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Cholangiocarcinoma: Advances in Pathogenesis, Diagnosis, and Treatment

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Cholangiocarcinoma (CCA) is an epithelial cancer originating from the bile ducts with features of cholangiocyte differentiation.¹ CCA is the second most common primary hepatic malignancy, and epidemiologic studies suggest its incidence is increasing in Western countries.² Advanced CCA has a devastating prognosis, with a median survival of <24 months.³ The only curative therapy is surgical extirpation or liver transplantation, but unfortunately the majority of patients present with advanced stage disease, which is not amenable to surgical therapies. Anatomically, CCA is classified into extrahepatic and intrahepatic forms of the disease. The extrahepatic form is more common, accounting for 80% to 90% of CCAs. It is further divided into proximal or perihilar and distal subsets depending on the location of the cancer within the extrahepatic biliary system. Perihilar disease is also frequently referred to as a Klatskin tumor. Three different growth patterns of extrahepatic CCA can be observed: (1) periductal infiltrating, (2) papillary or intraductal, and (3) mass forming.⁴ Intrahepatic CCA typically presents as an intrahepatic mass. In addition to their distinct morphology and clinical presentations, intrahepatic and extrahepatic CCAs differ in etiopathogenesis, molecular signatures, and management. In the last several years there have been significant new insights into the molecular pathogenesis of CCA. New diagnostic and therapeutic modalities have also been developed, resulting in improved detection rates and outcomes. In addition, we have now entered the era of targeted therapies for human cancers. Therefore, it is timely and topical to review these advances with a focus on promising targeted therapies for this disease. An additional goal is to stimulate further interest in this disease with the hope of improving outcomes for this still highly lethal malignancy.

Epidemiology of CCA

Hepatobiliary malignancies account for 13% of the 7.6 million annual cancer-related deaths worldwide and for 3% of the 560,000 annual cancer-related deaths in the United States. CCA accounts for 10% to 20% of the deaths from hepatobiliary malignancies. The prevalence of CCA shows a wide geographic variability, with the highest rates in Asia and the lowest in Australia.⁵ In the United States, the incidence of CCA has been reported to be 0.95/100,000 for intrahepatic forms and 0.82/100,000 for extrahepatic forms of the disease.⁵ Its prevalence in different racial and ethnic groups is heterogeneously distributed, with the highest age-adjusted prevalence in Hispanics (1.22/100,000) and the lowest in African Americans (0.17–0.5/100,000).⁶ In the last 4 decades, United States incidence rates of intrahepatic CCA have increased by 165%, whereas the extrahepatic CCA incidence has remained stable.^{7,8} The significant increase in age-adjusted incidence of intrahepatic CCA was confirmed after correction for a prior misclassification of hilar CCA as intrahepatic CCA.² Similarly,

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increasing incidence rates of intrahepatic CCA have also been reported in Western Europe and Japan.^{9,10} The cause for the increasing incidence has not been identified. We speculate that increased lipid mediators such as oxysterols may contribute to the current increased incidence in Western societies.¹¹ In Western nations, the median age at presentation is >65 years, and it is only rarely diagnosed in patients <40 years of age except in patients with primary sclerosing cholangitis (PSC).⁵ There is a slight male predominance for CCA.

Etiology of CCA

In the majority of cases, the etiology of CCA remains obscure. However, several conditions associated with inflammation and cholestasis have been identified as risk factors for CCA (Table 1). PSC is a common risk factor. The prevalence of CCA in this condition is 5% to 15%, and the annual incidence rate is 0.6% to 1.5%.^{12,13} The majority of PSC patients who develop CCA do so within the first 2.5 years following the diagnosis of PSC.^{12,13} Thus, the symptomatic patient who presents with their first diagnosis of PSC should be carefully screened for CCA. Hepatobiliary flukes—especially the species *Opisthorchis viverrini* and *Clonorchis sinensis*—are risk factors for CCA.¹⁴ They are endemic in portions of East Asia, where ingesting undercooked fish is common. Several case-control studies as well as animal models have confirmed the correlation between liver fluke infection and CCA.^{15–17} Another risk factor more commonly found in Asia than in Western countries is hepatolithiasis, for which an incidence rate of 10% for CCA has been described.^{18–20} Biliary malformations such as Caroli's disease and choledochal cysts carry a 10% to 15% risk for developing CCA.^{21–23} Hepatitis C and cirrhosis have also been reported as possible risk factors for CCA.²⁴ Biliary-enteric drainage procedures are associated with CCA in the presence of recurrent cholangitis.^{25,26} Finally, compounds such as thorotrast and dioxins have been correlated with an increased risk for CCA.²⁷ Although most patients have no identifiable overt risk factors for CCA, it remains possible that subclinical biliary tract inflammation underlies the pathogenesis of CCA in most patients.

