

Annexe 1

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Annexe 2

Rational for recommendations

General organization and methods for the guidelines

The relevant studies were analyzed using the Grade method (Grade of Recommendation Assessment, Development, and Evaluation), which establishes the level of scientific evidence through an internationally validated process based on the type of each study and its methodological quality. The level of evidence integrates the consistency of results among different studies, the direct or indirect nature of the evidence, and the magnitude of the benefit. A high overall level of evidence allows for the formulation of a "strong" recommendation ("it is recommended to do," "it is not recommended to do," Grade 1+ or 1-). A moderate, low, or very low overall level of evidence resulted in the drafting of an "optional" recommendation ("it is probably recommended to do" or "it is probably not recommended to do," Grade 2+ or 2-). When the literature was insufficient to propose a level 1 or 2, some recommendations were based on expert opinions ("experts suggest..."). After a first

presentation of the recommendations, each proposition was discussed with all the experts and then submitted to a vote. The experts participating in the vote were chosen by the scientific societies that participated in the development of the recommendations (ACABi, ACHBT, ANGH, CREGG, GERCOR, SFCC, SFED, SFP, SIAD Unicancer GI). Each expert independently rated all proposals on a scale from 1 (complete disagreement with the proposal) to 9 (complete agreement). The recommendation proposals were then presented at a dedicated meeting and discussed to identify points of agreement and points of disagreement or indecision. To validate a recommendation, at least 50% of the experts had to express an opinion that went in the same direction, while less than 20% expressed a contrary opinion. For a strong recommendation, at least 70% of the participants had to have an opinion that went in the same direction. In the absence of strong agreement, recommendations were reformulated and, once again, subjected to rating in order to achieve a consensus. Two rounds of rating were necessary to reach a consensual formulation of the recommendations presented here, ensuring that all recommendations have strong agreement.

Chapter 1: risk factors and screening

1/ What risk factors merit systematic investigation when cholangiocarcinoma is suspected?

Biliary carcinogenesis results from a complex interaction between the individual's specific genetic heritage and environmental risk factors. In Asia, especially in Northeast Thailand, the high incidence is linked to infection by *Clonorchis sinensis* and *Ophistorchis viverrini*, endemic in this region. The prevalence of *Ophistorchis viverrini* can reach 70% in some areas (1). Up to 10% of chronically infected individuals will develop CCA, especially intrahepatic (2). On the other hand, although its incidence is very low, primary sclerosing cholangitis (PSC) is the main pathology predisposing to pCCA in low-risk areas such as Europe and North America. Recent studies report an increase in incidence and mortality of iCCA in Western countries, especially Germany, France, the UK, and the USA (3), while the incidence of eCCA is stable or decreasing.

Risk factors may be common to different CCAs or specific to a tumor location on the biliary tree.

iCCA represents at least 10% of CCAs (4,5). As the second primary liver cancer, it shares many common risk factors with HCC, such as cirrhosis, metabolic steatopathy, alcohol, chronic hepatitis B or C.

pCCA represents 50-60% of CCAs (4,5). Its main risk factors are cystic and lithiasic pathologies of the biliary tract.

More general risk factors for carcinogenesis have also been identified. Type 2 diabetes, alcohol, and tobacco could contribute to the increased incidence of CCA. Clements' recent meta-analysis is the most comprehensive study of risk factors for iCCA and eCCA (6). Twenty-five studies were included, comprising 16,127 iCCA and 8,769 eCCA cases. Although this meta-analysis is international, very few European studies were included. Comparing risk factors between studies from Western countries (the United States, Denmark, and Italy) and Eastern countries (China, Taiwan, Japan, and South Korea) revealed few differences. In Eastern countries, cirrhosis and HBV conferred slightly more risk of developing iCCA than in Western countries.

2/ Is there a place for targeted screening, and if so, in what situations and with what protocols (type and frequency)?

2.1/ Should patients with low phospholipid-associated cholelithiasis (LPAC) syndrome be screened for cholangiocarcinoma?

The ABCB4 mutation is responsible for various cholestatic diseases, including the LPAC syndrome. Some cases of cholangiocarcinoma (CCA) have been reported in patients with the ABCB4 mutation and LPAC syndrome (7,8). In the only retrospective observational study of 67 patients with the ABCB4 mutation (9), 2 patients with LPAC syndrome developed CCA. The relationship between LPAC and CCA is not currently established. The impact of the ABCB4 mutation on the development of CCA is unknown.

2.2/ Should patients with Caroli disease be screened for cholangiocarcinoma?

Fibrocystic liver diseases constitute a heterogeneous group of rare conditions affecting the intrahepatic bile ducts, linked to an embryological developmental anomaly of the ductal plate. The origin of this anomaly is a dysfunction of proteins expressed in the primitive cilia of cholangiocytes, secondary to a mutation in a key gene such as PKD1-2, PKHD1, or PRKCSH. The transmission can be autosomal recessive or dominant. These conditions result in an increased risk of intrahepatic cholangiocarcinoma (iCCA) estimated to be 100 times that of healthy individuals (10). Carcinogenesis is promoted by biliary stasis, inflammation, and the formation of stones in malformed and abnormally dilated bile ducts. Among them, Caroli's disease is characterized by multifocal segmental dilation of small and large intrahepatic bile ducts, often associated with biliary microhamartomas. It is more common in Asia and predominantly affects women. If congenital hepatic fibrosis coexists, it is referred to as Caroli's syndrome. A study reports a very strong association with iCCA (Odds Ratio [OR] of 38.1; 95% CI 14.2-102.4) and extrahepatic CCA (OR of 96.8; 95% CI 51-183.7) (11). The incidence of CCA varies from 2.5% to 37% according to surgical series and is most often incidentally discovered during surgery (12,13). Congenital hepatic fibrosis alone appears to be rarely associated with iCCA (14). Rare cases of iCCA have been described in patients with hepatic cystic disease, showing images of cystic epithelium transformation (15–17). Simple bile cysts are not at risk of developing CCA.

2.3/ Should patients with biliary hamartomas be screened for cholangiocarcinoma?

Von Meyenburg complexes/biliary microhamartomas are millimetric periportal lesions, characterized by irregularly dilated bile duct structures often filled with bile within a fibrous tissue. They are observed in approximately 5% of individuals (18). They are frequently multiple and generally considered as benign lesions. Their histogenesis remains debated: a probable focal malformation of the ductal plate or a possible acquired lesion. They are consistently present in the livers of individuals with autosomal dominant polycystic kidney disease, where they give rise to cysts. They are asymptomatic and almost always discovered incidentally. The risk of cholangiocarcinoma (CCA) is controversial. Rare and controversial observations of transition to a small duct-type iCCA suggest that, exceptionally, they could be precancerous lesions for iCCA (19–22).

3/ What examinations should be done in the setting of cholangiocarcinoma screening for patients with PSC?

The occurrence of cholangiocarcinoma (CCA), which can be present at the diagnosis of PSC, is the most feared complication (30 to 50% of CCAs are diagnosed within the first year of PSC diagnosis), especially in patients with tight bile duct strictures. Large series suggest that the annual incidence (beyond the first year following PSC diagnosis) of CCA is approximately 0.6 to 1.5% (23). The only established risk factors are older age at PSC diagnosis and a history of colon cancer (23). Cholecystectomy is recommended for any gallbladder polyp > 8 mm due to a high risk of malignant transformation (24). A Danish retrospective cohort including 222 PSC-IBD and 8231 IBD without PSC showed significantly reduced overall survival for PSC-IBD patients compared to IBD controls, with a 10-year risk of CCA at 8%, 200 times higher than IBD without PSC (Hazard Ratio [HR]: 190, 95% CI 55-660) (25).

