Diplomarbeit

Benefit of second-line systemic chemotherapy for advanced biliary tract cancer: A propensity score analysis

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Florian Moik

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unter der Anleitung von

Dr.med.univ. Florian Posch, MSc.

Assoz.Prof. Priv.-Doz. Dr.med.univ. et scient.med. Armin Gerger, MBA

Dr.med.univ. Michael Stotz

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Graz, am 14.04.2018 *Florian Moik eh.*

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Abbrevations:

1LCTX.....*first line chemotherapy*
2LCTX.....*second line chemotherapy*
5-FU.....*5-fluorouracil*
aBTC.....*advanced biliary tract cancer*
AJCC.....*American Joint Committee on Cancer*
ALT.....*alanine aminotransferase*
ANA.....*antinuclear antibodies*
aOR.....*adjusted odds ratio*
AST.....*aspartate aminotransferase*
BilIN.....*biliary intraepithelial neoplasia*
BMI.....*body mass index*
BSC.....*best supportive care*
CA 19-9.....*carbohydrate antigen 19-9*
CBD.....*common bile duct*
CCA.....*Cholangiocarcinoma*
CEA.....*carcinoembryogenic antigen*
CHA.....*common hepatic artery*
CI.....*confidence interval*
CPV.....*cancer of the papilla vateri*
CRP.....*c-reactive protein*
CT.....*computer tomography*
CTX.....*chemotherapy*
CU.....*ulcerative colitis*
CUP-CCC.....*cancer of unknown primary with cholangiocellular differentiation*
DHC.....*ductus hepaticus communis*
DHD.....*ductus hepaticus dexter*
DHS.....*ductus hepaticus sinister*
DNA.....*desoxyribonucleic acid*
eCCA.....*extrahepatic cholangiocarcinoma*
EGFR.....*epidermal growth factor receptor*
ERCP.....*endoscopic retrograde cholangiopancreaticography*
EUS.....*endoscopic ultrasound*
FAP.....*familil adenomatous polyposis*
GBC.....*Gallbladder Cancer*
HCV.....*hepatitis-C virus*
HIV.....*human-immunodeficiency-virus*
HNPCC.....*heriditary nonpolyposis colorectal cancer*
HR.....*hazard ratio*
iCCA.....*intrahepatic cholangiocarcinoma*
IPMN.....*intraductal papillary mucinous neoplasm*
iTACE.....*irinotecan eluting beads-TACE*
LNN.....*lymph nodes*
MALT.....*mucosa associated lymphatic tissue*
MOR.....*mechanism of resistance*
MRI.....*magnetic resonance imaging*

OS ..... *overall survival*, *overall survival*
pANCA ..... *antineutrophilic cytoplasmatic antibodies with perinuclear staining pattern*
pCCA ..... *perihilar cholangiocarcinoma*
PDGF ..... *platelet derived growth factor*
PDGFR ..... *platelet derived growth factor receptor*
PFS ..... *progress free survival*
PS ..... *propensity score*
PSC ..... *primary sclerosing cholangitis*
PTC ..... *percutaneous transhepatic cholangiography*
PV ..... *portal vein*
RFA ..... *radiofrequency ablation*
RR ..... *relative risk*
SEER ..... *Surveillance, Epidemiology, and End Results*
SMA ..... *superioir mesenteric artery*, *smooth muscle antibodies*
SMD ..... *stndardized mean difference*
TACE ..... *transarterial chemoembolisation*
UICC ..... *Union internationale contre le cancer*
VEGF ..... *vascular endothelial growth factor*
VEGFR ..... *vascular endothelial growth factor receptor*
ΔS ..... *standardized mean difference*

Figures:

Tables:

Zusammenfassung

Hintergrund

Die Zweitlinien-Chemotherapie findet bei PatientInnen mit fortgeschrittenen Karzinomen der Gallenwege zunehmende Anwendung, obwohl es bisher keine randomisierten Studien gibt, die einen Vorteil dieser Therapie gegenüber allein palliativ-supportiven Maßnahmen belegen. In Ermangelung randomisierter Daten haben wir eine Propensity-Score-Analyse des Gesamtüberlebens bei PatientInnen mit fortgeschrittenen Gallenwegskarzinomen, welche mit palliativen Maßnahmen mit oder ohne zusätzlicher Zweitlinienchemotherapie behandelt wurden, durchgeführt.

Material und Methoden:

Es wurden retrospektiv 80 PatientInnen eingeschlossen, deren Galletraktkarzinom fortgeschritten (metastasiert, rezidiviert oder inoperabel) war (mittleres Alter: 68 Jahre, weibliches Geschlecht: n=38 (48%), ECOG-Performance-Status 1-2: n=27 (45%)). Von dieser Kohorte haben 38 PatientInnen (48%) eine Zweitlinien-Chemotherapie zusätzlich zu palliativen Maßnahmen erhalten (Fluoropyrimidin-basierte-Monochemotherapie: n=26 (68%), Fluoropyrimidin-basierte-Polychemotherapie: n=8 (21%), andere Chemotherapie-Regimente: n=4 (11%)). Der primäre Endpunkt der Studie war das 18-monatige-Gesamtüberleben. Um den ungleichen Verteilung prognostischer Faktoren zwischen den beiden Behandlungsgruppen Rechnung zu tragen, wurde eine inverse-Behandlungswarscheinlichkeits-gewichtete Analyse (inverse-probability-of-treatment-weighted-analysis (IPTW)) durchgeführt.

Ergebnisse

Während des medianen Beobachtungszeitraumes von 14.8 Monaten verstarben 49 der 80 PatientInnen. Die 6-, 12-, und 18-Monats-Kaplan-Meier-Gesamtüberlebens-wahrscheinlichkeiten waren 77%, 53% und 23 % in der Zweitlinienchemotherapie-Gruppe, und 29%, 21% und 14% bei jenen Patienten, die ausschließlich palliativ-supportive Therapiemaßnahmen erhielten (log-rank p=0.0003; HR=0.36, 95% CI:0.20-0.64, p=0.001). PatientInnen in der Zweitlinienchemotherapie-Gruppe hatten jedoch eine signifikant höhere Prävalenz prognostisch günstiger Variablen (besserer ECOG Perfomance-Status (p=0.05), metachrone Metastasierung (p=0.001), niedrigere Werte des C-reaktiven Proteins (p=0.04)).

Dieses Ungleichgewicht zwischen den Gruppen wurde durch die Gewichtung der Daten mittels IPTW drastisch reduziert. In der IPTW-adjustierten Analyse der Überlebenszeit blieb die Assoziation zwischen Zweitlinienchemotherapie und längerem Gesamtüberleben erhalten (adjustierte HR=0.42, 95%CI:0.18-0.96, p=0.04). In der IPTW-gewichteten Kaplan-Meier-Analyse waren die Gesamtüberlebenswahrscheinlichkeiten für 6, 12 und 18 Monate 74%, 57% und 33% in der Zweitlinien-Chemotherapie-Gruppe und 41%, 29% und 22% in der Palliativtherapie-Gruppe (log-rank p=0.04). Die positive Assoziation zwischen Gesamtüberleben und Zweitlinienchemotherapie wurde über den Beobachtungszeitraum tendenziell schwächer (Schönfeld-Test p=0.005). Der beobachtete Überlebensvorteil der Zweitlinienchemotherapie war in verschiedenen Subgruppen gleichermaßen gegeben, beispielsweise bei PatientInnen mit oder ohne objektives Ansprechen auf die Erstlinien-Chemotherapie sowie mit oder ohne Obstruktion des Galleabflusses.

Conclusio

Diese nicht-randomisierten Daten unterstützen die Hypothese, dass in der Behandlung von PatientInnen mit fortgeschrittenen Gallenwegskarzinomen eine Zweitlinienchemotherapie zusätzlich zu palliativ-supportiven Maßnahmen mit einem höheren Gesamtüberleben assoziiert ist als palliativ-supportive Maßnahmen alleine.

Abstract

Background

Second-line-chemotherapy (2LCTX) is increasingly applied in patients with advanced-biliary-tract cancer (aBTC), although no randomized trial has so far demonstrated the benefit of this intervention over best-supportive-care (BSC) alone. In the absence of randomized data, we conducted a comparative effectiveness analysis of survival outcomes in aBTC patients treated with BSC±2LCTX.

Methods

This single-center observational cohort-study includes 80 patients (median age: 68 years, female: n=38 (48%), ECOG 1-2: n=27 (45%) with aBTC (metastatic, recurrent, or inoperable) who completed 1st-line-chemotherapy at the department of Oncology of the Medical University of Graz (overall: 185 BTC-patients 2003-2016)). Thirty-eight of these patients (48%) received 2LCTX in addition to BSC (Fluoropyrimidine-based-monochemotherapy: n=26 (68%), Fluoropyrimidine-based-polychemotherapy: n=8 (21%), other regimens: n=4 (11%)). Primary endpoint was 18-month-OS. An inverse-probability-of-treatment-weighted-analysis (IPTW) was conducted to rigorously account for imbalances in prognostic variables between the two groups.