Pathogenesis of CCA

CCA likely results from malignant transformation of cholangiocytes, although transformation of epithelial cells within peribiliary glands and/or biliary stem cells may also contribute to its development. There is also evidence that subsets of CCA and mixed hepatocellular carcinoma/CCA originate from hepatic stem/progenitor cells.^{28,29} Etiologic and experimental evidence implicates inflammation and cholestasis as key factors in the pathogenesis of CCA. They create an environment that promotes damage in DNA mismatch repair genes/proteins, proto-oncogenes, and tumor suppressor genes.³⁰ Cytokines, growth factors, and bile acids, found in increased concentrations in inflammation and cholestasis, contribute to these molecular changes and augment the growth and survival of altered cells. Cytokines stimulate expression of inducible nitric oxide synthase (iNOS) expression in epithelial cells, and iNOS up-regulation is present in inflammatory cholangiopathies and CCA.³¹ Increased iNOS activity results in generation of nitric oxide and reactive nitrogen oxide species (RNOS) known to interact with cellular DNA and proteins. The interaction between RNOS and the cellular genome results in mutations and DNA strand breaks. Mutagenesis is further promoted by interaction between nitric oxide and RNOS with DNA repair enzymes such as human 8-oxoguanine glycosylase, which is directly inactivated by S-nitrosylation of its active site cysteine residues.³² A variety of oncogenic mutations have been identified in human CCA tissues. Their frequency depends on tumor stage, tumor type, anatomical location, etiology, and ethnic population. Although dysregulation of the proto-oncogene k-ras and the tumor suppressor gene p53 is commonly observed in malignancies, mutations of k-ras have only been described in 20% to 54% of intrahepatic CCA. This is in sharp distinction to pancreatic ductal carcinoma where k-ras mutations are present in >90% of cancers.^{33,34} Thus, despite shared developmental ontology

between the pancreatic ducts and the biliary tree, their adult cancers are different. Nuclear accumulation of p53 and up-regulation of the related protein mdm-2 and WAF-1 have been reported in 21.7% to 76% of CCAs.^{35–42} Other inactivated tumor suppressor genes include p16^{INK4a}, DPC4/Smad4, and APC.^{43–45} Correlation between these markers and prognosis varies among studies. Other dysregulated genes/factors involved in cell cycle regulation and found in CCA are listed in Table 2. The majority of these genetic changes were described in intrahepatic CCA. Given the paucicellular, desmoplastic nature of extrahepatic bile ducts, genetic analysis of these tumors will require careful laser capture microdissection of the CCA cellular elements—a tedious process that has seldom been applied to this tumor.

Interleukin-6 (IL-6) appears to be a critical signaling molecule in the pathogenesis of human cancers.⁴⁶ For example, IL-6 has recently been reported to promote cancer stem cell survival in human breast cancer by up-regulating expression of the stem cell survival regulator Notch-3.⁴⁷ In human lung cancer, epidermal growth factor receptor (EGFR)-activating mutations enhance IL-6 expression, promoting its autocrine/paracrine growth-promoting and survival properties.⁴⁸ Thus, IL-6 can be upstream or downstream of other potent oncogenes. IL-6 is also a key cytokine in the pathogenesis of CCA. It is a known mitogen, and its proliferative effect has been confirmed in CCA.⁴⁹ IL-6 is produced at high levels by CCA cells, and elevated IL-6 serum concentrations have been reported in CCA patients.^{50,51} IL-6 secretion by CCA cells is further enhanced by other inflammatory cytokines.⁵² In addition to autocrine and paracrine IL-6 stimulation, CCA cells overexpress the IL-6 receptor subunit gp130.⁵¹ The usual negative feedback regulation of IL-6 signaling is blocked by epigenetic silencing of suppressors of cytokine signaling 3 (SOCS-3).⁵³ Uninhibited IL-6 stimulation results in up-regulation of the antiapoptotic Bcl-2 protein Mcl-1, rendering CCA resistant to cytotoxic therapies.^{53–55} IL-6 has also been shown to increase telomerase activity in CCA resulting in inhibition of telomere shortening and thereby evasion of cell senescence.^{56–61} In CCA cells, IL-6 activates p44/p42 and p38 mitogen-activated protein kinases (MAPKs), both shown to be critical for CCA cell proliferation.⁵² Activated p38 MAPK decreases cyclin-dependent kinase inhibitor p21^{WAF1/CIP1}, a known negative cell cycle regulator.⁶² There is also cross-communication between IL-6 and other pathways (for example, IL-6-mediated overexpression of EGFR).⁶³ Mechanisms of IL-6 signaling in human CCA are depicted in Fig. 1.

