipharma	recruiting	BTC	phase 2 phase 3	298	NCT03873532
Niraparib	PARP	University of Florida	recruiting	cholangiocarcinoma	phase 2	57	NCT03207347
Olaparib	PARP	Academic and Community Cancer Research United	not yet recruiting	BTC	phase 2	36	NCT04042831
CB-103	NOTCH	Cellestia Biotech AG	recruiting	cholangiocellular carcinoma	phase I phase 2	165	NCT03422679
CX-2009	CD166	CytomX Therapeutics	recruiting	cholangiocarcinoma	phase I phase 2	150	NCT03149549
Bortezomib versus supportive care	proteasome inhibitor	Zhengang Yuan	recruiting	intrahepatic cholangiocarcinoma	phase 3	50	NCT03345303
Immunotherapy							
Pembrolizumab + CisGem versus placebo + CisGem	PD-1	Merck Sharp & Dohme	recruiting	biliary tract carcinoma	phase 3	788	NCT04003636

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Table 3. *Continued*

Drug	Target	Sponsor	Status	Condition or Disease	Phase	Enrollment	Trial Identifier
Durvalumab + CisGem versus placebo + CisGem	PD-L1	AstraZeneca	recruiting	Biliary tract neoplasms	phase 3	474	NCT03875235
Pembrolizumab	PD-1	Samsung Medical Center	recruiting	biliary tract cancer	phase 2	33	NCT03110328
Durvalumab + CisGem versus placebo + CisGem	PD-L1	AstraZeneca	recruiting	biliary tract neoplasms	phase 3	474	NCT03875235
Combined Therapies							
Systemic chemotherapy versus chemotherapy and radiation	chemotherapy, radiation	Tata Memorial Hospital	recruiting	cholangiocarcinoma	phase 3	155	NCT02773485
CisGem + pembrolizumab	chemotherapy, PD-1	EORTC	recruiting	BTC	phase 2	50	NCT03260712
Camrelizumab + apatinib versus camrelizumab + FOLFOX4 or GEMOX	PD-1, VEGF, chemotherapy	Jianssu HengRui Medicine	recruiting	advanced biliary tract carcinoma	phase 2	152	NCT03092895
Lenvatinib + pembrolizumab	PD-1, VEGF	Peking Union Medical College Hospital	recruiting	cholangiocarcinoma	phase 2	50	NCT03895970
Bintrafusp alfa + CisGem versus placebo + CisGem	PD-L1xTGF-ß	EMD Serono Research & Development Institute	recruiting	BTC cholangiocarcinoma	phase 2 phase 3	512	NCT04066491
XmAb22841.XmAb22841 + pembrolizumab	PD-l, CTLA-4 x LAG-3	Xencor, Inc	recruiting	advanced solid tumors including ICC	phase 1	242	NCT03849469
Rucaparib + nivolumab	PD-l, PARP	University of Michigan Rogel Cancer Center	recruiting	BTC	phase 2	35	NCT03639935
Pembrolizumab + olaparib	PD-l, PARP	Georgetown University	recruiting	cholangiocarcinoma	phase 2	29	NCT04306367
Pembrolizumab + sargramostim	PD-1, GM-CSF	Robin Kate Kelley	active, not recruiting	biliary cancer	phase 2	42	NCT02703714
Entinostat + nivolumab	PD-1, HDAC 1/3	Sidney Kimmel Comprehensive Cancer Center	recruiting	cholangiocarcinoma	phase 2	54	NCT03250273
Nivolumab + ipilimumab	PD-1, CTLA-4	National Cancer Institute	recruiting	cholangiocarcinoma	phase 2	818	NCT02834013
Durvalumab + tremelimumab versus durvalumab	PD-L1, CTLA-4	Institut fur Klinische Krebsforschung IKF GmbH	recruiting	intrahepatic cholangiocarcinoma	phase 2	50	NCT04238637
CisGem + nivolumab versus nivolumab + ipilimumab	chemotherapy, PD-1,CTLA-4	University of Michigan Rogel Cancer Center	active, not recruiting	biliary tract neoplasms	phase 2	64	NCT03101566
Durvalumab + tremelimumab versus durvalumab + tremelimumab + TACE versus durvalumab + tremelimumab+ RFA versus durvalumab + tremelimumab + cryo	PD-L1, CTLA-4, ablative therapies	National Cancer Institute	recruiting	biliary tract neoplasms	phase 2	90	NCT02821754
Nivolumab + radiotherapy versus nivolumab + ipilimumab + radiotherapy	PD-1, CTLA-4, radiation	Herlev Hospital	recruiting	metastatic BTC	phase 2	160	NCT02866383
M7824	anti-PD-L × TGF-β fusion protein	EMD Serono Research & Development Institute	recruiting	BTC cholangiocarcinoma	phase 2	141	NCT03833661
Pembrolizumab + oxaliplatin + capecitabine	PD-1, chemotherapy	National Cancer Institute	recruiting	biliary tract neoplasms, cholangiocarcinoma	phase 2	19	NCT03111732
Trastuzumab + CisGem	HER2, chemotherapy	Changhoon Yoo	recruiting	cholangiocarcinoma BTC	phase 2	15	NCT03613168