The radiological diagnosis of cholangiocarcinoma (CCA) is extremely challenging due to pre-existing abnormalities in the bile ducts. Radiological anomalies, in addition to the appearance of a tumor mass, include an intracanalicular polypoid gap of at least 1 cm, localized thickening exceeding 4 mm of the bile duct wall, and exacerbation of known bile duct dilation or stenosis. An MRI with contrast agent is systematically recommended within 6 months following PSC diagnosis if the initial MRI was performed without contrast agent (26). Annual systematic MRI is the standard practice in specialized centers. The most commonly used serum tumor markers are CA19-9 and carcinoembryonic antigen (CEA). The CA19-9 + (CEA x 40) index exceeding 400 U has poor specificity and sensitivity (27). Indeed, a moderate increase in CEA (<3N) can be observed in cases of chronic liver disease, and a significant increase in CA19-9 may be related to benign intra- or extrahepatic cholestasis (especially in cases of angiocholitis). Conversely, CA19-9 is not expressed in Lewis antigen-negative patients (5 to 10%) and therefore does not increase in the case of CCA. Its specificity is low; it is elevated in one-third of PSC patients without CCA. However, it is useful to have an initial value at the time of PSC diagnosis. The latest international recommendations no longer advocate regular and systematic CA19-9 testing due to its very low diagnostic performance (28). In the presence of clinical events (general deterioration, pain, itching, jaundice, or angiocholitis) or an increase in biological abnormalities, it is advisable, outside the framework of routine surveillance, to perform cross-sectional imaging with contrast injection to investigate CCA. The sensitivity of PET scans is high in mass-forming but low in infiltrating forms, and angiocholitis can lead to false positives. The use of PET scans is not recommended but may be considered as a second option (29).

4/ Is there a place for preventive interventions? Should patients with choledochal cysts undergo surgery?

The choledochal cyst, which can be associated with Caroli's disease, is an extremely rare congenital condition in the Western world, with an incidence of 1:13,000-1:150,000. In contrast, the incidence in Asia is high, estimated at 1:1000. It is characterized by one or more communicating cystic dilations of the bile ducts that can extend to the right and left hepatic ducts. Young Japanese and Asian women born in Asia are most affected. The pathophysiology is poorly understood. 80% of the cysts are congenital and present in children under 10 years old, mainly of type I (69.8%) and type IV (23.7%) according to the Todani classification. CCA mainly occurs in adulthood and mainly affects type I and type IV cysts with an incidence of 7.6% and 9.2%, respectively. Before the age of 18, this incidence is very low, estimated at 0.4%. After 30 years, the incidence increases with each decade, reaching 38.2% after the age of 60 (30). Preoperative diagnosis is exceptional. With a survival of less than 36 months, the prognosis is poor, especially for symptomatic cysts.

Chapter 2: diagnosis and pre-therapeutic work-up

2/ What should the differential diagnosis procedure comprise for iCCA

The challenge of differential diagnosis of iCCA varies according to the nature of the underlying liver.

In a healthy liver, which represents the most common situation, the primary differential diagnosis is the metastatic localization of an adenocarcinoma from another origin. The "simplified" phenotype of iCCA is CK7+ CK20-. CK19 has falsely emerged as a marker for iCCA; it is, in fact, a highly ubiquitous cytokeratin expressed by all adenocarcinoma metastases (colonic, gastric, pancreatic, pulmonary, mammary). CK19 staining is essentially useful for the differential diagnosis of iCCA versus hepatocellular carcinoma (HCC). Metastasis of colon adenocarcinomas are usually easily identifiable based on standard morphology and their immunophenotype CK7- CK20+ CDX2+ SATB2+ for most tumors. Rectal tumors and advanced-stage colon cancers can be CK7+ CK20+, and microsatellite instability-high (MSI-high) tumors CK7- CK20- (31,32). SATB2 expression in CCA is rare, even exceptional, and would be restricted to eCCA (33); moreover, SATB2 is more specific to a colorectal origin than CDX2, sometimes expressed in gastric or pancreatic cancers. The differential diagnosis with the metastatic localization of an adenocarcinoma of pancreatic, gastric, or extrahepatic biliary origin is extremely difficult, requiring the combination of several immunohistochemical markers.

Recently, several studies based on bioinformatic analysis of multiple markers have identified the most discriminating antibodies and proposed antibody panels best suited to differential diagnoses. In the study by Fernandez-Moro et al. (34) involving 409 biopsies or surgical specimens of pancreaticobiliary system tumors, the antibody panel: CK19, CK20, MUC2, MUC5AC, CA.19.9, mCEA, CA125, SMAD4 allows differentiation on surgical specimens as well as on guided biopsies with areas under the curve > 0.95 for ICCA, HCC, extrahepatic pancreaticobiliary adenocarcinomas, and ampullary adenocarcinomas of intestinal type.

The main new markers identified are: CRP, N-cadherin, CK17, S100P, pVHL, MUC5AC, mpsin, annexin A1, annexin A10 (35,36). CRP appears as a promising marker to differentiate ICCA from hepatic metastases of pancreatic and gastric adenocarcinomas; its performance is better than that of N-cadherin with a sensitivity and specificity for the diagnosis of ICCA of 93% and 88% on surgical specimens versus 80% and 88% for N-cadherin (35). It should be noted that these results are not always detailed according to the histological subtype of ICCA (large ducts or small ducts, with CRP being classically expressed by ICCA of the "small ducts" type) and are not confirmed on biopsy specimens. Furthermore, CRP expression would be associated with a better prognosis.

The interest in other markers such as CK7, CK17, vimentin, mpsin, and more recently annexin A1 and annexin A10 has been emphasized in the literature. The panel ANXA1, ANXA10, CK17 combines three markers expressed by pancreatic adenocarcinomas; the positivity of 2 of these markers allows the diagnosis of metastasis from a pancreatic adenocarcinoma versus an intrahepatic cholangiocarcinoma (ICCA) with a sensitivity of 83% and a specificity of 85% (36). The most discriminating antibody among the three is ANXA10. These various markers seem promising even though their performance still needs validation on guided liver tumor biopsies, i.e., in a clinical practice setting. In routine practice, these additional markers are still underutilized, and the phenotype CK7+ CK20- CRP+/- associated with the negativity of transcription factors CDX2 (intestinal or gastric origin), TTF1 (pulmonary origin), and GATA3 (breast origin) favors the diagnosis of CCA. These first-line panel markers are not infallible and lack both sensitivity and specificity. For example, triple-negative

breast carcinomas express GATA3 in only 30% of cases (37). Conversely, GATA3 can be expressed by ICCA: 3% and 9% according to Clark (38) and Miettinen (39) respectively. However, the signal would be weaker and less extensive in ICCA than in metastases. Similarly, about 28% of lung adenocarcinomas are negative for TTF1 (40). Today, TRPS1 staining could also be useful for the diagnosis of metastasis of breast cancers, including triple negative ones (more sensitive than GATA3) and to differentiate them from other metastasis (41); the specificity, even versus CCA, could be interesting, as shown on a series including 109 tissue microarray fragments of CCAs (175).

In complex situations (history of breast or lung cancer, tumor spread to multiple organs), it may be prudent to complement the basic panel with other more specific but sometimes less sensitive markers: napsin A (lung), GCFDP15, mammaglobin, and hormone receptors (breast). While the multiplication of markers can be useful for diagnosis, it is important not to forget to preserve the tissue sample to allow for later molecular analysis for theranostic purposes.