Receptor tyrosine kinases, which can be targeted pharmaceutically, are overexpressed in many cancers and modulate cancer biology. For example, inhibition of EGFR signaling has been shown to significantly suppress CCA cell growth.⁶⁴ EGFR can directly be activated by bile acids and promote CCA cell proliferation, a potential explanation for the tropism exerted by CCA for the biliary tree.^{65,66} EGFR activation is sustained in CCA by failure to internalize the ligand–receptor complex, a homeostatic mechanism essential for receptor inactivation.⁶⁴ EGFR phosphorylation results in activation of the downstream kinases p42/44 MAPK and p38 MAPK, which in turn increase cyclooxygenase 2 (COX-2) expression in CCA cells.⁶⁶ COX-2 plays an important role in CCA carcinogenesis through inhibition of apoptosis and growth stimulation.^{67–72} Additional induction of COX-2 is mediated by bile acids, oxysterols, and iNOS.^{11,66,70} Other COX-2-inducing molecules include the tyrosine kinase ErbB-2, which is overexpressed in CCA and involved in CCA carcinogenesis and progression.^{73,74} It is an EGFR homologue and is able to homodimerize or heterodimerize with other members of the EGF superfamily, resulting in activation of the Raf/MAPK-pathway. Also, hepatocyte growth factor and its receptor c-met are frequently over-expressed in CCA.^{51,74,75} Hepatocyte growth factor is mitogenic, and its increased secretion by CCA cells together with the overexpression of its receptor represents an autocrine mechanism for sustained growth stimulation by CCA.⁷⁶ In addition to the enhancement of these growth-promoting pathways, loss of growth inhibition has been demonstrated in CCA. Response to transforming growth factor- β 1 is aberrant in CCA, resulting in increased proliferative rates. In the presence of IL-6,

CCA cells are also resistant to activin-mediated growth inhibition.⁵¹ In summary, there is a complex net of different factors and pathways involved in CCA development, growth, and propagation.

Diagnosis of CCA

In the majority of cases, CCA is clinically silent, with symptoms only developing at an advanced stage. Once symptomatic, the clinical presentation depends on tumor location and growth pattern. Ninety percent of patients with extrahepatic ductal CCA present with painless jaundice, and 10% of patients present with cholangitis.^{77,78} Unilobar biliary obstruction with ipsilateral vascular encasement results in atrophy of the affected lobe and hypertrophy of the unaffected lobe.⁷⁹ Upon physical examination, this “atrophy–hypertrophy complex” phenomenon presents as palpable prominence of one hepatic lobe. Intrahepatic mass-forming CCA presents with symptoms typical for hepatic masses, including abdominal pain, malaise, night sweats, and cachexia. The tumor markers CA-125 and CEA can be elevated in CCA; however, they are nonspecific and can be increased in other gastrointestinal or gynecologic malignancies or cholangiopathies.⁸⁰ CA 19-9 is the most commonly used tumor marker for CCA.⁸¹ Its sensitivity and specificity for detection of CCA in PSC are 79% and 98%, respectively, at a cutoff value of 129 U/mL. Other investigators have identified a higher cutoff of >180 U/mL to achieve this degree of specificity.⁸² A change from baseline of >63 U/L has a sensitivity of 90% and specificity of 98% for CCA.⁸³ In patients without PSC, its sensitivity is 53% at a cutoff of >100 U/L and its negative predictive value is 76% to 92%.⁸⁴ CA 19-9 can also be elevated in bacterial cholangitis and other gastrointestinal and gynecologic neoplasias; patients lacking the blood type Lewis antigen (10% of individuals) do not produce this tumor marker.^{85–88} Ultrasound and computed tomography (CT) are only of limited value for detection of intrahepatic and extrahepatic CCA due to their low sensitivity and specificity, as well as their low accuracy in estimating tumor extent of intrahepatic and extrahepatic CCA.^{78,89–91} Their main role in CCA is detection of bile duct obstruction, vascular compression or encasement, tumor staging, and preoperative planning. For evaluation of tumor location and intraductal extent, cholangiography is the most important diagnostic modality, especially for extrahepatic CCA.⁹² Endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance cholangiopancreatography (MRCP), or percutaneous transhepatic cholangiography (PTC) can be used for this purpose.⁷⁸ ERCP and PTC allow therapeutic interventions (for example, placement of biliary stents) as well as collection of tissue samples for pathologic and cytologic analysis. MRCP/magnetic resonance imaging (MRI) provides information about intrahepatic location and tumor dimensions of intrahepatic CCA, ductal as well as periductal tumor extent of extrahepatic CCA, vascular involvement, and metastases (Fig. 2). Its sensitivity and imaging quality of tumor tissue can be increased significantly with ferumoxide enhancement.^{93,94} The most sensitive method for evaluation of regional lymphadenopathy is endosonography. Biopsy of lymph nodes via fine needle aspiration for further pathologic analysis can also be performed during the endosonographic procedure.⁹⁵ However, biopsy of hilar lesions during endosonography is discouraged, because it can result in tumor seeding.⁹⁶ In indeterminate cases, establishment of a diagnosis can be attempted with positron emission tomography (PET) with [18F]-2-deoxy-glucose.^{78,90} Sensitivity and specificity of integrated PET/CT in the identification of primary lesions has been reported as 93% and 80% for intrahepatic CCA and 55% and 33% for extrahepatic CCA.⁹⁷ For regional lymph node metastases, the sensitivity of PET/CT was 12% and the specificity was 96%.⁹⁷ False positive PET scans have been reported in the setting of chronic inflammation.⁹⁸ A recent report suggested that PET scanning in non-PSC patients can change management and therefore is useful in staging.⁹⁹