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Table 3. *Continued*

Drug	Target	Sponsor	Status	Condition or Disease	Phase	Enrollment	Trial Identifier
Immune Cell							
TC-210T cells	genetically engineered T cells	TCR2 Therapeutics	recruiting	cholangiocarcinoma	phase 1 phase 2	70	NCT03907852
MUC-1 CAR-T cell	target abnormal glycosylation MUC-1	Zhejians University Second Hospital	recruiting	intrahepatic cholangiocarcinoma	phase 1 phase 2	9	NCT03633773
Tumor-infiltrating lymphocytes	Tumor-infiltrating lymphocytes	Udai Kammula	recruiting	cholangiocarcinoma	phase 2	59	NCT03801083

CisGem, cisplatin + gemcitabine; EORTC, European Organisation for Research and Treatment of Cancer; FOLFIRINOX, irinotecan + oxaliplatin + 5-FU + leucovorin; FOLFOX, leucovorin calcium (folinic acid) + 5-FU + oxaliplatin; GEMOX, gemcitabine + oxaliplatin; GM-CSF, granulocyte-macrophage colony-stimulating factor; cryo, cryoablation; RFA, radiofrequency ablation; TACE, transarterial chemoembolization

According to the report at the European Society for Medical Oncology (ESMO), AG120 (ivosidenib) improved PFS from 1.4 months to 2.7 months compared with placebo (HR, 0.37; p < 0.001). 32% and 22% of ivosidenib-treated patients were progression free at 6 months and 12 months respectively, while all patients receiving the placebo had disease progression at data cutoff. The risk of disease progression or death was reduced by 63% with AG120 in ClarIDHy.[144] Overall, ivosidenib provides a significant improvement in PFS and OS and Agios plans to submit a supplemental approval application for the drug very soon. The company's another IDH targeting drug enasidenib (AG-221; an IDH2 inhibitor), which has been approved for IDH2 mutation-positive acute myeloid leukemia, has only been examined in one cholangiocarcinoma clinical trial (NCT02273739). Although the trial began in October 2014 and was completed in June 2016, no literature has yet been published about the results of this trial.

FT-2102, BAY 1436032. FT-2102 and BAY 1436032, which target IDH1 mutations, are currently undergoing clinical trials in solid tumors with IDH1 R132 mutations (NCT03684811, NCT02746081).