In parallel, it has recently been suggested that in situ hybridization of albumin mRNA could be an excellent test to differentiate a metastasis (negative) from an ICCA (positive in over 80% of cases), thanks to the use of a recent and highly sensitive in situ hybridization technique, applicable to fixed tissue sections (branch chain DNA platform). The specificity obtained in three large series (up to 445 metastases) was 99% or 100% on tissue microarrays or complete sections (42-45), but another recent study on complete sections involving 139 metastases found 7% positive metastases, including tumors of pulmonary, esophagogastric, and mammary origin (46).

In case of suspicion of chronic liver disease, performing a biopsy in non-tumoral liver tissue is essential because the detection of advanced fibrosis or cirrhosis guides diagnostic hypotheses differently. In this context, distinguishing between an intrahepatic cholangiocarcinoma (ICCA) and a hepatocellular carcinoma (HCC), especially in a squamous subtype, often positive for CK7 and/or CK19, can be challenging based on standard morphology and requires the use of hepatocellular markers: HepPar1, Glypican3, Arginase 1, Alpha-fetoprotein, and canalicular markers: ACE p, CD10, or BSEP. On surgical specimens, sufficient sampling of tumors is necessary to identify the two components of a possible hepatocholangiocarcinoma (47).

Another possible differential diagnosis for small duct-type CCAs and cholangiolocellular carcinomas is benign biliary lesions such as biliary adenomas and adenoma-like ductular reactions that can be observed in cirrhotic liver. The question of biliary adenomas remains somewhat theoretical as they are usually small lesions rarely biopsied (48). Some nodular foci of ductular reaction on cirrhosis can pose real challenges in surgical specimens with small CCAs. Poorly demarcated lesions, the presence of isolated infiltrating cells, nucleoli in the nuclei, and a high nuclear-cytoplasmic ratio are signs in favor of malignancy. Several markers have been identified (SerpH1, STIP1, 14-3-3 Sigma), but the most discriminant and easily achievable element in favor of a benign lesion is a proliferation index evaluated by Ki67 less than 10% with a sensitivity of 88% and a specificity of 100% (49,50). The negativity of P16 labeling would be another argument in favor of malignancy, as adenomas would always be positive (51).

5/ What are the recommendations for anatomical pathologists concerning the technical aspects of biliary cytology and the writing of reports?

PSC

PSC is characterized by chronic inflammation and fibrosis of the bile ducts, often diffuse, leading to a "dead tree" appearance of the bile ducts. It is particularly deceptive in advanced forms with predominant severe hilar stenosis. In surgical specimens, it would represent about 1.6% of benign lesions on pseudo-Klatskin stenosis. Characteristic lesions include pericanal inflammatory (lymphoplasmacytic) fibrosis in an "onion bulb" pattern thickening large ducts and a fibro-obliterative evolution, challenging to visualize on endoscopic biopsies. Dilations are possible, filled with more or less inflammatory bile material. In peri-hilar ducts, the bile epithelium may be ulcerated and show nonspecific inflammatory and dystrophic changes; dysplastic features may appear. Bile duct biopsy primarily aims to rule out CCA. In forms with predominant hilar stenosis, liver biopsy mainly reveals nonspecific obstructive signs; specific involvement of small intrahepatic ducts is rarely visible. Targeted samples under retrograde cholangioscopy have a very good diagnostic yield with sensitivity and specificity exceeding 90% in this indication (52), while brushings alone are not very sensitive. The moderate diagnostic sensitivity of endobiliary samples has led to the development of additional tests on bile duct brushing material and endobiliary biopsies to improve the diagnostic performance of these examinations. One of the first tests proposed was nuclear morphometry (Digital Image Analysis) after Feulgen staining; despite better sensitivity than standard cytological examination (53), this method has not been widely adopted. It is possible that in the future, the use of artificial intelligence on digitized images of bile cytology could be useful for diagnosis, as it appears to be for cholangioscopy images (54). The fluorescent in situ hybridization (FISH) technique proposed by the Mayo Clinic allows the detection of chromosomal abnormalities: trisomy 7, deletion of the 9p21 locus, and especially polysomy, suggestive of malignancy using a set of 4 probes (centromeres of 3, 7, 17, and locus 9p21) initially designed for bladder cancer diagnosis (Urovysion kit). This technique has been recommended by the Papanicolaou Society of Cytopathology for cytological diagnosis of bile duct strictures and has been used in the United States but little or not in France. Its performance remains mixed with a sensitivity of 30% to 55% according to studies that often include a majority of pancreatic carcinomas (55,56). The combination of cytological analysis and FISH achieves a sensitivity of 76% with a specificity of 92% (56). The use of more specific probes for pancreaticobiliary carcinomas could increase sensitivity by about 20% compared to UroVysion FISH (57). However, multi-color FISH is a complex and expensive method difficult to implement in routine practice, with a risk of interpretation errors (58). More recently, it has been proposed to apply new molecular techniques to peri-hilar cytological or biopsy samples: NGS exploring panels of genes specific to pancreaticobiliary carcinomas or, more anecdotally, searching for gene panel methylation (59,60) or miRNAs (61). These molecular analyses, which are not currently part of common practice, have the advantage of assisting in the diagnosis of cases where cyto- and histopathological examinations are inconclusive (material classified as atypical or suspicious), and for NGS, identifying therapeutic targets. From a technical point, DNA extracted from small samples (cytoblocks and biopsies) after fixation and paraffin embedding for biopsies is scarce and of sub-optimal quality, requiring the use of DNA enrichment techniques (62). DNA extracted from specimens preserved in Cytolyt is of inadequate quality in about 10% of cases. More recently, Singhi et al. used cytological and histological samples specifically reserved for the NGS technique and directly immersed in a lysis buffer, with optimal technical performance for DNA extraction and quality. The gene panels explored vary slightly from one center to another and include between 30 and 40 genes. The most frequently mutated genes are KRAS, TP53, SMAD4, CDKN2A, PIK3CA, GNAS, and BRAF. TP53 is mutated in grade III BillN and CCA, while SMAD4 can be mutated in grade I and II BillN. Studies by Dudley 2016 and Singhi 2020 report a diagnostic sensitivity gain (>70%) compared to cyto- and histopathological diagnosis with a specificity of 98 to 100% for NGS. However, the precise diagnostic contribution for CCAp is difficult to assess because these series include pancreatic carcinomas and a

majority of distal CCAs. In patients with primary sclerosing cholangitis, the sensitivity gain is even more pronounced, ranging from 8 to 83%. False negatives by NGS are largely explained by the absence or scarcity of tumor cells. The study by Bankov et al. from endobiliary biopsies of 16 patients, mostly showing dysplastic lesions, revealed significant genetic heterogeneity between different samples from the same patient, probably related to the presence of subclones, prompting caution in the interpretation of molecular results. Despite pre-analytical requirements, molecular analysis of endobiliary samples by NGS is becoming an increasingly accessible technique for a moderate cost, certainly lower than that of a multi-color FISH technique. Its routine use will require standardization of pre-analytical and analytical methods as a prerequisite.

IgG4-Related Sclerosing Cholangitis

IgG4-Related Sclerosing Cholangitis is a possible and deceptive manifestation of IgG4-related disease. Until 2010, up to one-third of patients with biliary involvement could be operated on with a diagnosis of CCAp (cholangiocarcinoma) (63). This rare systemic inflammatory disease, of unknown cause and complex immunological mechanisms, is responsive to corticosteroid therapy. It often affects the pancreas (IgG4-related pancreatitis or "type 1 autoimmune": 60%) and potentially almost all organs: salivary glands (34%), kidneys (23%), lacrimal glands (23%), peri-aortic peritoneum (20%), liver, bile ducts, and thyroid, in particular. It induces marked fibro-inflammatory changes in these organs with a distinctive appearance.