The pathologic diagnosis of CCA can be challenging due to the paucicellular desmoplastic nature of the periductal infiltrating form of CCA. Moreover, a cellular diagnosis of CCA is

confounded by common reactive changes in PSC, resulting in highly variable sensitivity and specificity of this technique in this patient population. Using a basket device may increase the diagnostic yield of conventional cytology compared with a brush.¹⁰⁰ Also, measurements of bile insulin-like growth factor can help distinguish malignant from benign strictures.¹⁰¹ The introduction of digital image analysis (DIA) and fluorescence *in situ* hybridization (FISH) have significantly increased the diagnostic yield of brush cytology. Both techniques depend on the identification of aneuploidy. The sensitivity and specificity of DIA for extrahepatic CCA are 39% and 77% compared with 18% and 98% with conventional cytology.¹⁰² In PSC patients, the sensitivity and specificity of DIA for CCA are 43% and 87%. FISH has a sensitivity of 47% and a specificity of 100% for detection of CCA in PSC. However, FISH analysis is complicated, and three subsets of chromosomal amplification occur: (1) trisomy 7, (2) tetrasomy or duplication of all chromosomes labeled, and (3) polysomy or amplification of at least three chromosomes. Trisomy 7 can occur in inflammatory diseases of the biliary tree, especially PSC.¹⁰³ This is a true amplification of chromosome 7, because EGFR, which is also located on this chromosome, is amplified. We do not consider trisomy 7 to be diagnostic of CCA, although it likely places the patient at risk for the development of CCA. Whether EGFR inhibitors can reverse this lesion or prevent subsequent CCA in PSC patients remains to be studied. Tetrasomy must be interpreted with caution, because high mitotic rates will yield tetrasomy during the M phase of the cell cycle. Polysomy remains diagnostic of cancer in the appropriate clinical context (for example, a biliary stricture). Recently, diagnostic modalities such as endoscopic/percutaneous flexible cholangioscopy, intraductal ultrasound, and radiolabeled imaging have become available. However, they are not part of the standard diagnostic algorithm and should be reserved for cases in which other techniques have failed to demonstrate CCA but the level of suspicion is high. In particular, the emerging role of choledochoscopy will need to be validated prospectively, and tissue/cytologic diagnosis will still remain the gold standard. In summary, the diagnosis of CCA is challenging and should be undertaken in a multimodality approach that includes clinical context and laboratory as well as radiologic and pathologic analysis. A diagnostic approach for ductal CCA is proposed in Fig. 3.

Staging of CCA

CCA has been classified using the International Union Against Cancer (UICC)/American Joint Committee on Cancer (AJCC) TNM (tumor-node-metastasis) system (Table 3). This classification is a pathologic staging system and therefore often requires surgical acquisition of the tissue. An optimal staging system should provide detailed information about disease extent, vascular involvement, and metastases without subjecting the patient to surgical treatment. It should also take into account treatment options, performance status, and age and correlate with meaningful clinical outcomes. There is an urgent need for such a validated staging system in hilar CCA. Without a staging system, stratification of patients for clinical trials is currently hampered.

Long-term survival in patients with hilar CCA critically depends on complete tumor resection with negative tumor margins.^{104–106} Therefore, evaluation for resectability of these tumors requires a staging system including parameters of biliary disease extent, vascular encasement, and hepatic lobar atrophy in addition to the information provided by a clinical TNM system. Such a staging system has been proposed by Memorial Sloan-Kettering Cancer Center (Table 4). Resectability, likelihood of curative or R0 resection, metastatic spread to N2 level lymph nodes, and survival correlated with the tumor stage of this modified classification.¹⁰⁷