Other Novel Targets. Vascular endothelial growth factors (VEGFs) were found to be overexpressed in 53.8% if ICCs.[145] Currently, several phase II or III clinical trials for treatments of BTCs targeting VEGFRs are ongoing, including ramucirumab (NCT02520141), apatinib (NCT03521219), and surufatinib (NCT03873532). For those with BRCA1/2 mutations (3%−5%) or BAP1 mutations (10%), poly(ADP-ribose) polymerase (PARP) inhibitors rucaparib (NCT03639935), olaparib (NCT04042831), and niraparib (NCT03207347) may provide some options. PARP inhibitors can compromise the DNA repair process, but this DNA single-strand damage can be converted into a double-strand break (DSB) and be repaired by homologous recombination. If the tumor cells have defects in homologous recombination repair (including the BRCA1/2 or BAP1 mutations), making DSB damage unable to be repaired, this can lead to the lethal effect of PARP inhibitors. For the approximately 5% of ICC patients who harbor PIK3CA mutation, the pan-class I PI3K inhibitor copanlisib plus CisGem is in a phase II clinical trial (NCT02631590).[146] In addition, binimetinib (MEK162), a potent inhibitor of MEK1/2, in combination with capecitabine was shown in a phase Ib clinical trial (NCT02773459) in RAS/RAF/MEK/ERK pathway activated BTC patients to have acceptable tolerability and encouraging antitumor efficacy, with response rate and disease control rate of 17.6% and 76.5%, and median PFS and OS 3.9 months and 8.0 months.[147]

Other clinical trials assessing the efficiency of CD166 inhibitor CX-2009 (NCT03149549), NOTCH transcription complex inhibitor CB-103 (NCT03422679), and proteasome inhibitor bortezomib (NCT03345303) are ongoing.

cHCC-ICC. To date, no standard molecular targeted therapy has been determined for cHCC-ICC. Sorafenib has been a standard of care for unresectable HCC. Because cHCC-ICC contains both the HCC and ICC elements, sorafenib has been used in some cHCC-ICC patients. Some studies suggests outcomes with sorafenib were poor compared with those with platinum-containing regimens.[9,10] However, in 2018, here was a clinical case of a patient with advanced cHCC-ICC who achieved complete remission after long-term sorafenib treatment and remained in remission after sorafenib was withdrawn.[148] The efficacy of sorafenib in cHCC-ICC needs to be further investigated in a large group of samples.

Immunotherapy. PD-1/PD-L1 antibodies are immune checkpoint inhibitors that help T cells to uncover the hypocritical veil of tumor cells and restore their recognition and killing of tumor cells.[149] PD-1 is a negative costimulatory molecule on T cells, and PD-L1 is the ligand of PD-1 and is expressed on tumor cells. After binding, the inhibitory signals are generated, which induces T cell apoptosis, inhibits T cell activation, and prevents T cells from attacking the "invaders" with full force, acting like a brake.[150] Blocking PD-1 or PD-L1, the restraints on T cells will be lifted, ensuring that T cells can fully fight cancer cells.[151]

HCC. *PD-1 Antibodies.* Nivolumab. The PD-1 antibody nivolumab is the first FDA-approved checkpoint inhibitor for HCC. On September 22, 2017, the FDA granted accelerated approval to nivolumab for HCC patients who have been previously treated with sorafenib. The confirmed overall response rate assessed by RECIST 1.1, was 14.3% (95% CI, 9.2, 20.8).[152] Currently, some phase III clinical trials of nivolumab are underway, both nivolumab monotherapy (NCT03383458, NCT02576509) and in combination with others (e.g., NCT04170556, NCT02423343, NCT03781960, NCT03510871).

Pembrolizumab. Pembrolizumab is another PD-1 antibody that was granted accelerated approval for second-line therapy of advanced HCC in 2018 based on KEYNOTE 224 (NCT02702414), a single-arm, multicenter trial enrolling 104 patients with HCC.[153] Based on the excellent data of phase II keynote 224, Merck went on to conduct the phase III keynote 240 trial. The trial included patients with HCC who had failed previous sorafenib treatment. The control group was treated with a placebo. The results showed that, compared with the control group, the OS results of the Keytruda group showed improvement, but did not reach a statistically significant difference (HR, 0.781; 95% CI, 0.611−0.998; p = 0.0238); PFS results also had an advantage, but did not reach statistically significant difference either (HR, 0.775; 95% CI, 0.609−0.987; p = 0.0186).[154] As the OS and PFS failed to achieve superiority, no formal evaluation of the key secondary endpoint ORR was performed.