Diagnosis is multidisciplinary and relies on a set of criteria, initially defined in Asia during the international consensus ICDC (64), then by the Japanese Pancreas Society (65), and more recently in the West: Mayo Clinic's HISORt criteria (66). In 2012, an international group of expert pathologists proposed recommendations for the anatomopathological diagnosis of this disease (67): affected organs are characterized by 1) a dense lymphoplasmacytic inflammatory infiltrate, 2) storiform fibrosis, and 3) obliterative phlebitis. Additionally, a large quantity of IgG4-positive plasma cells is present in inflammation and quantifiable through specific immunostaining. The diagnostic threshold varies depending on the organ, the type of sample (biopsy or surgical specimen), and the number of other signs present. Thus, the number of IgG4 cells alone is not sufficient for diagnosis. The proposed diagnostic algorithm leads to three possible levels of histological diagnosis: highly suggestive, probable, or unlikely. An elevation in the ratio of IgG4 cells to total IgG cells >40% in tissues would be an additional effective criterion. Eosinophils are often present in inflammation (68). In parallel, serum IgG4 levels are typically elevated: >140 mg/L (significant) or >300 mg/L (highly specific) (69). A moderate elevation may be seen in other biliary diseases (10% of PSC) and in some cases of CCA.

IgG4-related biliary involvement, called cholangitis or cholangiopathy (CIgG4), mainly affects large bile ducts and can clinically manifest in two forms: sclerosing cholangitis (usually diffuse) or hilar pseudotumor. CIgG4 is present in 60% of systemic forms but mainly affects distal bile ducts and the pancreas. Clinical practice recommendations for managing this disease have recently been published by the Japanese (70). Proximal canal involvement (hilar and intrahepatic) is reported in only 13% of cases (expected prevalence and incidence in Japan: 1.0 and 0.3/100,000, respectively), and isolated involvement of this part of the bile ducts is exceptional: 2% (71), potentially reaching 8% in tertiary care centers (72). These forms are highly misleading for CCAp. Conversely, over 95% of CIgG4 patients have pancreatic involvement, making the diagnosis of peri-hilar stenosis easier. In some recent series, CIgG4 would be the leading cause of operated pseudo-CCAp peri-hilar stenoses: 41% to 69% of cases (73,74).

The challenge in histological diagnosis is not to overlook CCAp and not to underestimate this medically treatable form of chronic cholangitis. The microscopic differential diagnosis with CCA generally does not arise if the samples are of good quality: the biliary epithelium is minimally altered in ClgG4. The occurrence of epithelial dysplasia and cancer in this context appears to be exceptional (described in 10 cases (75)). The typically affected large ducts have a diffusely thickened wall throughout their circumference (resembling a "pipe stem"), a narrow lumen, and well-preserved epithelium. Upstream dilation is moderate. The lesions are often multifocal, separated from healthy areas. The characteristic fibro-inflammatory lesions extend deeply (up to 5 mm or more) beneath this minimally altered epithelium. According to the international consensus, the microscopic diagnosis of ClgG4 is highly suggestive when there are more than 50 IgG4-positive plasma cells per high-power field (HPF) on a resection specimen when at least 2 of the other 3 histological criteria are also present; the diagnosis is only possible with this threshold if a single sign is present.

On relatively superficial and small biliary endoscopic biopsies, the morphological triad (pericanalicular lymphoplasmacytic infiltrate, storiform fibrosis, and obliterative phlebitis) is very rarely complete. According to the international consensus, the diagnosis can never be highly suggestive on canalicular biopsy; it is at best probable if there are more than ten IgG4-positive plasma cells/HPF and at least 1 of the 3 morphological criteria.

In the study by Oh, the morphological triad was not found to be complete in any of the 13 endoscopic biliary biopsies; however, the IgG4 infiltrate was significant (≥ 10 /HPF) in 9/13 (70%) of the biopsies (76). Another study found significant IgG4 staining on biopsy in 14/16 (88%) of cases. IgG4 staining of biopsies also appears to be more sensitive than serum levels since it was significant in 4/11 of affected patients with normal serum levels.

Given the possible presence of IgG4-positive plasma cells in other chronic inflammatory cholangitis, such as PSC, the ratio of IgG4-positive plasma cells to total IgG-positive plasma cells $>40\%$ in tissues would be even more specific for ClgG4. Simultaneous involvement of small intrahepatic bile ducts is observed in less than 30% of cases (77) and is never isolated. It leads to portal lymphoplasmacytic inflammation and an eosinophilic infiltrate, the formation of more or less storiform fibro-inflammatory portal nodules; however, obliterative phlebitis is rarely visible in the small portal spaces.

In the case of intrahepatic sclerosing cholangitis lesions, liver biopsy seems to be more contributive than bile duct biopsy: in 16 cases, the morphological triad was present in 2/3 of the biopsies, and IgG4 staining was significant for 3/3. The diagnosis of intrahepatic IgG4-related disease on liver biopsy is based on the same criteria, but the diagnosis will be at best probable if there are more than ten IgG4-positive plasma cells/HPF and at least 1 of the 3 morphological criteria; furthermore, an IgG4/IgG ratio $>40\%$ is suggestive.

Chapter 3: biliary drainage

What are the indications for biliary drainage in (potentially) resectable pCCA ?

The goal of biliary drainage is to improve liver function in the context of biliary obstruction. The decision to perform biliary drainage in a potentially resectable cholangiocarcinoma (CCA) should be made collectively, as suboptimal drainage or potential complications (especially infections) can complicate any potential curative surgical procedure. Biliary drainage, regardless of the method (radiological or endoscopic), should not be done before a complete staging evaluation and assessment of the resectability of the primary lesion because biliary stents and percutaneous biliary drains can create artifacts on imaging exams (CT, MRI), which can hinder the assessment of tumor extension.

Ideally, it should be done after discussing the case in a multidisciplinary meeting to select the best approach. In the preoperative setting, selective drainage and the choice of the hepatic segment should be discussed with a hepato-biliary surgeon. In cases of emergency (such as infectious cholangitis) when an optimal staging evaluation or discussion with a reference surgical team was not possible, drainage using removable material (plastic stents or external biliary drain) should be performed to avoid hindering potential curative surgery.

Preoperative biliary drainage applies to selected patients. Generally accepted indications include cholangitis or biliary sepsis, jaundice with comorbidities (malnutrition, liver failure, renal failure), and planned neoadjuvant treatment. It is also considered in cases of jaundice (bilirubin > 50 micromol/l) and planned extensive surgery, involving more than 50% of the total hepatic parenchyma (e.g., right hepatectomy) and/or portal vein embolization. In the case of left hepatectomy, drainage is not necessary and seems to increase morbidity due to infectious reasons.

Biliary drainage should be performed by an experienced operator following these rules: 1) drainage of any opacified segment to limit infectious complications, 2) no need to drain an atrophic segment, 3) drainage of at least 50% of functional hepatic parenchyma.

Biliary drainage does not increase the morbidity of hepato-biliary surgery. In a European multicenter study of 366 patients undergoing major hepatic resection for CCA, preoperative biliary drainage did not alter the mortality at day 90 or overall morbidity (9% and 68% in the drainage group, respectively, compared to 12% and 69% in the non-drainage group) (78). The specific morbidity related to drainage was 33% (cholangitis, hemorrhage, acute pancreatitis, biliary peritonitis).