Results

During a median follow-up of 14.8 months, we observed 49 deaths. Crude 6-, 12-, and 18-month Kaplan-Meier OS estimates were 77%, 53% and 23% in the BSC+2LCTX-group, and 29%, 21%, and 14% in patients in the BSC-group (log-rank p=0.0003; Hazard ratio (HR)=0.36, 95%CI:0.20-0.64, p=0.001). However, patients receiving 2LCTX+BSC had a significantly higher prevalence of favourable prognostic variables, such as a better ECOG-performance-status (p=0.05), metachronous metastasis (p=0.001), and lower C-reactive protein levels (p=0.04). These imbalances were strongly reduced by weighting of the data with the IPTW. In IPTW-adjusted analysis of time-to-death, the favourable association between 2LCTX and OS prevailed (Adjusted HR=0.42, 95% CI:0.18-0.96, p=0.04). In IPTW-weighted Kaplan-Meier analysis, 6-, 12-, and 18-month OS estimates were 74%, 57% and 33% in the BSC+2LCTX group, and 41%, 29% and 22% in patients in the BSC group

(log-rank p=0.04). The favourable association of 2LCTX with OS slightly weakened over time (Schoenfeld test p=0.005). Otherwise, the observed benefit of 2LCTX was consistent across several subgroups, such as patients with and without objective response to 1st-line chemotherapy, as well as patients with and without moderate biliary stenosis (indicated by serum bilirubin levels).

Conclusions:

Within the limitations of an observational study, our data support the concept that 2LCTX+BSC is associated with an OS benefit over BSC alone in patients with aBTC.

Publications and presentations based on this thesis

Moik F, Riedl J, Winder T, Bezan A, Rossmann C, Szkandera J, Kasparek AK, Schaberl-Moser R, Pichler M, Stöger H, Stotz M, Gerger A, Posch F. Benefit of second-line chemotherapy for advanced biliary tract cancer. (Scientific Reports, in Revision)

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Introduction

Classification and definition

Biliary tract cancer (BTC) is an aggressive orphan malignancy, embracing different cancers deriving from epithelial cells of the structures of the biliary tract, anywhere between the microscopic bile ductuli in the liver down to the Papilla Vateri, where the biliary drainage system meets the duodenum.(1) The term cholangiocarcinoma (CCA) is used for all these cancers, except for gallbladder cancer (GBC) and cancer of the Papilla Vateri (CPV). It is further subdivided into intrahepatic Cholangiocarcinoma (iCCA), with the limit of distal involvement just proximal of the confluence of the right and left hepatic ducts, and extrahepatic cholangiocarcinoma (eCCA). Extraheptic disease is sub-classified as perihilar (Klatskin tumor) and distal disease, with the locus of transition being just proximal of the confluence of the cystic duct and the common hepatic duct, posterior to the duodenum. (1) (**Table 1**). Advanced biliary tract cancer (aBTC) is commonly defined as carcinoma of the intra- or extrahepatic bile ducts, the gallbladder or cancer of the Ampulla Vateri that is either inoperable, recurrent, or metastatic and has a dismal prognosis. (2)

Table 1: Classification and crude Incidence of biliary tract cancer (BTC). (3)

Entity			Frequency	Incidence in Europe*
CCA	**iCCA**		**50%**	**0.3–3.5**
	eCCA	**Perihilar eCCA**	**10%**	
		Distal eCCA	**40%**	
Gallbladder Cancer				**1.6–2.0**
Cancer of the Papilla Vateri				**0.4-0.6**
CCA: Cholangiocarcinoma; iCCA: intrahepatic cholangiocarcinoma; eCCA: extrahepatic cholangiocarcinoma; **per 100.000.*				

Perihilar disease (pCCA) is further sub-classified with the Bismuth-Corlette-Classification according to the pattern of involvement of the different hepatic ducts and especially the confluence (**Table 2**). Type 1 affects the common bile duct distal of the confluence of the

left and right hepatic duct. As soon as the tumour affects the confluence, it is classified as Type 2. Type 3 is applied when the tumour extends up to the bifurcation of either the right (Type 3a) or the left (Type 3b) hepatic duct in combination with involvement of the confluence of the two. Type 4 means the (I) affection of both, the right and left hepatic duct up to their bifurcation in combination with involvement of the confluence or (II) multicentric disease of the perihilar biliary structures. This classification has prognostic value, with a higher rate of positive surgical margins and poorer overall survival after initial surgical treatment in Type 4 tumours compared to type 1-3. (4)

Table 2: Bismuth-Corlette-Classification of perihilar eCCA.

Classification	Involved Structures
Type 1	**DHC**
Type 2	**DHC + Confluence**
Type 3a	**DHC + Confluence + DHD**
Type 3b	**DHC + Confluence + DHS**
Type 4	**Confluence + DHS + DHD or multicentric**
DHC: Ductus hepaticus communis; DHD: Ductus hepaticus dexter; DHS: Ductus hepaticus sinister	

Epidemiology

Cholangiocarcinoma

Cholangiocarcinoma is rare, only accounting for 1% of all human cancers and 10-15% of primary liver malignancies. The incidence of cholangiocarcinoma in general is about 0.3-3.5/100.000 in Europe, Northern America and Australia.(5) Exceptions of that are related to regional exposure to certain risk factors, for example a higher incidence of liver fluke infections in south-east Asia, with the north-eastern regions of Thailand having the highest rates in the world with 90/100.000, rendering CCA with over 80% the most frequent primary hepatic malignancy in these areas.(6)

Over the past years, the epidemiology has slightly changed in most western countries with few exceptions (Denmark, Norway, Czech Republic) in the sense of rising incidence and mortality of iCCA and falling rates for eCCA.(7-9) The trends in Austria are in line with this development, with an increase in the incidence and mortality of iCCA in both sexes from 1990 to 2010 and a decrease of the incidence and mortality of eCCA in both sexes.(10) Several different factors have been suggested to contribute to this development, even though the changes in incidence have not been fully understood yet. Firstly, there are factors that changed the incidence of different subtypes independently of changes in the reality of the disease frequency in the population, such as better diagnostic capability in the workup of obstructive jaundice as well as misclassification during changes in the Staging system.(11) Secondly, there are changes in different factors, associated with a higher risk of the development of CCA, such as an increased rate of chronic liver disease (Hepatitis C, alcoholic hepatitis, non-alcoholic fatty liver disease),(12) the possible contribution of environmental factors as well as an increase in the rates of cholecystectomies, significantly reducing gallstone-disease.(12) Further, changes in the migration patterns over the last years in the west might have contributed to this development.(13)

The typical presentation of CCA occurs between 50 and 70 years of age, except for the cases associated with primary sclerosing cholangitis (PSC) or choledochal cysts, with a diagnosis typically being made 2 decades earlier.(5, 14) There is a slight male to female predominance (1.2-1.5:1), possibly reflecting the higher frequency of PSC in the male population.(15) This predominance stands in contrast to GBC, where there is a higher incidence in women.(16)

Gallbladder Cancer

In western countries such as the USA and Western Europe, Gallbladder cancer has a low incidence with 1.6-2.0 newly diagnosed cases yearly per 100.000.(17, 18) However, like cholangiocarcinoma, also the incidence of GBC varies strongly around the globe. It is a major health problem in Chile, Eastern Europe, India, Pakistan and Korea, with a peak Incidence of 24.3/100.000 in the Valdivia region of Chile in females.(19) These high risk areas share two of the major risk factors for gallbladder cancer, a high incidence of gallstones and salmonella typhi carriers. (18, 20)

Ampullary cancer

Primary ampullary cancer, cancer that derives from the epithelial cells of the Papilla Vateri, is rare, with an incidence of only 4-6 cases per million population.(21, 22) Despite their infrequency, they are responsible for 20% of all tumour related bile duct obstructions. The incidence seems to have increases in the last 30 years.(21) The incidence is increases by the factor 200-300 in patients with hereditary polyposis syndromes like familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC).(21, 23)

Prognosis

In general, biliary tract cancer has a very poor prognosis with differences among its subtypes.(24) According to the National Cancer Institute's SEER programme, BTC survival in patients diagnosed between 2000 and 2006, the 5-year overall survival for localized (Stage I), regionally advanced (Stages II-III) and disseminated (Stage IV) disease was 15%, 5% and 2% for intrahepatic and 30%, 25% and 2% for extrahepatic CCA.(25) The median overall survival in Austria for all entities of BTC combined was 4.8 months, 1 and 5 year overall survival was 31% and 10%.(10) The survival rates vary strongly according to respectability. Unfortunately, only 10-40% of patients with cholangiocarcinoma present with respectable disease. These patients have 5 year survival rates of 25-30%, compared to 5-10% in CCA in general.(24) Patients that underwent resection for CCA have a rate of recurrence of 50-60%, with a median disease free survival of 26 months. The most common organ of recurrence is the liver, with a frequency of 50-60%, followed by the regional lymph nodes in 20-25%.(24) This distribution underlines two of the risk factors for recurrence, which are positive surgical

margins as well as lymph node involvement.(26) Other factors elevating the risk of recurrence are tumor size, multiple tumors, and vascular invasion in iCCA.(24)

For gallbladder cancer, the 5-year survival rates for completely resected (R0) tumours with limited invasion to the mucosa or submucosa (TIS or T1, see “Staging” below) are excellent, with about 95%. Further invasion dramatically reduces the survival and correlates with the risk of distant metastasis, with a 5 year median survival of 0% in T3 tumours and a rise of distant metastasis from 16%-79% and nodal metastasis from 33% to 69% from T2-T4.(27)

Ampullary cancer has a slightly better prognosis than other forms of biliary tract cancer, probably due to the earlier development of clinical symptoms in these patients. Following resection, the 5-year survival rates range from 64%-80% for patients without lymph node positivity and 17-50% for node positive disease.(28, 29) The 5 year overall survival according to tumour stage (defined by the 2010 AJCC/UICC system, as survival data for the newest staging system is not yet available) is 84% for Stage I, 70% for Stage II, 27% for Stage III and 0% for Stage IV disease.(30)

Risk factors

General

Even though in most patients no underlying predisposing factor for cholangiocarcinoma can be determined, there are several well recognized risk factors for the development of this malignancy.(5, 31) One of the pathophysiological links between most of these risk factor and biliary tract cancer seems to be in their enhancement of a chronically inflamed state to the bile ducts. They serve as a good example of the role of chronic inflammation in the aetiology of malignancies in general. Therefore, I will discuss the role of inflammation in the promotion of cancer separately to the risk factors in cholangiocarcinoma.