Although the phase III trial of pembrolizumab is not satisfying, the researchers conducted an early trial (NCT03006926) of pembrolizumab combined with lenvatinib. The results are promising, with all patients except one showing tumor reduction.[155] Based on that, in 2019, the FDA granted the breakthrough therapy designation to pembrolizumab in combination with lenvatinib for the potential first-line treatment of patients with advanced unresectable HCC who do not respond to locoregional treatment. The phase III clinical trial of pembrolizumab plus lenvatinib is ongoing (NCT03713593, NCT04246177).

Camrelizumab, Sintilimab, Tislelizumab, and Toripalimab. Camrelizumab, sintilimab, tislelizumab, and toripalimab are four PD-1 inhibitors developed by Chinese pharmaceutical companies that all show great promise in recent clinical trials. According to the recent result published in Lancet Oncology, camrelizumab showed antitumor activity in pretreated Chinese patients with advanced HCC in an open-label multicenter phase II trial

(NCT02989922) and displayed manageable toxicities.[156] An objective response was displayed by 14.7% of patients (n = 32 of 217; 14.7%) and, among all patients, 6-month OS rate achieved 74.4% (95% CI, 68.0–79.7). On March 4, 2020, camrelizumab was officially approved by the National Medical Products Administration for patients with advanced HCC who have received sorafenib treatment and/or oxaliplatin-containing chemotherapy. This is the first PD-1 inhibitor approved for liver cancer indications in China.

Sintilimab received its first approval for the treatment of classical Hodgkin lymphoma in China in 2018.[157] Currently, sintilimab in combination with anlotinib (NCT04042805), lenvatinib (NCT04042805), and IBI305 (NCT03794440) are undergoing clinical trials for the treatment of various solid tumors, including HCC. Recently, Meihua Lin and colleagues reported an ICC patient who, after the first-line chemotherapy failed, achieved complete remission after three cycles of sintilimab treatment with only mild adverse reactions.[158] Unlike other PD-1 antibodies, tislelizumab is specifically designed to minimize binding to FcγR on macrophages[159] and to escape FcγR1-mediated effector function, because FcγR on macrophages impairs the antitumor activity of PD-1 antibodies by activating antibody-dependent macrophage-mediated T effector cell killing.[160] Phase Ia/Ib trials have shown promise for HCC.[161] A global, phase III clinical trial (NCT03412773) designed to evaluate the efficacy and safety of tislelizumab compared with sorafenib as a potential first-line treatment of unresectable HCC has been initiated.[162] Toripalimab, being developed by Shanghai Junshi Bioscience Co., Ltd, has received approval for the treatment of unresectable melanoma patients who failed previous systemic therapy in China.[163] Several clinical studies are currently being conducted to test the safety and efficiency of toripalimab in the treatment of HCC (NCT03412773, NCT04368078). These studies showed that the four domestic drugs showed great antitumor activity and efficiency and could be a first- or second-line treatment option for advanced HCC patients, even for a population with a high proportion of patients with HBV infection.

PD-L1 Antibodies. Atezolizumab, Bevacizumab. In July 2018, the PD-L1 monoclonal antibody atezolizumab in combination with bevacizumab was awarded the FDA designation of a breakthrough therapy in the treatment of advanced HCC based on a phase Ib clinical study (NCT02715531). In this study, patients with advanced unresectable or metastatic HCC were included. Atezolizumab 1200mg + bevacizumab 15 mg/kg was given once every 3 weeks. The median OS was 17.1 months, 6-month OS was 82%, and 12-month OS was 63%.[164] Just recently, the NEJM published an exciting phase III trial result,[165] in which the combination of atezolizumab and bevacizumab significantly improved OS and PFS (6.8 months versus 4.3 months) in patients with unresectable HCC with similar toxicity to that of sorafenib (grade 3 or 4 adverse events, 56.5% versus 55.1%). The 12-month OS rate of the patients increased to 67.2% compared with 54.6% with sorafenib, breaking the long-standing bottleneck in liver cancer treatment. The combination is currently under review by the FDA, and it is possible that it will become the new standard of care later in 2020.