Regarding the technical modalities of drainage (percutaneous radiological approach, retrograde endoscopic approach, number/type of stents), the scientific literature up until recently has been of moderate quality, with many single-center studies, often retrospective, with small sample sizes or larger sample sizes but including pancreatic tumors. The durations of the studies, often long, lead to many biases, including heterogeneity in practices, making the results difficult to interpret. Several meta-analyses have been conducted, with controversial results. Two meta-analyses showed a higher complication rate with endoscopic drainage and a higher risk of conversion and postoperative cholangitis (79,80). Another recently published meta-analysis (including 8 studies, 692 patients) showed no significant difference in terms of therapeutic success, complication rate, and mortality at J30 between percutaneous and endoscopic drainage (81).

More recently, a high-quality randomized controlled multicenter trial compared endoscopic drainage to radiological drainage in 54 patients with resectable CCA (82). The study was prematurely terminated due to higher mortality in the percutaneous drainage group (41%, 11/27 patients, vs. 21%), while the rate of drainage-related complications was similar in both groups (63% vs. 67%), marked by cholangitis, pancreatitis, and prosthetic dysfunction. These results should be taken with caution due to the small sample sizes (27 patients in each group), but it is the first prospective, randomized study in this specific indication (excluding distal CCA) and it seems reasonable to propose endoscopic drainage by experienced operators in cases of potentially operable CCA as a first-line approach. However, the complication rates related to the procedures in both groups confirm that preoperative drainage should not be systematic in cases of resectable pCCA but should be discussed on a case-by-case basis in a multidisciplinary meeting.

Regarding the type of stents, in the absence of histological diagnosis, or in a curative or potentially curative context, biliary stents should be removable: plastic stents, or covered metal stents for Klatskin I or II tumors.

What are the indications for biliary drainage in palliative care?

Biliary drainage in palliative situations aims to drain an adequate hepatic volume (50% or more), either unilaterally or bilaterally. Evaluation with cholangio-MRI helps specify the drainage strategy and select the segments to be drained. The indication for biliary drainage should be discussed in a multidisciplinary meeting.

In palliative situations, radiological drainage and endoscopic drainage appear to be equivalent in terms of technical success, complications, and effectiveness, although the literature data are quite limited (retrospective studies, often single-center, with long study periods) (83). About 25% of patients required a different drainage modality after the initial approach failed (endoscopic or radiological).

Uncovered metal stents have shown their superiority over plastic stents in non-resectable pCCA, in a randomized controlled trial (better technical success, better survival) (84). In two meta-analyses, the patency of metal stents was superior to that of plastic stents (HR 0.73, 0.56-0.93) and the complication rate was lower with metal stents (OR 2.92, 1.65-5.17) (85,86). There was no significant difference between unilateral and bilateral drainage in terms of technical success, complications, or survival.

Permanent metal stents should only be used in cases where histological proof is obtained, and surgical management is contraindicated.

In cases of insufficient retrograde biliary drainage, additional drainage of the left ducts under endoscopic ultrasound, via transgastric approach, or of the right ducts via radiological percutaneous approach, can be considered. Drainage under endoscopic ultrasound should be favoured as a second-line approach when possible (87). It has shown its equivalence to radiological drainage in a randomized trial, in terms of technical success, with fewer complications (31% in the radiological drainage group, versus 9% in the echo-endoscopic drainage group) (88). Cases should be discussed with teams with expertise in both radiological percutaneous and echo-endoscopic drainage to choose the most appropriate complementary drainage approach for the patient

Chapter 5: Locoregional treatment

1/What is the place of interventional radiologic locoregional treatments in CCA?

ICCA, radioembolization, intra-arterial chemotherapy injection) with different mechanisms of action. They are offered to patients with unresectable intrahepatic cholangiocarcinoma (CCAi), either as a first-line option or after tumor progression during systemic chemotherapy. The level of evidence regarding the efficacy and oncological benefit of these treatments is low, as the available data are extracted from heterogeneous series (patient selection, role of LRT in the therapeutic strategy, variability in the techniques used, numerous outcome criteria), often analyzed retrospectively.

A systematic review by Edeline et al., published in September 2021, provides the most comprehensive overview of the available literature (89). The authors identified 93 studies (101 cohorts, 3390 patients) evaluating a variety of LRT in the treatment of CCAi. Overall, 69 (74%) were retrospective, 70 (75%) were single-center studies, 86 (93%) did not include an

adequate control group, and 18 (19%) were only available as abstracts. Only one study was a randomized controlled trial, but the results were only available as an abstract. Overall, the authors concluded that 79 studies (85%) had a high risk of bias, 14 (15%) had an intermediate risk of bias, and no study had a low risk of bias. The median number of patients per included treatment cohort was 25, ranging from 10 to 183. Patient characteristics differed based on treatment modalities (chemotherapy or previous surgery), the size and number of treated tumors, and the presence of macrovascular invasion or extrahepatic disease.

Data and studies available in the review by Edeline et al. include:

- 15 cohorts of patients treated with an ablation technique (645 patients). Radiofrequency was the most commonly used LRT (7/15 cohorts).
- 27 cohorts of patients treated with radioembolization (1232 patients). Concurrent systemic chemotherapy was administered to 30% of the patients.
- 22 cohorts of patients treated with chemoembolization (1145 patients). Conventional chemoembolization was most commonly used (7/19 studies) followed by drug-eluting beads (6/19 studies). Concurrent systemic chemotherapy was administered to 74% of the patients.
- 16 cohorts of patients treated with intra-arterial infusion of chemotherapy (331 patients). The molecules varied (floxuridine, FUDR, gemcitabine, etc.), and concurrent chemotherapy was administered to 96% of the patients.

Results:

The available results are highly heterogeneous. Ablations need to be analyzed separately because the evaluation criteria are different. The results below are presented as grouped and weighted data extracted from the patient cohorts described above.

- Ablation techniques are associated with an overall complete response rate of 94% and an average overall survival of 30.2 months (95% CI: 21.8–38.6). These results are similar to those of surgical series, but the treated populations are different. Ablation targets smaller tumors and is often performed after previous surgery (51% in the review by Edeline et al.) and more frequently in patients with cirrhosis or those who are inoperable (due to comorbidity, fragility).
- Intra-arterial treatments are associated with objective response rates of 23%, 26%, and 41% for radioembolization, chemoembolization, and intra-arterial chemotherapy infusion, respectively. The average progression-free survival is estimated at 7.8, 15.0, and 10.1 months, respectively, and the average overall survival at 14.1, 15.9, and 21.3 months, respectively. Comparing different intra-arterial treatments is challenging, once again, due to the significant heterogeneity of the included populations. Note that the results of the only randomized trial comparing gemcitabine-cisplatin combined with drug-eluting bead chemoembolization to irinotecan versus gemcitabine-cisplatin are promising in favor of the combination: the secondary resection/ablation rate was higher (25% vs. 8%, $P < 0.005$), and the average overall survival was longer (33.7 vs. 12.6 months, $p = 0.048$).

2/ What is the place of endoscopic ablation techniques?

Photodynamic therapy and endobiliary radiofrequency are two endoscopic destructive treatments via ERCP for the treatment of perihilar cholangiocarcinoma (pCCA). Regarding

photodynamic therapy, two older randomized trials (in 2005 and 2003) had suggested a survival benefit of this technique compared to biliary drainage alone (90,91). However, the control arms lacked chemotherapy, histological proof was not systematic, and the quality of biliary drainage was questionable. The PHOTOSTENT 2 study, with more rigorous methodology (histological proof in all included patients and an effective biliary drainage protocol), published in 2018, showed lower overall survival in the group treated with photodynamic therapy + biliary stents vs. biliary stents + conventional chemotherapy (92).