The risk factors can be grossly separated into inflammatory causes as their malignancy-promoting factor (such as PSC, parasitic infections, fibropolycystic liver disease and hepatolithiasis), toxic exposure, genetic disorders and others (**Table 3**).

Primary sclerosing cholangitis

PSC (Primary sclerosing cholangitis) is defined as a chronic inflammation of the bile ducts, resulting in fibrotic changes and strictures.(32) It is strongly associated with inflammatory bowel disease and especially with ulcerative colitis.(32) Patients with PSC suffer from symptomatic colitis in 40 - 50 %, with an incidence of up to 90 % when also taking into account only histological colitic changes. The other way around, PSC is estimated to be present in around 5% of patients with CU and 3.4 % in Crohns' disease.(32, 33)

As of its strong association with inflammatory bowel disease and the finding of unspecific autoantibodies such as antinuclear antibodies (ANA) or smooth muscle antibodies (SMA) and especially the high prevalence of very specific anti-neutrophilic cytoplasmatic antibodies with perinuclear staining pattern in immunofluorescence (pANCA) in up to 80%, PSC is thought of as an autoimmune disease. (34, 35)

The association with CCA is very well established, with almost 30 percent of all CCA being diagnosed in patients with PSC, especially perihilar disease. The lifetime risk of the development of CCA in PSC is 5-15 % with an annual risk of 0.5-1.5%.(36, 37) The patients at risk typically present at a younger age (30-50 years) with a rapid clinical deterioration in a previously stable state of chronic disease, with the development of jaundice and abdominal discomfort.(33) Except for alcohol consumption and smoking as well as certain genetic polymorphisms, there is no established risk factor for the development of CCA in PSC and

therefore unfortunately also no predictive marker for that.(38-40) The timing of the malignant transformation can vary strongly, with reports having found one third of patients presenting in their first two years after the diagnosis of PSC and others having stated that 37% only develop after 10 years of diagnosed PSC.(33, 39) The cancer is typically hard to diagnose, because of the changes in the biliary system caused by PSC can mimic malignancy in imaging and is often only found in an advanced stage.(39)

Parasitic infection

This certain risk factor is responsible for the epidemiological differences of cholangiocarcinoma in Asia, in particular in Thailand, and the rest of the world.(6) In the northern regions of Thailand, the ingestion of undercooked fish can lead to the ingestion of liver flukes of the genera Opisthorchis and Clonorchis, which leads to the colonialization of the bile ducts of an adult worm, laying its eggs there. This causes a chronic inflammatory state that can lead to malignant transformation of the epithelial lining in the proximal biliary tree. Cofactors further promoting this process are thought to be carcinogens produced by bacteria in some foods such as fish, alcohol consumption, smoking and chronic hepatitis B virus infection.(41-44) Beside liver flukes, also chronic biliary giardiasis was described as a risk factor for biliary carcinogenesis.(22)

Helicobacter pylori positivity

The association of H. pylori infection and CCA was proposed by a study conducted in the Thai population. The prevalence of H. pylori in this study was 66.7 % in the CCA group and 25 % in the control group ($P<0.05$).(45)

Fibropolycystic liver disease

This entity includes the congenital diseases of Caroli-syndrome, characterized by multiple cystic dilatations of the intrahepatic bile ducts, congenital hepatic fibrosis and choledochal cysts. Despite the rare incidence of these disease themselves, they carry a significant risk of malignant change of around 15 % with an average age at diagnosis of 34 years.(46-48) The suspected factors for malignant transformation are biliary stasis and reflux of pancreatic juice, creating an environment of chronic inflammation, as well as abnormalities in bile salt transporters, leading to altered bile content, and possibly deconjugation of carcinogens previously processed by the liver.(49)

Biliary stone disease and cholecystitis

The association of cholangiocarcinoma and cholelithiasis and cholecystitis is not as severe as with gallbladder cancer, but there still has been an observation of increase in risk.(20, 50) The entity of hepatolithiasis, also called recurrent pyogenic cholangitis, a disease rarely seen in western countries, endemic in certain parts of south-east Asia, is associated with cholangiocarcinoma.(51) The cause of this disease is yet to be found, even though congenital duct abnormalities and chronic inflammation due to bacterial or parasitic infection have been implicated.(52) The resulting biliary stones are suspected to cause biliary stasis, enhancing chronic inflammation as a possible link to malignancy. The incidence of hepatolitihiasis in patients undergoing resection of a cholangiocarcinoma is as high as 50 – 70 % in Taiwan and 6 -18 % in Japan.(52-54)

Toxic risk factors

The role of toxic carcinogens contributing to the development of cholangiocarcinoma seems reasonable, given the nature of the hepatobiliary system being an excretory body system, exposed to a variety of substances. The association of cholangiocarcinoma with several occupations, including the auto, rubber, chemical and wood-finishing industries, supports this concept.(12, 55) Despite the elevated risk of CCA in PSC in smokers and drinkers, the role of alcohol and smoking as a risk factor in general has not been proofed, due to conflicting study results.(55, 56) Historically, the radiologic contrast agent Thorotrast, banned in the 1960s, showed a clear association with cholangiocarcinoma, with malignancy usually developing 30 to 35 years after being exposed.(57, 58)

Genetic risk factors

There are several genetic syndromes associated with an increased risk of cholangiocarcinoma. Firstly, Lynch syndrome (hereditary nonplyposis colorectal cancer), an autosomal dominant disorder of the DNA mismatch repair genes (MMR), increases the risk of not only colonic cancer but also a variety of extracolonic cancers, including CCA.(59, 60) Secondly, the rare disease of multiple biliary papillomatosis, being characterized by multiple adenomatous polyps of the biliary tract with clinical manifestation of obstructive jaundice, abdominal discomfort and pain as well as recurrent periods of cholangitis, is considered a premalignant condition.(61) One study found 83 % of the lesions undergo malignant transformation to CCA.(62) Further, familial adenomatous polyposis and cystic fibrosis have been described as a risk factor for biliary tract cancer.(22)

Summary of other independent risk factors

The conditions listed in Table 3 are examples for further risk factors of biliary tract cancer.

Table 3: Examples of study findings on the correlation of CCA with other independent risk factors.

Risk factors		iCCA	eCCA
Hepatic diseases	HCV positivity (63)	3.40 (aOR)	1.90 (aOR)
	Nonspecific cirrhosis (12)	27.2 (aOR)	
	Alcoholic liver disease (12)	7.4 (aOR)	
Elevated blood glucose	Diabetes (64)	1.60 (RR)	
	Sweet beverages* (65)	1.69 (HR)	1.79 (HR)
Metabolic syndrome** (66)		1.56 (aOR)	
HIV positivity (12)		6.4 (aOR)	

**Consumption of 200 mL per day of sweetened beverages (sugar and artificial)*
*** Three of the following: elevated waist circumference (central obesity), dyslipidemia, hypertension, impaired fasting glucose*
iCCA: intrahepatic cholangiocarcinoma; eCCA: extrahepatic cholangiocarcinoma; HCV: hepatitis-C-virus; HIV: human-immunodeficiency-virus; aOR = adjusted odds ratio; RR = relative risk; HR = hazard ratio;

Risk factors for gallbladder cancer

The risk factors for gallbladder cancer vary from those of cholangiocarcinoma, as described above. The strongest risk factor for GBC is gallstone disease. Others are porcelain gallbladder, a descriptive term used to account for the macroscopic appearance of a chronically inflamed, calcified gallbladder,(17, 22) gallbladder polyps, PSC, obesity, congenital malformations resulting in cholestasis as well as chronic infections with salmonella typhi. This correlation seems reasonable, as the infectious reservoir for Salmonella typhi is the human gallbladder. (22, 67, 68)

Molecular pathogenesis

The biological relatedness of biliary tract cancers to pancreatic cancer is reflected in the similarity of its precursor lesions, being intraductal papillary mucinous neoplasm (IPMN), a macroscopic lesion of the bile ducts, and the more common biliary intraepithelial neoplasia (BilIN), graded based on the severity of cellular atypia. (69, 70) A stepwise accumulation of genetic abnormalities in the development of malignancy through these precursor lesion seems likely, however the level of understanding of these changes is less profound as it is in other cancers, such as colorectal cancer.(71, 72) A number of different genetic alterations, both in the group of oncogenes and tumour suppressor genes have been described in biliary tract malignancies (**Table 4**). For example, p53 was found to be overexpressed in one third of tumours, implying mutations in this tumour suppressor gene. Further, abnormal expression of KRAS is present in 45 – 54 % of iCCA and 10 – 15 % of eCCA.(71) These alterations seem to be associated with a more aggressive biological behaviour of the tumour.(73)

Table 4: Examples for genes affected by molecular defects in specimens of BTC.