Durvalumab. Durvalumab is an FDA-approved immunotherapy first used for locally advanced or metastatic urothelial carcinoma and non-small cell lung cancer developed by Medimmune/AstraZeneca. It is a human immunoglobulin G1 kappa monoclonal antibody that blocks the interaction of PD-L1 with PD-1. Based on the promising results in other solid tumors, several phase II/III clinical trials are being conducted for HCC. Durvalumab in combination with bevacizumab (NCT03847428) and with tremelimumab (NCT03298451) are currently under phase III evaluation.

CTLA-4 Antibodies. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) is another a co-inhibitory molecule that functions to regulate immune responses.[166] Antibodies that block the interaction of CTLA-4 with its ligands B7-1/B7-2 can enhance T cell activation as well as antitumor efficacy.[167] Two CTLA-4 antibodies that are currently under clinical investigation are tremelimumab and ipilimumab.

Tremelimumab. Tremelimumab is a human monoclonal CTLA-4 antibody. In 2020, the FDA granted an orphan drug designation to tremelimumab plus durvalumab for the treatment of patients with HCC. The combination is being tested in a phase III clinical trial (NCT03298451) to evaluate durvalumab alone and in combination with tremelimumab compared with standard sorafenib in 1,310 patients with unresectable, advanced HCC who have not received prior systemic treatment and are ineligible for locoregional therapy.[168] Results from an early phase II trial combining tremelimumab and durvalumab demonstrated a safety and a promising antitumor activity both in HCC and BTC.[169] The tremelimumab and durvalumab combination holds great promise in becoming a new first-line treatment for liver cancer.

Ipilimumab. Ipilimumab is another CTLA-4 monoclonal antibody intended to activate the immune system. On 10 March 2020, the FDA granted the first combination therapy accelerated approval for treatment of HCC to the combination of ipilimumab and nivolumab for HCC patients intolerant to sorafenib. The approval was based on the favorable ORR and duration of response (DoR) from cohort 4 of the CHECKMATE-040 (NCT01658878) trial, which included a total of 49 patients who received nivolumab in combination with ipilimumab. The ORR was 33% (n = 16; 95% CI, 20, 48) with four complete responses and 12 partial responses, and DoR ranged from 4.6 to 30.5+ months, with 31% of responses lasting at least 24 months.

Neoantigen-Based Therapy. New and rapidly growing cancer immunotherapy treatments include the development of personalized tumor vaccines that target neoantigens. In most tumor patients, there are certain specific T cells that can recognize short peptide antigens presented by major histocompatibility complex (MHC) on the surface of cancer cells. This short peptide antigen, which can induce specific T cells to eliminate cancer cells, does not exist in normal tissues, and is thus called a tumor-specific antigen or neoantigen.[170] Unlike traditional vaccines, which are limited by the dual restrictions of human leukocyte antigen (HLA) diversity and expression, personalized neoantigen vaccines target each patient's tumor tissue mutation antigen, combining precise gene detection and tumor immunotherapy. This approach uses specific tumor gene mutations to design vaccines that stimulate patients' autoimmunity to eventually kill tumor cells.[171,172] Neoantigens are mostly caused by errors in the DNA replication process of cancer cells, and some are caused by environmental factors such as viruses, radiation, and chemicals.[173] Although personalized tumor vaccines are still in the exploratory stage, the currently reported clinical trials of individualized neoantigen vaccines have shown encouraging results, especially in the treatment of melanoma, with high accuracy and low side effects.[174–180]

Dendritic cells are the most effective antigen-presenting cells in the body. After recognizing the antigen, dendritic cells are activated and enhance the antitumor immune response through T cells and natural killer (NK) cells.[181] Currently, several neoantigens based on personalized dendritic cells vaccines for HCC patients are under investigation in multiple ongoing clinical trials (NCT03674073, NCT04147078, NCT03942328) (summarized in Table 2). We expect that follow-up trials can achieve good results and realize its potential to bring patients an efficient, safe, and truly personalized tumor vaccine as soon as possible.