Surgical Therapy of CCA

Surgical treatment is the only curative therapy for CCA and is therefore the treatment of choice if feasible. Solitary intrahepatic CCAs are managed by segmentectomy or lobectomy. Five-year survival rates are 22% to 44% and correlate with R0 (negative margin) resection, absence of lymph node metastases, and vascular invasion.^{108–114} Survival rates after surgical treatment of intrahepatic as well as extrahepatic CCA have significantly improved in the last decade, possibly reflecting a more careful patient selection, thereby achieving higher rates of R0 resection.¹¹⁵ Surgical resection is also the treatment of choice for extrahepatic CCA in the absence of PSC. However, resection should only be attempted with curative intent, because there is no significant survival benefit of noncurative or debulking resection compared with patients not treated surgically.¹⁰⁷ Exclusion criteria for surgical resection of hilar CCA are outlined in Table 5.^{116,117} Local lymph node metastases (N1) are not an absolute contraindication to surgical treatment, because they do not significantly influence outcomes in hilar CCA.¹⁰⁷ Five-year survival rates after R0 resection for hilar CCA are 11% to 41% and for distal extrahepatic CCA are 27% to 37%.^{105,107,114,118–120} However, overall R0 resection rates are <50%.¹¹⁸ Survival rates may be higher for *en bloc* resected patients; however, this approach is technically not feasible for cancers originating from or with significant involvement of the left hepatic duct.¹²⁰ In PSC, outcomes of surgical resection are complicated by advanced liver disease in the majority of these patients, recurrent cholangitis with a biliary–enteric anastomosis, the multi-focal nature of the cancer, and their increased risk for further CCA. Because a biliary–enteric anastomosis is a risk factor for *de novo* CCA,^{25, 26} creating a biliary–enteric anastomosis in a PSC patient should be viewed with caution, and informed consent regarding the potential development of additional CCA should be discussed. Patients with PSC plus CCA may be better evaluated as potential liver transplant candidates (*vide infra*). Perioperative morbidity following resection of hilar CCA is 31% to 85%, and postoperative mortality is 5% to 10% at major referral centers.^{107,121–123} Several techniques have been evaluated for their potential to increase resectability, including preoperative portal vein embolization plus extended hepatectomy.^{124,125} The goal of portal vein embolization is to induce hyperplasia of the nonembolized lobe increasing the volume of the remnant liver following an extended hepatectomy. This strategy achieved increased resectability in patients with hilar CCA and marginal remnant liver volumes.^{124,126} Adjuvant and neoadjuvant treatments for extrahepatic CCA—including chemotherapy, radiation therapy, and photodynamic therapy—cannot be recommended, because studies either failed to show significant effects or were statistically underpowered, nonrandomized, or restricted to short-term follow-up.^{127,128}

Results of liver transplantation for intrahepatic CCA are discouraging with 5-year survival rates of 0% to 18%, and therefore cannot be recommended.^{108,129–132} Outcomes of liver transplantation for extrahepatic CCA were similarly disappointing, with 5-year survival rates of 23% to 26%.^{133,134} However, the development of new liver transplantation protocols for extrahepatic CCA at the Mayo Clinic and the University of Nebraska yielded highly promising results.^{135,136} Based on their initial experiences as well as on analysis of outcomes and correlated risk factors, strict selection criteria have been developed (Table 6), and neoadjuvant treatment has been optimized to its current form.^{137–139} This protocol includes neoadjuvant therapy with external beam radiation therapy concurrent with 5-FU chemotherapy, followed by brachytherapy and chemotherapy with capecitabine. Prior to transplantation, patients undergo explorative laparotomy for restaging. Survival analysis of patients treated according to the Mayo Clinic protocol has yielded 1- and 5-year survival rates of 91% and 76%, respectively.^{138,140} Predictors for tumor recurrence include older age, CA 19-9 >100 U/mL on the day of transplantation, prior cholecystectomy, mass on cross-sectional imaging, residual tumor >2 cm in explant, tumor grade, and perineural invasion in explant.¹³⁸ For highly selected

patients with *de novo* perihilar CCA, unresectable CCA, and CCA superimposed on PSC, liver transplantation can be curative and is the treatment of choice.

Palliative Treatments for CCA

CCA causes significant morbidity related to cholestasis and its complications, abdominal pain, cachexia, and bacterial cholangitis. Therefore, palliative therapies are quite important in the management of this disease. Endoscopic stent placement is as successful as surgical choledochojejunostomy or hepaticojejunostomy for restoration of biliary drainage and relief of cholestasis.^{141–143} Unilateral hepatic duct stent placement has been shown to be equivalent to bilateral hepatic duct stenting for biliary drainage.¹⁴⁴ Metal stents have higher patency rates and are more cost-effective for patients, with an expected survival of >6 months. Plastic stents require exchange every 2 to 3 months due to occlusion, migration, or cholangitis and are preferred in patients who are candidates for surgical treatment or whose life expectancy is <6 months.^{78,145,146} In cases where endoscopic stent placement is not feasible, PTC can be employed for biliary drainage.