Oncogenes	RAS, ERBB2, BRAF, EGFR, PIK3CA, CTNNB1
Tumor suppressor genes	p53, SMAD4, CDKN2A

There is no clear data, suggesting a link of genetic alterations to certain risk factors such as chronic inflammation, except for some data suggesting that point mutations in the p16INK4a promoter play a role in the development of CCA in the setting of PSC.(74) Interestingly,

mutations in the gene encoding isocitrate dehydrogenase 1 (IDH1) have been found in 25 % of iCCA but not eCCA or GBC, underlining the biological difference between the subtypes of biliary tract cancer as well as suggesting a potential therapeutic target in this pathway.(75)

Due to the limited understanding of molecular changes in the biology of this malignancy, at the moment there are no molecular markers used for selection of treatment or stratification of prognosis.(76)

Chronic Inflammation and Cancer

Background

Chronic inflammation of the bile ducts is a key component of several of the established risk factors for the development of biliary tract cancer, such as PSC or liver fluke infestation.(31, 34, 37, 44) With chronic inflammation being a recognized risk factor for many different types of cancer, the knowledge of the correlation and also principles of this link has grown tremendously in the last couple of years.(77) The suspected causality between these two states, inflammation and malignancy, was supported by several different findings and established evidence. First, besides BTC, up to 20% of all cancers in general develop under the condition of chronic inflammation, well known examples are chronic viral hepatitis, predisposing for hepatic cancer, Helicobacter pylori for gastric cancer and MALT-Lymphoma, Schistosoma for bladder cancer or chronic reflux esophagitis, elevating the risk of oesophageal cancer via Barrett´s metaplasia.(78) Further, it was found that patients with chronic inflammation, as well as with autoimmune diseases are predisposed to a higher risk of cancer in general.(79) The tumour tissue itself is populated by a variety of immune-competent cells and mediators of inflammation, an observation that has gained a lot of attention recently but was already described by the German pathologist Rudolf Virchow in the 19th century.(78, 80) Finally, the long-term treatment with non-steroidal anti-inflammatory drugs was found to decrease the risk of several cancers.(81) This growing understanding of the role of tumour promoting inflammation has led to its inclusion in the Hallmarks of Cancer by Hanahan and Weinberg in 2011.(77)

Intrinsic pathway of inflammation

The link of chronic inflammation and cancer is not only limited to cancers being substantially promoted by an underlying inflammatory process, which is referred to as the extrinsic pathway of inflammation in cancers,(77) it also play a role in tumours in which chronic inflammation does not play a role as an etiological factor. In these cancers, the activation of oncogenes and inactivation of tumour-suppressors not only leads to uncontrolled proliferation, it also enhances the transcription of pro-inflammatory genes such as chemo- or cytokines, recruiting cells of inflammation into the tumorous tissue and thereby fuelling an inflammatory state, providing a continuous environment of cell proliferation. This enhancement of inflammation by the tumour itself underlines the importance of an

inflammatory micro-environment for tumour development and growth. This process was classified as the intrinsic pathway of inflammation by Hanahan and Weinberg. (77)

Mechanisms of cancer-promoting inflammation

The biological mechanisms are complex and diverse. One of the key mechanisms involved in these processes is the production of soluble pro-inflammatory mediators, creating a constant environment of proliferation, promoting tumours in their natural behaviour. Those are either produced by cells of the immune system, such as macrophages, or by the tumour itself, as described above. Two of those mediators, Interleukin-1 (IL-1) and tumour necrosis factor (TNF), play a key role in these processes. Through specific receptors, the cytokines activate a cellular response leading to the transcription of NF-kB, which acts as a major regulator of the inflammatory response. In cancerous cells, it activates anti-apoptotic genes (BCL2, c-FLIP) and proliferation-regulating genes (c-Myc, cyclins) whereas in macrophages it leads to the activation of genes encoding for pro-inflammatory cytokines and enzymes that are essential for inflammation, such as COX-2. This activation amplifies the immune response and further enhances the inflammatory state within the tumour in the sense of a self-promoting system. (78, 79, 82)

Another important mediator in this setting is Interleukin-6 (IL-6). This cytokine promotes the transcription of STAT-3, which, in analogy to the description above, activates different pathways, depending on the cell type. In cancer cells STAT-3 enhances survival and further proliferation as well as invasion. In contrast to that, STAT-3 activation in macrophages leads to functional exhaustion and therefore local immunosuppression. (82, 83)

This inflammatory microenvironment leads to enhancement of proliferation due to the overweight of cytokines, activating cellular reproduction. It also leads to the recruitment of many immunocompetent cells, releasing proteolytic enzymes which further enhances tumour invasion and spreading. In addition to that, the recruitment of macrophages is important for the formation of new vessels inside the tumour, as macrophages produce, among other cells, key growth factors for this process, vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF).(82)

Pathology

Macroscopic appearance

Intrahepatic cholangiocarcinoma can be sub-classified into four categories, according to its macroscopic appearance. This classification was recently proposed by the Liver Cancer Study Group of Japan.(84) These categories include:

- I: Mass-forming type (MF): This type is characterized by a firm tumour mass that is non-capsulated and grey or white in colour. There is no connection to a macroscopic bile duct.
- II: Periductal-infiltrating type (PI): The tumour growth tends to spread along the intrahepatic portal tracts. Usually, the involved bile duct tends to get obstructed, resulting in dilatation and inflammation of the proximal bile duct.
- III: Intraductal growth type (IG): This type is characterized by papillary or polypoid tumour growth inside the lumen of the bile duct, which normally is grossly dilated.
- IV: Mixed pattern type: This subclass consists of tumours that show combined features of the categories above.(84, 85)

Extrahepatic cholangiocarcinoma are, like their intrahepatic counterpart, mostly firm nodules and grey in colour. They tend to infiltrate nearby structures.(86) Gallbladder cancer normally presents as an unspecific thickening of the wall, either papillary or diffuse, often within the presence of gallstones. The tumour might infiltrate the liver or other adjacent structures.(87) Ampullary cancer often appears as a tumour bulge, infiltrating the duodenal lumen. The common bile duct is often times dilated.(21, 87)

Histology

Most cholangiocarcinomas are adenocarcinomas, with over 90% in frequency, followed by the less common squamous cell differentiation, followed by less common histological variants such as adenosquamous-, mucinous- signet-ring cell-, clear cell-, lymphoepitheliom-like-, neuroendocrine type and sarcomatous carcinoma.(85, 86) Adenocarcinomas themselves are subclassified according to their microscopic appearance and biological behaviour in (I) the most common sclerosing type, characterized by an intense desmoplastic reaction and tendency to early infiltration of the bile duct and adjacent structures, (II) nodular CCA, presenting typically as an annular lesion, constricting the bile duct and (III) papillary tumours, which are the rarest form of CCA, typically causing

obstruction by an bulging mass inside of the common bile duct.(88) The special histological finding of both cholangiocellular and hepatocellular differentiation is commonly staged as cholangiocarcinoma.(89) Gallbladder cancer histologically is very similar to CCA, with adenocarcinoma being the most common histological subtype (>90%), being subdivided into infiltrative, nodulary and papillary type, in close accordance to CCA.(90) In contrast to the pancreatobiliary differentiation of cholangiocarcinoma and gallbladder cancer, carcinoma of the Ampulla Vateri often times are of intestinal differentiation. In one study, the most common histological findings were intestinal adenocarcinoma (47%), followed by pancreatobiliary adenocarcinoma (24%) and poorly differentiated adenocarcinomas (13%).(91) Immunohistochemistry is used to verify the tissue of origin in cholangiocarcinoma, with biliary tract cancer being a diagnosis of exclusion, even though cytokeratin-7 (CK-7) can be a typical finding in these cancers, even though it is also expressed by cancers of the lungs and breast, but can play an important role in excluding colorectal metastasis.(85)

Tumor dissemination

The typical biological behaviour of CCA, GBC and CPV includes direct infiltration of the bile duct or gallbladder itself as well as of adjacent structures such as the liver. These tumours also tend to perineural growth and lymph-vessel infiltration, leading to lymphatic local dissemination. Right sided iCCA and GBC typically spread to the hilar, periduodenal and peripancreatic nodes, left sided iCCA to the lesser curvature of the stomach. For iCCA, periaortic or pericaval lymph node spread is considered to be distant metastasis. For perihilar tumors, the regional lymph nodes are hilar and pericholedochal nodes. Distal CCA and CPV typically spread to its regional lymph nodes, which are the same as for exocrine cancers of the pancreatic head, namely the nodes along the common bile duct, the nodes anterior and posterior to the duodenum and pancreas as well as the nodes along the portal vein, the common hepatic artery and the superior mesentery artery. Distal CCA and CPV can, due to anatomical proximity, directly infiltrate the duodenum, pancreas stomach, omentum or colon. Distant metastasis most commonly affects the liver, followed by the peritoneum. Metastasis to the bone, lung or pleura occur less frequently.(1, 92, 93)

Staging

Tumour staging should include histological verification of the cholangiocellular differentiation, local extend of the disease as well as assessment for lymph node and distant metastasis, in order to select patient for respectability and potential cure. T-stage is assessed by MRI, further improved in specify by ERCP or PTC. N-stage should be assessed via MRI and endoscopic ultrasound (EUS), distant metastasis should be excluded with a thoracic CT. Another important diagnostic tool in order to better assess respectability is contrast-enhanced CT, which is effective in defining the proximity and involvement of vascular structures next to the tumour, such as the common hepatic artery and the portal vein. The TNM- staging of biliary tract cancers is different in every subtype, as listed below (**Table 5**).(92, 94)