ICC. Tumors with mismatch repair (MMR) pathway deficiency have been demonstrated to have favorable responses to PD-1 blockade immunotherapy.[182] MMR-deficient (dMMR) tumors cause high levels of microsatellite instability (MSI) and can generate neoantigens that make the cancer cells susceptible to inhibition of the PD-L1/PD-1 interaction and sensitive to immunotherapy.[183] MSI is most commonly seen in colorectal and endometrial cancers; however, cholangiocarcinoma has also been reported to exhibit MSI with a frequency above 10%.[184,185] Several clinical immunotherapies for ICC are currently in use, including the PD-1 antibodies pembrolizumab and nivolumab, the PD-L1 antibody durvalumab, and the CTLA-4 antibodies ipilimumab and tremelimumab (Figure 2).[186]

Pembrolizumab. On May 23, 2017, pembrolizumab was granted accelerated approval by the FDA for the treatment of patients with microsatellite instability-high (MSI-H), or dMMR solid tumors. This was the first time that the FDA approved a drug based on the genetic profile instead of the primary

tumor site.[187] The phase II, multicohort KEYNOTE-158 study (NCT02628067) evaluated the antitumor activity and safety of pembrolizumab in patients with advanced solid tumors, including 104 cholangiocarcinoma patients.[188] Median PFS was 1.9 months versus 2.1 months, median OS was 7.2 months versus 9.6 months, and ORR was 6.6% versus 2.9% in patients with PD-L1 combined positive score CPS ≥1 versus CPS < 1. All responders were not MSI-H. Pembrolizumab in another phase Ib study (NCT02054806) with 24 BTCs also showed durable antitumor activity regardless of PD-L1 CPS and had manageable toxicity.[188] Currently, several pembrolizumab clinical trials are ongoing, both monotherapy (NCT03110328) and in combination with others therapies, which include the standard first-line care drug, CisGem (NCT03260712); the PARP inhibitor, olaparib (NCT04306367); the bispecific antibody that simultaneously targets immune checkpoint receptors CTLA-4 and LAG-3, XmAb22841 (NCT03849469); and immune cell therapy (NCT03937895).

Nivolumab. In a Japanese multicenter, open-label, phase I trial, researchers found nivolumab showed activity against BTCs that have progressed on prior systemic therapies, with a manageable safety profile in patients with unresectable or recurrent BTC.[189] The median patient age was 64.5 years old. Two-thirds of the patients (64.7%) had intrahepatic cholangiocarcinoma, 2.9% had extrahepatic cholangiocarcinoma, and 32.4% had tumors of the gall-bladder. The median OS was 5.2 months in nivolumab monotherapy and 15.4 months in nivolumab plus CisGem, with the median PFS 1.4 months and 4.2 months, respectively. A phase II study (NCT02829918) also found nivolumab to have promising efficacy with tolerated toxicity, including durable responses lasting 2 years in BTC.[190] These initial assessments of nivolumab for the treatment of advanced BTC provide supportive evidence for future larger randomized studies of nivolumab in this refractory cancer.

Durvalumab and Tremelimumab. A phase I, open-label, multicenter study (NCT01938612) evaluated the safety, tolerability, and pharmacokinetics of durvalumab and tremelimumab in Asian patients with advanced solid tumors including BTC.[191] Patients were enrolled in durvalumab (n = 42) and durvalumab plus tremelimumab cohorts (n = 65). Promising clinical efficacy was observed in both groups with no unexpected toxicities. Currently, a phase II trial of durvalumab and tremelimumab (NCT04238637) is ongoing.

The first randomized, double-blind, international phase III clinical trial to evaluate immunotherapy plus chemotherapy in patients with BTC in the first-line setting is also in progress, testing durvalumab in combination with CisGem (NCT03875235).[192]

cHCC-ICC. Currently, few studies in the literature or clinical trials have focused on the use of immunotherapy for treatment of cHCC-ICC. Therefore, it will not be discussed in this review.