Few studies are available regarding endobiliary radiofrequency. The results are satisfactory in terms of feasibility and safety profile in a recent meta-analysis (93). Only two randomized trials with conflicting results have been published. The first, in 2018, suggested an increase in the overall survival of patients with unresectable perihilar cholangiocarcinoma treated with plastic stents + radiofrequency compared to the group treated with plastic stents alone (94). The lack of oncological treatment and biliary drainage by plastic stents makes it difficult to extrapolate these results. The second study, published more recently in 2020, combined drainage by uncovered metal stents with radiofrequency versus drainage by metal stents alone. There was no significant difference in the primary endpoint (stent patency) or secondary endpoints (overall survival, complications, stent patency at 90 days). However, the calculation of the necessary sample size was questionable, as was the inclusion of distal perihilar cholangiocarcinomas and tumors of the pancreatic head (95). These data do not advocate for the use of endoscopically delivered tumor-destructive treatments as a first-line approach. These alternative treatments should only be proposed in clinical trials.

3/ What is the place of radiation therapy in inoperable CCA?

Data mostly come from comparative or non-comparative retrospective studies, including both intrahepatic and extrahepatic cholangiocarcinomas (iCCA and eCCA). In these series, radiotherapy is delivered in a conventional fractionation with a total dose ranging between 45 and 54 Gy, often combined with platinum-based chemotherapy and 5FU, with acceptable toxicity. Median overall survival (OS) varies from 11 to 15 months across the series.

Two retrospective studies based on national registries (Surveillance Epidemiology End Results) conclude in favor of the benefit of radiotherapy:

- Torgeson's study included 2996 patients with eCCA, showing a median OS benefit of 14.5 months for patients treated with chemoradiotherapy versus 12.6 months for those treated with chemotherapy alone ($p < 0.001$) (96).
- Liu's study included 1706 patients with unresectable iCCA between 2010 and 2013, demonstrating a specific survival benefit for patients treated with radiotherapy (97).

In France, the randomized phase 2 study FFCD 9902 compared Gemox chemotherapy to chemotherapy followed by chemoradiotherapy. This study included 34 patients and did not reach the planned number of patients due to recruitment difficulties. It showed no significant difference in terms of progression-free survival: 11.0 and 5.8 months (HR: 0.65 [CI: 0.32-1.33]) or OS: 19.9 and 13.5 months (HR: 0.69 [CI: 0.31-1.55]) between the two treatment arms of chemotherapy and radiochemotherapy (98).

Interestingly, the observational retrospective study by Tao on 79 patients treated with radiotherapy demonstrated a clinical benefit at 3 years associated with the delivered dose level, with a threshold at 80.5 Gy (biologically equivalent dose): better OS at 3 years (73% vs. 38%, $p=0.017$) and a better local control rate at 3 years (78% vs. 45%, $p=0.04$) (99). This dose level is not achievable in standard conformal radiotherapy but is possible with

stereotactic radiotherapy, proton therapy, or by adding a dose supplement with brachytherapy to standard conformal chemoradiotherapy.

- Brachytherapy as a dose supplement after chemoradiotherapy

Several retrospective studies suggest an effect on local control with a brachytherapy dose supplement. The largest comparative retrospective study with propensity score matching included 209 patients treated with radiotherapy or radiotherapy + brachytherapy and showed a benefit in local control for the brachytherapy dose supplement (RT+BT = 65%; RT: 35% ($p=0.09$)) without a difference in overall survival (100).

- Stereotactic Radiotherapy

The number of publications on stereotactic radiotherapy has exponentially increased in recent years (18 studies in the last 10 years and a meta-analysis of 10 studies (101)). This meta-analysis, including 231 patients, showed a one-year local control of 83% (95% CI: 76–89%), a one-year overall survival of 57% (range: 45–58%) for CCAi and 81% (range: 80–83%) for CCAe. The toxicity remains low for CCAi, while dose constraints to the digestive hollow organs can be limiting for CCAe.

- Proton Therapy

Six proton therapy studies with biologically equivalent doses ranging between 68 and 76 Gy have shown local control rates equivalent to stereotactic studies. During the management of patients treated for CCAe, episodes of biliary stent obstruction are frequent. Some retrospective studies demonstrate an extension of the duration of biliary stent patency with radiotherapy (external or brachytherapy) at limited doses ranging between 40 and 50 Gy.

4/ What is the place of radiation therapy in the perioperative treatment of pCCA?

Neoadjuvant

Neoadjuvant chemoradiotherapy has been studied either before resection or before liver transplantation. Chemoradiotherapy before surgery has been evaluated in three older studies, leading to R0 resection rates ranging from 83% to 100%. Nelson et al retrospectively compared patients treated with neoadjuvant chemoradiotherapy versus adjuvant. In this study, neoadjuvant chemoradiotherapy showed a trend toward improved overall survival (5-year OS rate 53% vs. 23%, $p = 0.16$) with similar rates of surgical morbidity grade 2-3 (102). Neoadjuvant radiotherapy has also been studied in combination with liver transplantation in patients with pCCA in small single-center studies, using brachytherapy, stereotactic radiotherapy, or concurrent chemoradiotherapy. In a multicenter American study published by Darwish Murad et al., 293 pCCA patients were treated with neoadjuvant radiotherapy followed by liver transplantation (103). The 5-year post-transplant recurrence-free survival rate was 65%.

Adjuvant

Studies on adjuvant radiotherapy for pCCA are mostly retrospective, with a low level of evidence and contradictory results. In the non-randomized phase II trial SWOG S0809 (104), 79 patients with pCCA or gallbladder carcinoma received adjuvant chemotherapy with gemcitabine plus capecitabine followed by adjuvant chemoradiotherapy with concurrent capecitabine. The median overall survival was 35 months. The resection margin status (R0

or R1) did not influence the prognosis. For reference, the median OS was 51 months in the adjuvant capecitabine arm in the phase III BILCAP trial (105). The meta-analysis by Horgan et al. published in JCO in 2012 included 20 studies conducted between 1960 and 2010, involving 6712 patients (106). The authors concluded a borderline significant overall survival benefit of any adjuvant treatment (chemotherapy, radiotherapy, or chemoradiotherapy) compared to surgery alone ([HR]: 0.74; $p=0.06$). The benefit of chemotherapy and chemoradiotherapy was significantly greater than that of radiotherapy alone ([OR]: 0.39, 0.61, and 0.98, respectively; $p=0.02$). The adjuvant treatment benefit became significant in the case of N+ status ([OR]: 0.49; $p=0.004$) or R1 status ([OR]: 0.36; $p=0.002$). A second meta-analysis published in 2020 included 21 studies with 1465 patients and also concluded a benefit in terms of 5-year overall survival and local control for patients with nodal involvement or positive margins (107). These results should be interpreted cautiously due to the retrospective nature of most studies. Adjuvant chemotherapy and chemoradiotherapy have never been compared by a prospective trial

Chapter 6: systemic treatment

Which systemic treatments are recommended in first line?

The results of available randomized trials favor the superiority of a platinum-based combination chemotherapy, primarily with gemcitabine, over monotherapy with fluoropyrimidine or gemcitabine, or exclusive best supportive care:

- In a Scandinavian phase III trial involving 90 patients with advanced biliary or pancreatic cancer, a chemotherapy regimen of 5FU and folinic acid (plus etoposide for patients in good general condition) was not significantly superior to exclusive supportive care in the subgroup of patients with biliary cancer, and the toxicity was significant (grade 3-4: 41%) (108).
- An Indian phase III monocentric trial in 81 patients with advanced gallbladder carcinoma showed an overall survival benefit for a gemcitabine-oxaliplatin combination compared to exclusive supportive care, as well as compared to a 5FU-folinic acid combination (9.5, 4.5, and 4.6 months respectively, $p=0.039$) (109).
- An Italian phase II randomized trial demonstrated the superiority of a combination of 5FU, folinic acid, and oxaliplatin (FOLFOX regimen) over a 5FU-folinic acid combination (overall survival: 13.0 vs. 7.5 months; $p=0.001$) (110).