Photodynamic therapy (PDT) and radiation therapy have been evaluated as palliative therapies. Highly variable results have been reported with radiation therapy in largely uncontrolled studies, precluding a consensus opinion on the effectiveness of this modality. Radiation is also associated with significant morbidity, including gastrointestinal bleeding, biliary strictures, intestinal obstruction, and hepatic decompensation.^{147–150} Therefore, radiation therapy cannot be unconditionally recommended for palliative or adjuvant therapy of intrahepatic and extrahepatic CCA. In PDT, a photosensitizing agent (such as hematoporphyrin) is administered followed by illumination at a wavelength corresponding to the absorption spectrum of the agent resulting in reactive oxygen species–induced cell death, tumor–vessel thrombosis, and tumor-specific immune reactions.^{151–154} PDT treatment can reduce cholestasis and improve quality of life.^{155–157} Complication rates are low and include sensitization to skin phototoxicity and acute cholangitis.¹⁵⁸ Several studies including two randomized controlled trials indicate a survival benefit with PDT in hilar CCA.^{157,159–162} In summary, PDT is a reasonable and recommendable approach for palliation of hilar CCA.¹⁶³ Studies evaluating PDT as an adjuvant treatment have been uncontrolled, precluding a recommendation for PDT in this context.^{128,164}

Medical Treatment for CCA

There are no curative medical therapies for CCA. The most studied chemotherapeutic drugs are 5-FU and gemcitabine; the latter was approved for CCA in 2002 by the U.S. Food and Drug Administration.¹⁶⁵ Both drugs have been tested in combination with a variety of other drugs, including cisplatin, oxaliplatin, docetaxel, paclitaxel, mitomycin-C, doxorubicin, epirubicin, lomustine, and interferon- α .^{166–177} However, none of the studies was randomized, and most studies were statistically underpowered, based on case reports, or demonstrated poor response rates. In conclusion, there is currently no randomized study showing a clear survival benefit for a specific chemotherapeutic regimen. With the advent of targeted therapies, it is apparent that survival is a more legitimate endpoint than response rates. For example, response rates with sorafenib for renal cell carcinoma are minimal, although life expectancy is prolonged.¹⁷⁸ Given this evolving information, future trials will need to have a comparison arm. Randomized trials are urgently needed.

Targeted Therapy of CCA in Preclinical Studies

The growing understanding of the molecular pathogenesis of CCA opens new therapeutic options for molecular targeting. The majority of these strategies target antiapoptotic and growth-stimulating pathways. The use of methylation inhibitors is able to restore SOCS-3

expression, thereby reducing protein expression of the prosurvival bcl-2 family protein Mcl-1 and sensitizing CCA cells to tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)-induced apoptosis.^{53,63} Other strategies for inhibiting Mcl-1 expression include the use of the multikinase inhibitor sorafenib and overexpression of mir-29 microRNA.^{179,180} Small molecules such as obatoclox are also in development to inhibit Mcl-1.¹⁸¹ Sensitization of CCA cells to TRAIL was also achieved through the use of a γ -secretase inhibitor to inhibit Notch signaling.¹⁸² Tyrosine kinase inhibitors have been used to target EGFR signaling and reduce tumor cell growth.^{64,183,184} Inhibition of IL-6 pathways by anti-IL-6 neutralizing antibodies or MAPK inhibitors results in growth inhibition of human CCA cell lines.⁵² Other strategies—including COX2 inhibitors, hepatocyte growth factor antagonists, ErbB-1/2 inhibition, and telomerase inhibition—achieved growth inhibition and/or induction or sensitization to apoptosis *in vitro*.^{61,68,69,185–188} These examples show the promising potential of these new therapeutic approaches. However, the vast majority of these studies were conducted *in vitro*. Though the preliminary *in vivo* results are promising, the majority of *in vivo* studies have focused on tumor inhibition rather than on treatment of established tumors.^{187,189–191} Better *in vivo* models of CCA will be necessary to study targeted therapies.^{16,60,73,192–195}

New *In Vivo* Models of CCA

In vivo evaluation of therapeutic compounds is an essential step in the preclinical development of effective antineoplastic therapies. Several subcutaneous xenograft systems have been described for CCA.^{183,196,197} However, preclinical data derived from these xenograft systems correlate only poorly with clinical outcomes, resulting in an increasing trend toward the use of organ-specific *in vivo* cancer models.^{198–201} Early hepatobiliary CCA models were restricted to hamsters and rats, which develop tumors after treatment with carcinogens [*N*-nitrosobis(2-oxopropyl)amine, methylazoxymethyl acetate, dimethylnitrosamine, furan, thioacetamide] or infection with *O. viverrini*.^{16,60,192,193,202–204} Recently, several new genetic CCA models have been described. Liver-specific combined deletion of the tumor suppressor genes *Smad4* plus *PTEN* results in formation of CCA in mice.¹⁹⁵ Another model of intrahepatic mass-forming CCA is achieved by treating p53-deficient mice with carbon tetrachloride (CCl₄).²⁰⁵ Sirica and colleagues developed two models of CCA in which malignant transformation of explanted rat cholangiocytes followed by direct biliary inoculation of these cells resulted in CCA formation in 56% to 100% of animals.^{73,206} In summary, new models of CCA have been developed that resemble human CCA in many aspects. The majority of these models represent intrahepatic CCA, however, and genetic models of hilar CCA still need to be developed.