Currently, the drug development field for liver cancer is mainly dominated by antibody drugs, of which PD-1/PD-L1and CTLA-4 are the main targets, and VEGFR and BRAF are the main small-molecule inhibitor targets. Among the first-line treatment research and development drugs, the combination of Roche's atezolizumab and bevacizumab is the most promising first-line treatment for HCC globally, while Suzhou Zelgen Biopharmaceuticals' donafenib is expected to become the first-line treatment in China. Among the drugs developed for second-line treatment, Hengrui Medicine's apatinib can significantly improve the OS of HCC patients, and is expected to become a new second-line therapy for HCC.

Future Perspective. Although many clinical drugs have been approved or tested in advanced HCC and ICC, the median PFS and OS remain dismal. One of the reasons is the acquired drug resistance due to the intra-tumor heterogeneity or the continuous diversification during treatment, which allows certain tumor cells to survive and eventually develop a drug-resistant phenotype. This remains a huge hurdle for the long-term use of targeted therapies for PLC.[193,194] It is therefore necessary to further explore the mechanism of drug resistance. Recently, Tang et al.[195] reported a novel somatic mutation in OCT4 (c.G52C) associated with sorafenib resistance. Further work in this vein will allow us to understand the mechanism and the exact gene mutation responsible for the drug resistance, allowing for targeting of specific mutation sites, thereby hopefully overcoming drug resistance.

Another challenge for targeted therapies in PLC is lack of precise targets and biomarkers. Unlike breast cancer, which has the precise biomarker HER2, PLC has a high degree of heterogeneity and genomic diversity, with no accurate biomarkers. Although many high-frequency mutant genes such as TERT, TP53, CTNNB1, and KRAS have been confirmed in PLC, it is still not clear whether they play the role of "driver gene" or "passenger gene" in the progression of liver cancer, which limits the development of targeted drugs. There is therefore an unmet need to comprehensively understand the genomic architecture, define the mutation landscape, and identify novel biomarkers and driver genes in order to develop new therapeutic interventions. With this information, future clinical trials could employ precision medicine to treat patients based on specific genetic mutation and drivers. Another point of concern is that PLC has a high recurrence rate; more than 70% of patients will relapse within 5 years after surgery.[196] Thus, whether the genetic features remain the same in the primary and recurrent tumors is also worth exploring.

In recent years, immune checkpoint inhibitors (ICIs) have been of increased research interest. The 2018 Nobel Prize in Physiology or Medicine was awarded to American immunologist James Allison and Japanese immunologist Tasuku Honjo for their contributions to the tumor immunity field, leading to the development and progression of PD-1/CTLA-4 inhibitors and other immunotherapy drugs. However, the overall response rate for ICIs has not been very high (10%−20% in PLCs),[152,153] which means the majority of patients cannot benefit from ICIs. It has been the main issue for ICIs. Fortunately, recent studies suggest that ICIs combined with other treatments, especially VEGF/VEGFR inhibitors, can significantly improve the overall response rate, with prolonged median PFS and OS. For example, the overall response rate of the atezolizumab (a PD-L1 inhibitor) and bevacizumab (a VEGF inhibitor) combination was 62% in the phase Ib clinical trial and 27% in the phase III trial. Combined therapies are therefore under more study currently. Combination therapies (Table 2) including pembrolizumab (PD-1 inhibitor) plus lenvatinib (VEGFR inhibitor) (NCT03713593), atezolizumab (PD-L1 inhibitor) plus cabozantinib (VEGFR inhibitor) (NCT03755791), durvalumab (PD-L1 inhibitor) plus bevacizumab (VEGF inhibitor) (NCT03847428), CS1003 (PD-1 inhibitor) plus lenvatinib (VEGFR inhibitor) (NCT04194775), and camrelizumab (PD-1 inhibitor) plus apatinib (VEGFR inhibitor) (NCT03764293) are all in phase III clinical trials for HCC, and we are eagerly waiting for the results.