Rates of tumour stage at presentation varies strongly according to tumour type, reflecting the differences in clinic symptoms and how early they develop in each particular tumour type. For example in iCCA, only a minority of 15% of the patients presents with initially respectable disease.(24) Most patients with iCCA (69%) are diagnosed with either stage III or IV disease, compared with only 46% in eCCA, probably due to the lack of early symptoms in iCCA.(95) In Gallbladder cancer, in a large retrospective analysis, 36.6% of the patients presented as Stage IV. A total of 47% were incidental findings at laparoscopic cholecystectomy.(16)

Table 5: TNM classification of bile duct cancer

TNM classification according to the AJCC/UICC staging, 8th edition. (92)

	iCCA	pCCA	dCCA	GBC	CPV
T: Primary Tumous					
TIS	Cis				
T1	Solitary tumour without vascular invasion	Confined to bile duct (Muscle or fibrous layer)	Confined to bile duct	T1a: Lamina propria T1b: Muscular layer	Limmited to ampulla or Musculus sphincter oddi
T2	T2a: Solitary tumour + vascular invasion	T2a: Invasion beyond bile duct (surrounding adipous tissue)	Invasion beyond wall of bile duct	Invasion of perimuscular connective tissue	Invasion duodenal wall
	T2b: Multiple tumours +/- vascular invasion	T2b: Invasion beyond bile duct (adjacent hepatic parenchyma)			
T3	Perforation of visceral peritoneum / direct invasion local extrahepatic structures	Invasion of unilateral branches portal vein / hepatic artery	Invasion of adjacent structures (gallbladder, duodenum, pancreas)	Perforation of serosa (visceral peritoneum) / invasion of adjacent structure (liver, stomach, duodenum, colon, pancreas, omentum, extrahepatic bile ducts)	Invasion pancreas

T4	Periductal invasion	Invasion PV / bilateral branches (PV) / CHA / biliary radicals (2nd order) bilaterally / bilary radicals (2nd order) unilat. + contral. PV or CHA	Invasion of coeliac axis / superior mesenteric artery	Invasion PV / CHA / ≥2 adjacent extrahepatic structures	Invasion peripancreatic soft tissue or other adjacent structures or organs
N					
Nx	Regional lymph nodes cannot be assessed				
N0	No regional lymph nodes				
N1	Metastasis in regional lymph nodes	Regional lymph node metastasis	Metastasis in regional lymph nodes	Nodes along cystic duct, CBD, CHA, PV	Metastasis in regional lymph nodes
N2		Periaortal / pericaval / sup. mesenteric artery / coeliac artey LNN		Periaortal, pericaval, SMA coeliac artery LNN	
M					
M0	No distant metastasis				
M1	Distant metastasis				
PV: Portal vein; LNN: Lymph nodes; SMA: Superior mesentery artery; CHA: Common hepatic artery; CBD: Common bile duct;					

Table 6: Tumour stages

Staging according to the AJCC/UICC staging, 8th edition. The table only displays the distinguishing features of each stage. (92)

	iCCA	**pCCA**	**dCCA**	**GBC**	**CPV**
Stage					
O	TIS				
I	T1	T1	**IA**: T1 **IB**: T2	T1	**IA**: T1 **IB**: T2
II	T2	T2a-b	**IIA**: T3 **IIB**: N1	T2	**IIA**: T3 **IIB**: N1
III	T3	**IIIA**: T3 **IIIB**: N1	T4	**IIIA**: T3 **IIIB**: N1	T4
IV	**IVA**: T4 / N1 **IVB**: M1	**IVA**: T4 **IVB**: N2 / M1	M1	**IVA**: T4 **IVB**: N2 / M1	M1
iCCA: intrahepatic cholangiocarcinoma; pCCA: perihilar cholangiocarcinoma; dCCA: distal cholangiocarcinoma; GBC: gallbladder cancer; CPV: cancer of the papilla vateri;					

Clinical signs, symptoms and findings

There are some differences between the type of presentation of cholangiocarcinoma, ampullary cancer and gallbladder cancer, strongly depending on whether the bile duct typically gets obstructed in the specific disease. Extrahepatic cholangiocarcinomas and carcinomas of the ampulla Vateri usually cause symptoms when the tumour obstructs the biliary drainage.(88) These symptoms include jaundice in 90% of the patients at presentation, pruritus, dark urine and clay-colored stool. Hepatomegaly (25%), a mass in the right upper quadrant (10%) and fever (2-14%) occur less likely at presentation. Additionally, patients often complain of abdominal pain (30-50%) and weight loss (30-50%).(88) The well-known physical exam finding of palpable gallbladder combined with jaundice (Courvoisier´s sign) is neither sensitive, nor specific for malignancy, as also benign causes such as chronic pancreatitis, parasitic obstruction and other biliary obstructions can cause these combined symptoms.(96) The findings and complaints of intrahepatic cholangiocarcinoma and gallbladder cancer are even more unspecific, with right upper quadrant tenderness, weight loss and rarely fever being typical symptoms, often mimicking cholelithiasis or cholecystitis in GBC.(88) Actually, most cases of GBC are diagnosed in the setting of diagnostic evaluation for cholelithiasis, with a tumour being present in 1-2% of these cases.(97, 98)

Paraneoplastic syndromes

Paraneoplastic syndromes may rarely be caused by cholangiocarcinoma and should therefore be included in the further workup of unexplained paraneoplastic findings such as acute febrile neutrophile dermatosis (Sweet´s syndrome),(99) acanthosis nigricans maligna,(100) Leser-Trélat-sign (sudden onset of multiple seborrheic keratosis),(100) porphyria cutanea tarda(101) and persistent erythema multiforme.(102)

Laboratory abnormalities

Tumours causing biliary obstruction typically cause elevation of alkaline phosphatase (AP), total bilirubin and direct bilirubin. Intrahepatic CCA typically cause a raise in AP with normal bilirubin counts. Especially in eCCA, initially the aminotransferases may be within normal range.(93) Rarely, patients present with signs of paraneoplastic hypercalcemia.(103)

Tumour markers

CA 19-9: Carbohydrate-Antigen 19-9 (CA 19-9) is a glycoprotein used as a tumour marker for biliary tract cancer in the diagnostic as well as in the surveillance setting, despite its wide range in reports of sensitivity (50-90%) and specificity (54-98%), as it is also elevated in benign biliary disease.(104) Another useful setting for CA 19-9 testing is the screening for CCA in patients with PSC.(33, 34) The tumour marker also has prognostic impact, with elevated levels prior to treatment being associated with a poorer prognosis and an elevation of over 1000 units/mL being suggestive for advanced disease and peritoneal involvement. (104, 105)

CEA: Another serum marker for biliary tract cancer is carcinoembryogenic antigen (CEA), improving diagnostic accuracy in combination with CA 19-9. Despite CA 19-9, there seems to be no prognostic value in the preoperative elevation of CEA. Additionally, CEA is rather unspecific, with an elevation also occurring in other gastrointestinal malignanciess, such as stomach, colon and pancreatic cancer (104)

CRP: Another unspecific serum marker used in prognostic stratification with proven value in iCCA is C-reactive protein (CRP), a member of the family of the acute phase proteins, being an independent indicator of prognosis. Low CRP values (<1 mg/dl) are associated with a favourable prognosis.(104)

Treatment

As the only chance for cure in BTC is the complete resection of the tumour, adequate staging taking into account information regarding respectability must be obtained in every patient, newly diagnosed with BTC.(94) The criteria for respectability include (I) the absence of retropancreatic and paraceliac nodal metastases or distant liver metastases, (II) the absence of major vessel invasion (portal vein and main hepatic vein) (III) the absence of extrahepatic adjacent organ invasion and (IV) the absence of disseminated disease.(106) Further, the patients' performance status and possible functional outcome of a radical operation must be taken into account in the decision making.(3)

Local treatment

Surgery

The surgical treatment in BTC depends on the type of cancer and the local extend of the disease. In CCA, lymph node involvement is a key prognostic feature, therefore routine lymphadenectomy should be performed.(107) In pCCA, respectability often can only be assessed by surgical exploration. The role of pre-imaging stenting is controversial, due to the radiological impairment in evaluating the extent of the disease.(108) For iCCA and pCCA, portal vein embolization might be conducted preoperatively in order to induce hypertrophy of the non-resected liver parts. The role of liver transplantation in locally unresectable disease in combination with neoadjuvant chemoradiotherapy is currently being investigated and no standard of care.(94) In contrast to iCCA and pCCA, as described above, the removal of pancreatic head is necessary in dCCA and ampullary cancer, normally being performed as a partial duodeno-pancreatectomy with extended bile duct resection.(94) GBC, as described above, often times is found incidentally in the specimen after laparoscopic cholecystectomy.(16) In very early stages (TIS and T1a), cholecystectomy might be an adequate treatment. For T1b and higher stages, reoperation including partial hepatectomy and lymphadenectomy of the hepato-duodenal ligament should be performed.(98) In general, GBC resection can also be performed in locally advanced stages such as T4, especially when the cancer is located in the fundus of the gallbladder.(94)

Other local approaches

Further local strategies for tumour control, especially in iCCA, include radiofrequency ablation, Y-90 radioembolisation, radiotherapy and transarterial chemoembolisation. In most of these approaches, prospective data is lacking.