Potential Targeted Therapies for Human CCA

The chemoresistance of CCA is not completely understood. There is evidence that expression of multidrug resistance genes as well as up-regulation of antiapoptotic bcl-2 proteins are involved in the poor response rates of CCA to chemotherapeutics.²⁰⁷ New targeted therapies may overcome these barriers. Several drugs, targeting essential pathways in CCA pathogenesis, are already approved by the U.S. Food and Drug Administration and are in clinical use for other cancer types. Examples include EGFR inhibitors (Cetuximab, Erlotinib, and Gefitinib), Raf-kinase inhibitors (Sorafenib), Her-2–directed inhibitors (Trastuzumab and Lapatinib), and vascular endothelial growth factor–directed inhibition (Sorafenib and Bevacizumab). The National Institutes of Health reports several ongoing clinical trials evaluating the COX-2 inhibitor celecoxib and the receptor tyrosine kinase inhibitors Sorafenib, Erlotinib, and Bevacizumab as monotherapy or in combination with other agents in CCA.²⁰⁸ Other potential combinations that have not been evaluated yet include drugs inhibiting drug-resistance genes/proteins or agents down-regulating antiapoptotic signals. Glutathione-*S*-transferase- π inhibitor C16C2 decreased the IC₅₀ of adriamycin and cyclophosphamide *in vitro* and enhanced the

tumor suppressive potential in a CCA xenograft murine model.²⁰⁷ Another strategy involves sensitization to TRAIL-induced apoptosis by down-regulation of Mcl-1 (for example, by treatment with Sorafenib).²⁰⁹ These are just a few examples, but they represent the possibilities for targeting molecular pathways in human CCA.

Conclusion

Advances have been made in the diagnosis and management of CCA. From the clinical perspective, advanced cytologic techniques for the diagnosis of CCA have increased the use of brush cytology for the diagnosis of this neoplasm. *En bloc* surgical resection and liver transplantation have advanced the surgical treatment of this disease, and PDT has emerged as an important palliative therapy. The development of *in vivo* animal models for CCA will permit more rigorous development of targeted therapies for this disease. A clinical stratification system is necessary prior to the implementation of multicenter trials. Although progress on CCA has been continuous, more work is needed to cure and prevent this disease.

Abbreviations

CCA, cholangiocarcinoma
 CT, computed tomography
 DIA, digital image analysis
 EGFR, epidermal growth factor receptor
 ERCP, endoscopic retrograde cholangiopancreatography
 FISH, fluorescence in situ hybridization
 IL-6, interleukin-6
 iNOS, inducible nitric oxide synthase
 MAPK, mitogen-activated protein kinase
 MRCP, magnetic resonance cholangiopancreatography
 MRI, magnetic resonance imaging
 PDT, photodynamic therapy
 PET, positron emission tomography
 PSC, primary sclerosing cholangitis
 PTC, percutaneous transhepatic cholangiography
 RNOS, reactive nitrogen oxide species
 TRAIL, tumor necrosis factor-related apoptosis-inducing ligand

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Fig. 1.

IL-6 signaling and therapeutic targets in CCA. A schematic overview of IL-6 signaling and its downstream effectors as well as examples of potential therapeutic interventions is depicted. IL-6 binds to its receptor, which consists of the common receptor unit gp130 and the IL-6-specific subunit gp80. Activation of this receptor complex results in downstream activation of the JAK/STAT, PI3K/Akt, and MAPK signaling pathways. Activation of the JAK/STAT pathway results in nuclear translocation of phosphorylated STAT3 and transcriptional up-regulation of target genes, including the antiapoptotic Mcl-1. In addition, IL-6 increases EGFR expression by decreasing its promoter methylation. Activation of phosphoinositide 3-kinase (PI3K) results in phosphorylation and activation of Akt kinase, which in turn inhibits proapoptotic factors and facilitates cell growth. Activation of p38 MAPK decreases negative cell cycle regulators and is critical for CCA proliferation. Inhibitors of IL-6 receptor, Akt, MAPK, or JAK signaling, as well as bcl-2 protein inhibitors, have the potential of inhibiting these pathways.

Fig. 2. Imaging of CCA. A gadolinium-enhanced MRI scan of the liver with ferumoxide of a patient with hilar CCA is depicted. The arrow inserted into the T2-weighted MRI scan points out the biliary tumor.

Fig. 3.