In a systematic review of 104 first-line chemotherapy studies reported between 1985 and 2006 (5 to 65 patients per study, 2810 patients analyzed in total), all non-randomized except for three (two phase II and one phase III on 47 patients), the highest rates of objective response and tumor control were achieved with regimens combining gemcitabine and platinum (cisplatin or oxaliplatin) (111). An update of data involving 6337 patients included in 161 studies, with an extension to targeted therapies, was published in 2014 by the same authors (112). The pooled analysis showed objective response and tumor control rates of 26% and 63%, respectively, and median progression-free survival and overall survival of 5.3 months and 9.5 months in patients treated with a gemcitabine-platinum regimen.

The phase III British trial ABC-02 demonstrated in 410 patients (ECOG PS 0-1: 88% and total bilirubin < 1.5 N) the superiority of the cisplatin-gemcitabine combination (CISGEM, cisplatin 25 mg/m², gemcitabine 1000 mg/m², days 1 and 8, every 3 weeks) administered for 24 weeks over gemcitabine alone (overall survival: 11.7 vs. 8.1 months; HR: 0.64; CI: 0.52-0.80; $p < 0.001$), regardless of the stage (locally advanced or metastatic) and location of

biliary cancer (113). It should be noted that in subgroup analysis, the combination therapy provided a significant benefit only in patients PS 0-1. The combination's tolerance was acceptable, and the low unit dose of cisplatin (25 mg/m² on day 1 and day 8, day 1 = day 21) required only reduced hydration. These results were supported by those of the randomized Japanese phase II trial BT-22 in 84 patients, combined with ABC-02 in a pooled analysis (114,115). It is worth noting that doubling the administration duration in the BT-22 trial (48 weeks) did not seem to improve the results.

The GEMOX regimen has been studied in numerous studies, including several prospective trials (116–118). In a systematic review of 33 studies involving 1470 patients, median overall survival was 9.7 months in the CISGEM group and 9.5 months in the GEMOX group, with median progression-free survival of 6.3 and 4.9 months, respectively (119). CISGEM chemotherapy was associated with significantly higher toxicity (asthenia, diarrhea, hepatic and hematologic toxicity). A randomized Indian phase III non-inferiority trial compared a modified GEMOX regimen (mGEMOX: gemcitabine 900 mg/m², oxaliplatin 80 mg/m², days 1 and 8, every 3 weeks, maximum 6 cycles) to the CISGEM regimen (maximum 8 cycles) in 260 patients with advanced vesicular carcinoma with PS 0 to 2 (120). Peripheral neuropathy and thrombocytopenia were more common with mGEMOX, and nephrotoxicity with CISGEM. Despite a numerically higher median overall survival in the mGEMOX arm (9.0 months vs. 8.3 months; HR: 0.78; CI95%: 0.60-1.02; p=0.057), this trial failed to demonstrate the equivalence of the two regimens (median difference: 0.8 months, upper bound of CI95% greater than 2 months [-1.1-2.7]), nor a possible superiority of mGEMOX.

A randomized phase III non-inferiority trial compared in the first-line treatment of 222 patients with advanced biliary cancer CAPOX (capecitabine-oxaliplatin) and another modified GEMOX regimen (gemcitabine 1000 mg/m², days 1 and 8, oxaliplatin 100 mg/m² on day 1, every 3 weeks), for a total of 8 cycles (121). Median progression-free survival was 5.8 months in the CAPOX arm compared to 5.3 months in the GEMOX arm, with progression-free survival at 6 months (the primary endpoint of the trial) being 47% and 44% respectively (non-inferiority demonstrated). There were no differences in terms of response rates and overall survival.

The Phase II/III AMEBICA PRODIGE 38 trial (NCT02591030) compared chemotherapy with modified FOLFIRINOX (5FU, folinic acid, irinotecan, and oxaliplatin) to the standard CISGEM. The study was negative for its primary Phase II endpoint, 6-month progression-free survival (51% with modified FOLFIRINOX compared to 59% with CISGEM). Therefore, the evaluation of FOLFIRINOX will not be continued in Phase III (122).

Single-arm Phase II studies evaluated nab-paclitaxel-based regimens (gemcitabine-nab-paclitaxel, gemcitabine-cisplatin-nab-paclitaxel) with interesting median overall survival (12.4 and >20 months, respectively) and progression-free survival (7.7 and 11.4 months, respectively). However, these require evaluation in Phase III compared to CISGEM (currently ongoing) (123,124).

Similarly, the compound NUC-1031 (derived from gemcitabine) was evaluated in combination with cisplatin in 14 patients, with a response rate of 64%, and is currently being assessed in a Phase III trial compared to CISGEM (125).

The NIFE AIO trial was a randomized, non-comparative Phase II trial in which chemotherapy-naïve patients with intrahepatic or perihilar cholangiocarcinoma and an ECOG PS of 0-1 received either CISGEM or a combination of 5FU/leucovorin with nal-IRI (liposomal irinotecan) in 93 patients recruited from 21 German centers (126). The primary endpoint was achieved with a 4-month progression-free survival rate of 51% with 5FU/leucovorin plus nal-

IRI (compared to 59% with CISGEM). The median progression-free survival was 6.0 months (2.37-9.59) with 5FU/leucovorin plus nal-IRI and 6.9 months (2.46-7.82) with CISGEM. The median overall survival (data not yet mature) was 15.9 months and 13.6 months, and the overall response rate was 24% and 12%, respectively. A differential effect was observed depending on the location of the primary tumor, with a median progression-free survival of 3.5 months with 5FU/leucovorin plus nal-IRI versus 7.7 months with CISGEM (median OS 14.2 versus 16.4 months, n = 66), and 9.6 months versus 1.8 months in extrahepatic cholangiocarcinoma (median OS 18.2 versus 6.3 months, n = 25).

In Asia, where the compound S-1 is available, a randomized Phase II South Korean trial showed comparable efficacy of gemcitabine-cisplatin combinations (a different schedule than CISGEM) and S-1-cisplatin (127).

The gemcitabine-S-1 combination was shown to be superior to S-1 alone, but not to gemcitabine, in three Asian randomized Phase II trials (128–130). A Phase III study with 354 patients demonstrated the non-inferiority of the gemcitabine-S-1 combination compared to the gemcitabine-cisplatin combination (median OS: 15.1 versus 13.4 months; median progression-free survival: 6.8 versus 5.8 months) (131). Finally, a Phase III study with 246 patients showed the superiority of the gemcitabine-cisplatin-S-1 triplet over the gemcitabine-cisplatin doublet (median OS: 13.5 versus 12.6 months, HR 0.79, 95% CI 0.60-1.04, p = 0.046; median progression-free survival: 7.4 versus 5.5 months, HR 0.75, 95% CI 0.58-0.97; p = 0.0015; response rate: 41.5% versus 15.0%)(132).

However, the unavailability of S-1 in France limits the practical relevance of these results.

Regarding immunotherapy, the results of only one randomized controlled trial (TOPAZ-1) are currently available (133). The results of the interim analysis of this international Phase III trial show a benefit in response rate, progression-free survival, and overall survival of durvalumab compared to a placebo in combination with first-line chemotherapy by CISGEM in patients with advanced biliary cancer in good general condition (performance status 0 or 1). The magnitude of this benefit was moderate to modest (absolute benefit in response rate < +8%; HR 0.75 for progression-free survival; 0.80 for overall survival); notably, the suggestion of an apparently increasing benefit in long-term survival is currently impossible to confirm due to a still short follow-up and low patient numbers at these time points. In subgroup analysis, the benefit seemed smaller in non-Asian patients and in cases of gallbladder carcinoma. Anti-PD-L1 immunostaining did not seem to discriminate a population responding better to immunotherapy, including, notably, for high scores. The results of another international Phase III randomized controlled trial (KEYNOTE-966) are available. This study compared pembrolizumab (200 mg every 3 weeks, maximum 35 cycles) and placebo in combination with rdds (134).