- **RFA**: Retrospective analyses showed a survival benefit of RFA in patients with iCCA who were not eligible for surgery.(109)
- **Y-90-radioembolisation**: A pooled analyses of 12 studies found an objective response rate of 28% and a median overall survival of 15.5 months in treated patients. Importantly, in 3 studies, 7 of 73 patients were converted into respectable disease, offering a possible way of secondary curative diseases for initially unresactable patients.(94)
- **Radiotherapy**: The role of radiotherapy has also not been assessed in a randomized prospective fashion yet, neither in the adjuvant nor in the palliative setting. In a meta-analysis, adjuvant radiochemotherapy was associated with a survival benefit compared with surveillance alone in patients with node-positive disease and positive surgical margins (R1). However, there were some major limitations to the study, such as selection bias, heterogeneity in treatment regimens and the quality of data.(110) Further, the direct comparison between the two modalities of chemotherapy and radiotherapy alone has not been conducted yet.(94) In the locally advanced setting, the role of radiotherapy is also not clear. In non-randomized analyses, chemoradiotherapy seemed to be a possible option for these patients, with a median overall survival of 9 to 14 months. However, a recent phase III trial comparing chemotherapy with chemoradiotherapy (50 Gy radiation + concurrent 5-FU and cisplatin vs. gemcitabine + oxaliplatin) was closed before completion due to an advantage in the chemotherapy study-arm in PFS (median: 11 vs 5.8 months) and OS (median: 20 vs 13.5 months).(94, 111)
- **Transarterial chemoembolization (TACE)**: There were reports of prolonged overall survival of patients with TACE after curative surgery with early recurrence.(112) Further, a prospective study reported the safety and efficacy of irinotecan-eluting beads-TACE (iTACE). When compared with conventional TACE with mitomycin-C, iTACE was superior in OS and PFS but non-superior to chemotherapy with oxaliplatin and gemcitabine in unresectable iCCA with normal liver function.(113)

Chemotherapy

Adjuvant chemotherapy

The high likelihood of local and distance recurrence, as described above, raises the question of the role of adjuvant therapy. The role of adjuvant chemoradiotherapy is still not clear.(94) Also, chemotherapy alone has not yet been proven effective in a randomized, prospective trial, but is currently under investigation in several phase III studies.(94) There seems to be a benefit in the administration of adjuvant treatment as proposed by a meta-analysis of retrospective data, comparing surgery alone with chemotherapy or chemoradiotherapy. The analysis showed a benefit in the subgroup of node positive disease and positive surgical margins, as mentioned above.(110) There were two prospective trials, comparing chemotherapy alone with surveillance. Firstly, Takada et al compared mitomycin C and 5-FU followed by 5-FU alone until recurrence in high risk (node positive) patients. Patients with bile duct carcinoma had no survival benefit (5-year OS 27 vs 24%), but patients with Gallbladder cancer had a better disease free survival (20.3 vs 11.6% and overall survival (26 vs 14%). The survival benefit was not statistically significant as the trial was underpowered.(24, 110) The second randomized trial assesses the role of chemotherapy (5-FU or gemcitabine) in resected periampullary adenocarcinomas. There was a statistically significant survival benefit, especially for gemcitabine (median OS 43 vs 35 months).(24, 114) In summary, most guidelines and expert groups suggest the use of adjuvant chemotherapy after resection, especially in node positive disease or in patients with positive surgical margins. Typically used agents are cisplatin and gemcitabine, with the combination being superior to gemcitabine alone, with a lack of direct comparison to other gemcitabine-containing regimens.(24)

Palliative chemotherapy

While first-line systemic chemotherapy (1LCTX) in palliative intent is an accepted treatment strategy for improving survival and quality-of-life in these patients,(115-117) the benefit of second-line systemic chemotherapy (2LCTX) is less clear.(118)

- **1st Line-data:** The role of palliative chemotherapy being active in aBTC was suggested in 1997 by a randomised trial comparing BSC alone with 5-FU based chemotherapy regimens, showing superiority in OS (6 vs 2.5 months) and quality of life in the chemotherapy study group.(119) Several studies and a meta-analysis of two randomized trials comparing Cisplatin + Gemcitabin with Gemcitabin alone

showed superiority of the combination therapy in PFS (HR=0.64) and OS (HR=0.65) and established the combination of cisplatin and gemcitabine as the standard first line treatment in biliary tract cancer.(116, 117) The median overall survival, achieved with this combination, was 11.7 months, compared to 8.1 months for gemcitabine alone.(94, 116) The benefit was independent of age, gender, stage, primary tumour site and prior therapy. The treatment was the least beneficial in patients with poor performance status.(94) Reasonable alternatives for patients with a less favourable performance status are single agent Gemcitabine, Leucovorin-modulated FU or capecitabine monotherapy.(120, 121) Capecitabine monotherapy seems to be more active in gallbladder cancer then in cholangiocarcinoma.(120)

- **2nd line-data:** Both best supportive care alone (BSC) and BSC+2LCTX are practicable options in the second-line setting,(122) but considerable uncertainty exists to date about which of these two treatment approaches will benefit patients most. Observational evidence in support of the 2LCTX+BSC approach is based on retrospective cohort studies of aBTC patients treated with fluoropyrimidine-based mono- or polychemotherapy.(118, 122-131) These retrospective single-arm studies have reported favourable OS outcomes in aBTC patients treated with systemic chemotherapy in the second-line setting. For example, Kim and colleagues recently observed a median OS of 6.5 months in 321 patients treated with fluoropyrimidine-based mono- or polychemotherapy.(126) Other median OS estimates in patients undergoing 2LCTX for aBTC ranged from approximately 7 months in the large cooperative studies by Brieau et al. (6.5 months), Fornaro et al (6.6 months) and Walter et al. (7.5 months),(118, 122, 128) and the meta-analysis by Lamarca et al. (7.2 months),(132) to 13.8 months in a single-center chart review study by Rogers and colleagues.(124) However, due to their single-arm design, these studies cannot address the overall benefit of 2LCTX over BSC. The issue of patient selection for second line chemotherapy has been addressed in two studies, trying to find predictive parameters for a beneficial influence of 2LCTX. These studies found that patients with a good performance status, disease control during the first-line chemotherapy, a relatively low level of CA 19-9 and previous surgery on their primary tumour seem to benefit the most of 2LCTX(118, 133). Importantly, a systematic comparison of outcomes in patients receiving 2LCTX+BSC versus patients treated with BSC alone is currently lacking.(130, 131)

Chemoresistance in biliary tract cancer

The overall poor prognosis of biliary tract cancer seems to have a variety of reasons, for example the late stage at presentation in many patients as well as its anatomical proximity to vital structures and the tendency of BTC to invade these structures.(24, 94) Another reasons seems so be the poor and often times short-lasting response to chemotherapy, possibly due to mechanisms of chemoresistance. These mechanisms are diverse in their nature but act not only synergistic, but are also present in other hepatic malignancies such as hepatocellular carcinoma and hepatoblastoma.(134) The stimulation of the expression of several of the many genes described as genes responsible for mechanisms of resistance (MOR) is enhanced by chemotherapeutic drugs and leads to only a short period of chemotherapeutical effectiveness in BTC and other hepatic malignancies.(135) The identification of these genes and their mechanisms of action are subject of recent investigations. Some of the mechanisms have already been identified, namely a decrease in uptake of the chemotherapeutic drug into the tumour cell, an enhancement in efflux of the drug, an elevation in drug inactivation (represented by an altered ratio between prodrug, active drug and inactive metabolites), alteration in the molecular targets of the drugs, an enhanced ability of the tumour cells to repair DNA-damage caused by the drug and as well as an impairment in the balance between apoptosis and cell-survival.(136)

Targeted therapies

In the light of the devolvement in the recent years towards more personalized approaches in oncology, using targeted therapies according to specific tumour biology, there have been several studies evaluating the role of targeted therapies in biliary tract cancer. Most of them targeted the axes of the epidermal growth factor receptor (EGFR) or the vascular endothelial growth factor (VEGF).

EGFR-axis targeted therapy

Cetuximab (monoclonal antibody targeted against EGFR) added to the combination of Gemcitabin and Oxaliplatin in patients with advanced (locally or metastatic) BTC seemed to be effective in a phase II study conducted in Austria, with an objective response rate of 63%, with 9 of the 30 patients enrolled in the study undergoing potentially curative resection after responding to the therapy.(137) Unfortunately, these promising results of the addition of Cetuximab to Gemcitabin and Cis-/Oxaliplatin was not being translated into prolonged

PFS or OS in a randomized phase II study.(138) Other agents, targeting the EGFR pathway such as Erlotinib (selective inhibitor of the tyrosine kinase of the EGFR) and Panitumumab (monoclonal antibody against EGFR) showed similar negative results, independent of k-ras mutation status, being essential for efficacy of EGFR targeted therapy in colorectal cancer, and EGFR overexpression.(94)

VEGF-axis targeted therapy

Another agent assessed in a clinical trial was sorafenib, an oral multikinase inhibitor, targeting, amongst others, VEGFR, which showed no benefit when added to single agent gemcitabine in patients with advanced BTC in a randomized, double blinded phase II study.(139) Further, cediranib, another oral multi-tyrosine kinase inhibitor (targeting VEGFR-1, -2, -3, PDGFR and c-kit) added to gemcitabine and cisplatin showed no beneficial results for palliative treatment in aBTC in another randomized phase II trial.(140)