The immunotolerance of the liver protects it from autoimmune damage caused by foreign antigens,[197] but also helps liver cancer cells to escape immune cells hunting. A decrease in NK cell number or impairment of function, accumulation of regulatory T cells, and exhausted CD8+ T cells have all been seen in HCC tumors, implicating an immunosuppressive microenvironment.[198] Many patients cannot respond to immunotherapy with a low response rate due to an insufficient immune activation. Thus, how to turn a "cold tumor" (immune tolerant) into a "hot tumor" (immunogenic) remains a major challenge for current tumor immunotherapy research and development. Future efforts in immunotherapy should be made in two directions: boosting the existing immune response and stimulating a *de novo* immune response.

For the first, more combination strategies such as ICIs combined with VEGFR, CTLA-4, or CDKs should be developed. In addition, immunoregulating the function of regulatory T cell (Treg) and CD8+ T cell function is also of great importance. For example, previous studies have shown that TGF-β promotes tumor immune escape by inducing Treg cell differentiation. The mouse model confirmed that TGF-β inhibitor SM-16 administration reduces Treg cell frequency, resulting in a reduced development of HCC,[199] providing a mechanistic rationale for the combination of TGF-β inhibitor and ICI in liver cancer.

Cyclic peptide C25 targeting human LAG-3 protein is reported to be able to significantly stimulate CD8+ T cell activation in human peripheral blood mononuclear cells, resulting in inhibition of tumor growth in CT26-, B16-,

and B16-OVA-bearing mice.[200] The use of C25 and blockade of the LAG-3/HLA-DR interaction may also provide an alternative method for cancer immunotherapy. The bispecific anti-PD-1/LAG-3 antibodies are also promising in future cancer treatment.

For stimulating the *de novo* immune response, cell-based immunotherapies such as adoptive cell therapies, including CAR-T cell therapies (e.g., NCT03993743, NCT04121273, and NCT03941626), T cell receptor T cell therapies (e.g., NCT03441100), and the vaccine-based therapies such as neoantigen-based vaccines (e.g., NCT03674073), peptide vaccines, and oncolytic virus drugs (NCT03071094, etc.), are currently being evaluated in HCC. We look forward to seeing the evaluation. Also, more research and studies are expected to be conducted in the future.

Recent studies have begun to unveil the complex hepatic immune microenvironment.

Further work is required to decipher the intricate immune microenvironment of liver cancer, such as the function and subtype of diverse immune cell subsets in liver, including T cells, B cells, macrophages, neutrophils, DCs, myeloid-derived suppressor cells, NK cells, and cancer-associated fibroblasts, as well as the dynamic interaction between the immune cells and the tumor ecosystem. Solving these will help us take a deep look inside the tumor microenvironment and understand patients' responses to immune therapy, and develop more immunotherapy options.

Aside from these mainstream treatments, some novel therapies have also been proposed in the management of PLC. For example, René Bernards and his team recently elaborated some new ideas about combined therapies by devising a "one-two punch" method (named after the effective combination of two rapid consecutive moves in boxing).[201] The first punch makes use of specific mutations (like TP53) in tumor cells to specifically induce it to a certain state, like cell senescence, and then the second punch precisely removes aging tumor cells. Therefore, although these two drugs are not used at the same time, they have synergistic effect with reduced toxicity and high precision.

The treatment of PLC is gradually shifting away from traditional chemotherapy and toward targeted therapy, including immunotherapy and especially the combination therapy. These new approaches have shown great potential in clinical trials, and there is a need to develop more combination strategies or try novel combinations of the previously studied drugs.

Despite these past and ongoing trials investigating PLC treatments, publications and clinical trials regarding systematic treatments of the rare cHCC-ICC are still extremely limited. Further study is undoubtedly required to further improve current diagnosis, as well as to better understand the genomic profile and pathogenesis of cHCC-ICC in order to develop novel therapeutics.

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DECLARATION OF INTERESTS

The authors declare no competing interests.