Witch systemic treatments are recommended in second line

The available studies, predominantly non-randomized and mostly retrospective, report low response rates (3-12%) and modest median progression-free survival (1.9-4.0 months) and overall survival (6.2-11.0 months) (135–140). These studies have not identified one chemotherapy regimen as more effective than others. In particular, the superiority of combination chemotherapy over monotherapy (especially with fluoropyrimidine) is not established (141). A Phase II Italian randomized trial with 57 patients with PS 0-2 showed similar 6-month progression-free survival rates with either single-agent capecitabine (8%) or combined with mitomycin C (10%) (142). In a Chinese randomized Phase II trial comparing 60 patients in good general condition (ECOG PS 0-1) with advanced biliary cancer that had

progressed after first-line gemcitabine and cisplatin, the combination of capecitabine and irinotecan (irinotecan 180 mg/m² on day 1, capecitabine 1000 mg/m² twice daily on days 1 to 10, every 14 days) was compared to irinotecan alone. The median progression-free survival was 3.7 vs 2.4 months (p=0.036), the 9-month survival rate was 61% vs 32% (p=0.045), and the median overall survival was 10.1 vs 7.3 months (p=0.107) (143).

Conversely, the randomized Phase II GB-SELECT trial comparing capecitabine-irinotecan (capecitabine: 1700 mg/m²/day from day 1 to 14; irinotecan: 200 mg/m²) to irinotecan alone (240 mg/m²), every 21 days until progression, in 98 patients with advanced gallbladder cancer whose disease had progressed after previous gemcitabine-based chemotherapy, showed no difference in terms of 6-month overall survival (38.4% vs 54.2%) and median overall survival (5.16 vs 6.28 months, HR 1.02; 95% CI, 0.64-1.49, p=0.93); a greater number of patients had to adjust their dose in the CAPIRI group compared to the irinotecan group (13 [27%] vs 4 [9%], respectively, P = 0.03) (144).

Results from the British Phase III ABC-06 trial were reported at the 2019 ASCO congress (145). This study compared an FOLFOX regimen combined with best supportive care versus supportive care alone in 162 patients with advanced biliary cancer, in good general condition (ECOG PS 0-1), whose disease had progressed on (or after) CISGEM. Patients treated with FOLFOX had an improvement in overall survival (primary endpoint; median: 6.2 vs. 5.3 months, HR: 0.69 [95% CI 0.50-0.97], p=0.031); 12-month overall survival favored the FOLFOX arm (26% vs. 11%). FOLFOX was also as effective or even more effective in platinum-resistant patients (progression during or within 3 months following first-line CISGEM). The response rate was low, around 5%. The chemotherapy's tolerance was acceptable, but with more neutropenia, asthenia, and infections, and three toxic deaths. Quality of life results are not yet available.

A South Korean Phase II randomized trial compared, in the second line after CISGEM, mFOLFIRI (irinotecan 150 mg/m², leucovorin 100 mg/m², 5FU 2400 mg/m² over 46h) and mFOLFOX (oxaliplatin 100 mg/m², leucovorin, and 5FU same as mFOLFIRI) every 2 weeks in 118 patients with advanced biliary cancer and ECOG performance status 0 to 2 (146). There was no difference between mFOLFOX and mFOLFIRI in terms of objective response rate (6% vs 4%, p=0.66) or disease control rate (67% vs 64%, p=0.78), progression-free survival (median: 2.8 vs 2.1 months; p=0.97), or overall survival (median: 6.3 vs 5.7 months, p=0.68; 54% vs 44% at 6 months).

The Phase II NIFTY randomized trial, presented at ASCO 2021, reported initial results of the combination of 5FU/leucovorin and nal-IRI in advanced CCA in the second line in an Asian population, with an improvement in progression-free survival (median: 7.1 vs 1.4 months, HR 0.56 [95% CI: 0.39-0.81], p=0.0019), overall survival (median: 8.6 vs 5.5 months HR 0.68 [95% CI: 0.48-0.98], p=0.0349), and overall response rate (15% vs 6%) compared to 5FU/leucovorin alone (147). Results from the Phase II NallIRICC study in the European population are awaited.

On the other hand, results from the Vogel et al. study (ESMO 2022) do not show any benefit of 5FU/leucovorin and nal-IRI chemotherapy compared to 5FU-Leucovorin, neither in progression-free survival (median 2.63 vs 2.3 months HR 0.87), nor in overall survival (median 6.9 vs 8.1 months, HR 1).

What is the place of molecular screening for guiding patient care?

All randomized trials reported to date have failed to demonstrate a survival benefit of combining targeted therapy with chemotherapy in unselected patients with advanced biliary cancer, including anti-EGFR (148) or anti-angiogenic agents (149–151).

High-throughput sequencing studies have shown the molecular heterogeneity of biliary cancers and their richness in potential therapeutic targets (IDH1/2, FGFR2, BRAF, HER2, MSI, NTRK...) (154–156). The MOSCATO-01 trial provided the first evidence that high-throughput sequencing molecular profiling was feasible and could be beneficial for these patients (157). The success rate for detecting at least one targetable molecular alteration was about 70%. Administering targeted treatments for these anomalies showed a clinical benefit (defined by the ratio of progression-free survival with targeted therapy to progression-free survival with the previous line > 1.3) in 80% of patients and an objective response rate of 33%.

The results of the Phase III ClarIDHy study showed that ivosidenib, an oral drug targeting the mutation of isocitrate dehydrogenase 1 (IDH1), detected in about 20% of patients with advanced cholangiocarcinoma (predominantly intrahepatic), significantly improved progression-free survival compared to placebo (2.7 vs 1.4 months; HR: 0.37; 95% CI: 0.25-0.54, $p < 0.001$) in 185 patients with advanced cholangiocarcinoma, who had failed one to two lines of systemic treatment and had preserved general condition (ECOG PS 0-1) (158). The median progression-free survival rates at 6 and 12 months were 32% and 22%, respectively, with ivosidenib, compared to 0% in the placebo group. The improvement in overall survival (10.8 vs 9.7 months; HR: 0.69, $p = 0.06$) was significant after statistical analysis accounting for patient crossover to ivosidenib after progression in the placebo arm (57% of patients; 10.8 vs 6.0 months; HR: 0.46, $p = 0.0008$). Ivosidenib was generally well-tolerated. Fusions involving one of the three tropomyosin receptor kinase (NTRK) genes were identified in 1 out of 28 (3.5%) patients with intrahepatic cholangiocarcinoma (159). A Phase 1-2 trial evaluated larotrectinib, a selective NTRK inhibitor, in 55 adult and pediatric patients with 17 types of tumors harboring NTRK fusions (160). The response rate was 75% (including one of the two patients with intrahepatic cholangiocarcinoma) and was independent of patient age or tumor type.

Several Phase II trials and some retrospective series suggest the potential of targeting alterations in FGFR2, BRAF, or HER2 in advanced biliary cancers (161–172). Phase III trials for FGFR2 inhibitors are currently underway.

Biliary tract cancers with microsatellite instability (MSI) are responsive to anti-PD1 treatments (173,174). Regardless of MSI status, studies are also underway to evaluate the effectiveness of immunotherapies in these tumors.

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