Immunotherapy and checkpoint-blockade in biliary tract cancer

With traditional chemotherapy and targeted therapies having shown only limited or no effect in biliary tract cancer, new therapeutic approaches are needed. There is a variety of ongoing investigation towards alternative methods of treatment, for example using methods of immunotherapy with antigen-pulsed dendritic cells or cytotoxic T-cells are, showing some promising early clinical results.(141) As in many cancers, there is some ongoing investigation towards the immunoescape-mechanisms of the tumour, targeting the PD-L1 PD-1 interaction, an interaction that directly inhibits the adoptive immune system from acting towards the tumour cells. An upregulation of PD-L1 and PD-1 was reported in iCCA, suggesting a potential role for checkpoint-inhibition in the treatment.(142) Preliminary results from an ongoing phase 1b study, conducted by Bang et al, assessing pemprolizumab (monoclonal antibody against PD-L1) in patients with pretreated aBTC, expressing PD-L1, showed an objective response rate of only 17.4%.(142)

Summary and future perspective

In summary, the role of targeted therapies and immunotherapy as not been established in aBTC. Patients should be enrolled in clinical trials for further investigation of these approaches.(94) Further, despite the disappointing results in targeted therapies in studies assessing aBTC in general, there are other ways of conducting research on targeted therapy, tailored for specific genetic modifications of tumours. For example, there recently was a

report of a basket trial, also enrolling patients with cholangiocarcinoma, targeting TRK-fusion in different cancers positive for this biological feature with the agent larotrectinib, showing promising results. This enhances the future of tumour agnostic, genetic and tumour biology specific therapy in targeted therapies in oncology, giving orphan malignancies such as aBTC a perspective for possible therapeutic strategies in the future.(142)

Aims of this study

As described above, randomised, prospective data on the efficacy of palliative chemotherapy in patients suffering from aBTC is limited to the first line of treatment.(115) After the termination of first line therapy, either due to progression of the disease or discontinuation because of non-tolerability of the first line regimen by the patient, both the patient as well as the treating physician are confronted with the difficult decision whether to continue treatment in the sense of second line chemotherapy or to focus solely on best supportive care measures. As possible side effects of chemotherapeutic drugs can often severely affect the quality of life, especially in already terminally ill patients,(115) the decision to continue treatment should be backed by solid evidence of efficacy, according to the Hippocratic principle "primum nihil nocere".

This evidence in aBTC is limited due to several reasons. Due to its low incidence, conducting studies in a prospective, randomized fashion is severely complicated by the fact that it is difficult to recruit a sufficient number of patients in order to properly power the study. To conduct such a study in a reasonable timescale, the recruitment of patients in a great number of centres would be mandatory, which in reality is hard to implement.(143) Further, the rarity of aBTC itself leads to the subsumption of different subtypes of cancer, possibly confounding the effects of investigated interventions.(144) Lastly, most of the advances in the past decades in the field of oncology have been made in more frequent cancers, caused by the priorisation of these cancers by research conducted by pharmaceutical companies. This approach seems reasonable on the one hand, as advances in these cancers have favourable effects on a maximum of patients and on the other hand more common cancers are a more promising potential market. On the other hand, this leaves rare cancers to still have a poor prognosis that is, in contrast to more common cancers, mostly not improving.(143)

All these reasons led to single-arm retrospective analyses being the only evidence of efficacy of 2LCTX in aBTC at the time. This study aims to approach this lack of solid evidence in a "pseudo-randomized" fashion with IPTW-analysis, as described below. This approach cannot fully replace a prospective, randomized trial but, in the event of its unavailability, still can add some valuable information to the therapeutic effect. This is due to the principle of this method, trying to eliminate the major point of critics for a retrospective analysis,

namely its high likelihood of selection bias, leading to an uneven contribution of prognostic variables in the two study groups.(145)

This study aims to address this issue and provide evidence for the patient in the sense of a basis for informed consent as well as the physician in the difficult decision-making process that is the initiation of 2LCTX. Beyond that, the assessment of the beneficial effect in different covariates such as subentity or performance status might lead to the identification of subgroups that either profit more or less from the therapeutic intervention and therefore might help identify patients who benefit the most of another line of chemotherapy and to spare those patients who might benefit the least from an intervention that might reduce their quality of life.

Methods

Study Population and Design

In this single-center, observational, historical cohort study, we included all consecutive patients with histologically-confirmed aBTC who completed 1LCTX at the Division of Oncology, Medical University of Graz, Austria (n=80). This patient population was drawn from the greater population of patients with non-advanced and advanced BTC treated at our Department between 2003 and 2016 (n=185). From this population, we excluded patients who did not progress after resection/treatment in curative intent (n=79), patients who did not receive 1st-line chemotherapy (n=18), and patients who were lost-to-follow-up (n=1). Further 7 patients were excluded because they died during 1st-line chemotherapy (n=3), or due to missing data already in 1st-line chemotherapy (n=4). Baseline and outcome data for the 80 remaining patients were retrieved retrospectively from a prospectively-maintained in-house electronic health care database as reported previously.(146, 147) The baseline date was defined as the date of progression in 1st-line chemotherapy for both groups. In case patients did not progress during 1st-line chemotherapy, but 1st-line chemotherapy was terminated and BSC alone initiated due to poor performance status, we selected the end date of the last chemotherapy cycle as the baseline date. Primary endpoint of this study was death-from-any-cause within 18 months of follow-up. Data collection and analysis was approved by the local institutional review board (Ethics Committee of the Medical University of Graz, Austria; document number No. 25–458 ex 12/13).

Statistical methods

All statistical analyses were performed using Stata (Windows version 14.0, Stata Corp., Houston, TX, USA). Differences in means and proportions between patients in the 2LCTX+BSC and BSC group were quantified using standardized mean differences (SMDs),(145) and further evaluated with Wilcoxon's rank-sum tests, $\chi 2$-tests, and Fisher's exact tests, respectively. SMDs >0.20 were considered to indicate potentially relevant imbalance between the two study groups.(145) Median follow-up was computed with the reverse Kaplan-Meier estimator according to Schemper & Smith,(148) whereas OS was

computed with the traditional Kaplan-Meier estimator. Log-rank tests were used for comparing overall survivor functions between the two study groups. For uni- and multivariable modeling of time-to-death-from-any-cause, Cox proportional hazards models were fitted. For each patient, the propensity score e was defined as the probability of receiving 2LCTX+BSC conditional on baseline covariates, and the inverse-probability-of-treatment-weight (IPTW) was defined as the inverse of the probability of receiving the treatment that the patient received.(145) We calculated the propensity score using a multivariable logistic regression model including all covariates reported in Table 8. For this model, we imputed missing baseline covariates using a chained equations algorithm (Stata routine mi impute chained; a list with the conditional imputation models is available on request from the corresponding author).(149) To explore whether IPTW yielded balance on baseline variables between the two study groups, SMDs were re-estimated after weighing of the data with the IPTWs following best-practice recommendations.(145) IPTW-weighted Kaplan-Meier estimators and Cox proportional hazards models were fitted for analyzing the "imbalance-adjusted" association between 2LCTX and OS, respectively.(150) In a sensitivity analysis, we used a "trimmed" IPTW excluding patients <5th and >95th percentile of the IPTW's distribution.(151) Because we observed strong evidence for a violation of the proportional hazards assumption in the time-to-death analysis (as indicated by Schoenfeld test p-values and the interaction hazard ratio between study group and follow-up time), analyses were re-performed with flexible parametric models allowing for time-varying associations of study group and time-to-death (Stata routine stpm2).(152) Subgroup analyses were performed by fitting interactions between treatment assignment and the subgroup/covariate of interest. Here, interaction p-values (rather than subgroup p-values) were considered as measures for testing the hypothesis of a differential association of 2LCTX and OS between the pertinent subgroups.(153) The full analysis code is available on request from the corresponding author.

Ethics statement

Data collection and analysis was approved by the local institutional review board (Ethics Committee of the Medical University of Graz, Austria; document number No. 25–458 ex 12/13) before any patient-related activities were performed. Written informed consent was not obtained from individual patients, because the local ethics committee specifically granted a "waiver of consent" for this retrospective database study. All investigations took place in accordance with the principles embodied in the declaration of Helsinki.

Results

Baseline characteristics and crude overall survival estimates

Eighty patients were included in the analysis (**Table 8**). At baseline, the median age of the cohort was 68.0 years [25th-75th percentile: 60.0-73.0], 38 patients (48%) were female, and the median Karnofsky Index was 90% [80-90]. Most patients' tumors were moderately differentiated (tumor grade G2: n=43 (96%)) and were adenocarcinomas (n=78 (98%)). During first-line chemotherapy, 20 patients (25%) had received Cisplatin/Gemcitabine, 45 (56%) had received Gemcitabine monochemotherapy, and 15 patients (19%) were treated with other regimens. The objective response rate in 1st-line CTX was 20% (95%CI: 12-30). After baseline, patients were followed-up for a median interval of 14.8 months (25th-75th percentile: 5.0-24.6). During this interval we observed 49 deaths (61%), of which 46 (94%) were adjudicated to aBTC. Causes-of-death not adjudicated to aBTC were cardiorespiratory arrest (n=1), heart failure (n=1), and acute bleeding from esophageal varices (n=1). The 3-, 6-, 12-, and 18-month OS estimates were 69% (95%CI: 57-78), 54% (42-65), 38% (26-50), and 19% (9-31), respectively (**Figure 1**).

Table 8: Baseline characteristics of the study population (n=80).