Diagnostic evaluation of hilar CCA. In patients with clinical suspicion of hilar CCA, CA 19-9 serum analysis, endoscopic retrograde cholangiopancreatography, and conventional as well as molecular cytologic analysis of endoscopically obtained biliary brushings of suspicious areas should be performed. In cases where these tests are negative, observation is recommended. In cases in which a dominant stricture is identified, Ca 19-9 serum levels are >129 U/mL, biopsy/cytology are positive for carcinoma and/or polysomy is shown, management for CCA should be initiated. In indeterminate cases, gadolinium-enhanced MRI of the liver with ferumoxide should be performed. If a mass lesion and/or vascular encasement are identified, management of CCA should be initiated. If the MRI scan is negative but there is significant concern for CCA, PET can be performed. If "hot spots" are identified, further management should be directed toward CCA. If the PET scan is negative, careful observation is recommended. In cases with a negative MRI scan and minimal concern for CCA, patients can be observed.

Table 1
CCA Risk Factors

Definite risk factors
Primary sclerosing cholangitis
Liver fluke infection (<i>Opisthorchis viverrini</i>)
Hepatolithiasis
Biliary malformation (choledochal cysts, Caroli's disease)
Thorotrast
Probable risk factors
Hepatitis C
Cirrhosis
Toxins (dioxin, polyvinyl chloride)
Biliary–enteric drainage procedures

Table 2**Molecular Pathology of CCA**

Malignant Phenotype	Dysregulated Genes/Pathways
Proliferation	IL-6, IL-6 receptor (gp130) HGF/Met ErbB-2 K-ras BRAF COX-2
Apoptosis/evasion	Caspase-9 Mcl-1 Bcl-2 Bcl-XL COX-2
Senescence/evasion	Telomerase
Cell cycle dysregulation	Cyclin D1 p21 ^{waf1/cip1} p27 ^{kip1} pRb p53 mdm-2 DCP4/Smad4 p16 ^{INK4a}
Invasion/metastases	E-cadherin $\alpha\beta$ -cadherin Aspartyl (asparaginy) β -hydroxylase WISP1v
Angiogenesis	Matrix metalloproteinases VEGF TGF- β

Table 3
TNM Classification and UICC Staging System for Intrahepatic CCA

TNM Stage		Tumor Spread	
T1	Solitary tumor without vascular invasion		
T2	Solitary tumor with vascular invasion		
	OR		
	Multiple tumors ≤ 5 cm		
T3	Multiple tumors > 5 cm		
	OR		
	Tumor involving major branch of the portal or hepatic veins		
T4	Tumors with invasion of adjacent organs other than gallbladder		
	OR		
	Tumors with perforation of visceral peritoneum		
N0	No regional lymph node metastases		
N1	Regional lymph node metastases		
M0	No distant metastases		
M1	Distant metastases		
UICC Stage	Tumor	Node	Metastases
I	T1	N0	M0
II	T2	N0	M0
IIIA	T3	N0	M0
IIIB	T4	N0	M0
IIIC	Any T	N1	M0
IV	Any T	Any N	M1

Table 4

Memorial Sloan-Kettering Cancer Center Staging System for Hilar CCA

Stage	Criteria
T1	Tumor involving biliary confluence ± unilateral extension to 2° biliary radicles
T2	Tumor involving biliary confluence ± unilateral extension to 2° biliary radicles AND Ipsilateral portal vein involvement ± ipsilateral hepatic lobe atrophy Tumor involving biliary confluence + bilateral extension to 2° biliary radicles OR Unilateral extension to 2° biliary radicles with contralateral portal vein involvement
T3	OR Unilateral extension to 2° biliary radicles with contralateral hepatic lobe atrophy OR Main or bilateral portal venous involvement

Table 5
Exclusion Criteria for Surgical Resection of Hilar CCA

Bilateral lobal extension involving the secondary biliary radicles
Unilobar disease with encasement of the contralateral portal vein or hepatic artery branch
Bilateral portal vein or hepatic artery branch encasement
Intrahepatic or distant metastases
Distant lymph node metastases
Significant comorbidities

Table 6
Mayo Clinic Criteria for Liver Transplantation for Hilar CCA

Diagnostic criteria
Positive (transluminal) biopsy
Positive conventional cytology
Stricture plus FISH polysomy
Mass lesion on cross-sectional imaging
Malignant-appearing stricture and persistent CA 19-9 > 130 U/mL in the absence of cholangitis
Exclusion criteria
Resectable by conventional surgery
Prior radiation therapy or chemotherapy
Uncontrolled infection
Intrahepatic metastases
Extrahepatic or distal lymph node metastases
Other malignancy within 5 years of CCA diagnosis
Age <18 or >65 years
Comorbidities forbidding chemotherapy or radiation therapy, liver transplantation
Hilar mass on cross-sectional imaging with a radial diameter >3 cm