Distribution overall and by treatment assignment to 2LCTX+BSC versus BSC. Continuous variables are summarized as medians [25th percentile (Q1) – 75th percentile (Q3)], whereas categorical variables are reported as absolute frequencies and percentages. *p-values for difference 2LCTX+BSC vs. BSC alone are from Pearson's chi-squared tests (categorical variables with expected cell counts ≥5), Fisher's exact tests (categorical variables with expected cell counts <5), or Wilcoxon rank-sum tests (continuous variables); Abbreviations: n (%miss.) – number of patients with fully observed data (% missing from a total of 80 patients), BSC – best supportive care, 2LCTX+BSC – 2nd-line chemotherapy and BSC, ΔS – Standardized mean difference (SMD), ΔS-IPTW – IPTW-weighted SMD, BMI – body mass index, ECOG – Eastern Cooperative Oncology Group performance status, CUP-CCC – cancer of unknown primary with cholangiocellular differentiation, CTX – chemotherapy, AST – Aspartate aminotransferase, ALT – Alanine aminotransferase, CEA – carcinoembryonic antigen, CA 19-9 – Cancer antigen 19-9.

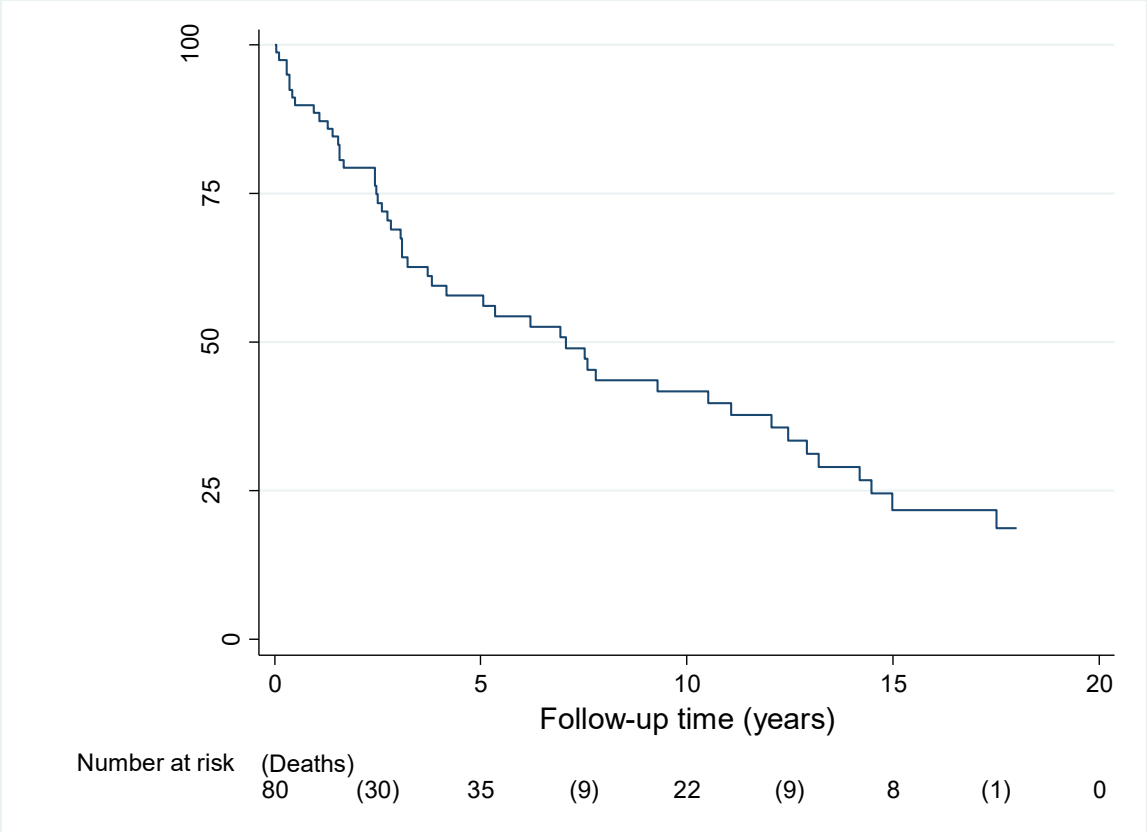
Table 7: Baseline characteristics of the study population.							
Variable	**n (% miss.)**	**Overall (n=80)**	**BSC only (n=42)**	**2LCTX+ BSC (n=38)**	**p***	**Δs**	**Δs-IPTW**
Demographics							
Age (years)	80 (0%)	68.0 [60.9-73.0]	69.5 [63.0-73.7]	67.1 [57.5-72.3]	0.16	0.26	0.26
Female Gender	80 (0%)	38 (48%)	24 (57%)	14 (37%)	0.07	0.41	0.08
BMI (kg/m²)	70 (13%)	24.4 [22.3-26.9]	24.0 [21.8-27.1]	24.8 [22.4-26.6]	0.49	0.15	0.03
History of smoking	72 (10%)	19 (26%)	11 (31%)	8 (22%)	0.42	0.19	0.18
Charleson Comorbidity Index	80 (0%)	8 [7-10]	9 [7-10]	8 [7-9]	0.69	0.02	0.01
Synchronous aBTC	71 (12%)	36 (51%)	10 (29%)	24 (71%)	0.001	0.83	0.36
Performance status	60 (25%)	/	/	/	/	/	/
Karnofksy Index	/	90 [80-90]	80 [70-90]	90 [80-90]	0.0001	1.21	0.20
---ECOG 0	/	33 (55%)	10 (34%)	23 (74%)	0.002	0.85	0.21
---ECOG 1-2	/	27 (45%)	19 (66%)	8 (26%)			
Tumor location	80 (0%)	/	/	/	/	/	/
---Gallbladder	/	23 (29%)	17 (40%)	6 (16%)	0.006	0.56	0.01
---Intrahepatic	/	30 (38%)	17 (40%)	13 (34%)		0.13	0.04
---Perihilar	/	9 (11%)	2 (5%)	7 (18%)		0.43	0.56
---Distal/ Ampulla	/	14 (18%)	3 (7%)	11 (29%)		0.58	0.43
---CUP-CCC	/	4 (5%)	3 (7%)	1 (3%)		0.21	0.86
Tumor histology	80 (0%)	/	/	/	/	/	/
---Adeno-carcinoma	/	78 (98%)	41 (98%)	37 (98%)	0.99	0.02	0.03
---Others	/	2 (3%)	1 (2%)	1 (3%)			
Tumor grade	75 (6%)	/	/	/	/	/	/
---G1 or G2	/	46 (61%)	22 (56%)	24 (67%)	0.60	0.21	0.10
---G3	/	29 (57%)	17 (44%)	12 (33%)			
1st line CTX data	80 (0%)	/	/	/	/	/	/
---Cisplatin/ Gemcitabine	/	20 (25%)	10 (24%)	10 (26%)	0.47	0.06	0.32

---Gemcitabine mono	/	45 (56%)	26 (62%)	19 (50%)		0.24	0.44
---Other CTX regimens	/	15 (19%)	6 (14%)	9 (24%)		0.24	0.21
Objective response	80 (0%)	16 (20%)	8 (19%)	8 (21%)	0.82	0.05	0.21
Number of cycles	79 (1%)	4 [2-6]	3 [2-6]	4 [3-5]	0.10	0.36	0.44
2nd line CTX data	38 (0%)	/	/	/	/	/	/
---Fluoro-pyrimidine mono	/	N/A	N/A	26 (68%)	N/A	N/A	N/A
---Fluoro-pyrimidine-based combinations	/	N/A	N/A	8 (21%)	N/A	N/A	N/A
---Other CTX regimens	/	N/A	N/A	4 (11%)	N/A	N/A	N/A
Objective response rate	38 (0%)	N/A	N/A	1 (3%)	N/A	N/A	N/A
Number of cycles	36 (5%)	N/A	N/A	4 [2-8]	N/A	N/A	N/A
Laboratory parameters	/	/	/	/	/	/	/
Haemoglobin (g/dL)	78 (3%)	11.7 [10.1-12.6]	10.2 [9.8-12.1]	12.4 [11.3-13.1]	0.002	0.62	0.57
Leukocyte count (G/L)	78 (3%)	7.4 [5.2-9.4]	7.7 [5.7-10.4]	6.9 [5.1-9.1]	0.26	0.39	0.11
Neutrophil count (G/L)	78 (3%)	4.8 [3.0-6.4]	5.5 [3.5-8.3]	4.4 [2.8-5.4]	0.06	0.41	0.56
Lymphocyte count (G/L)	78 (3%)	1.4 [1.1-1.7]	1.3 [0.9-1.7]	1.4 [1.1-2.1]	0.12	0.47	0.45
Platelet count (G/L)	78 (3%)	225 [138-343]	225 [134-307]	224 [141-428]	0.45	0.33	0.30
C-reactive protein (mg/dL)	77 (4%)	21.6 [7.7-47.1]	32.5 [11.4-62.0]	12.6 [5.2-34.2]	0.03	0.45	0.04
Bilirubin (mg/dL)	77 (4%)	0.6 [0.4-1.3]	0.8 [0.4-2.0]	0.5 [0.4-0.9]	0.06	0.49	0.07
Gamma-GT (units/L)	77 (4%)	311 [102-577]	336 [99-613]	245 [113-394]	0.43	0.12	0.35
Alkalic Phosphatase (units/L)	77 (4%)	168 [106-352]	208 [113-378]	152 [96-268]	0.32	0.34	0.14
AST (units/L)	77 (4%)	46 [30-80]	47 [33-83]	37 [27-66]	0.12	0.38	0.20

ALT (units/L)	77 (4%)	34 [20-62]	34 [19-70]	32 [20-59]	0.74	0.16	0.05
Albumin (g/dL)	77 (4%)	3.7 [3.2-4.0]	3.4 [2.9-3.9]	3.9 [3.6-4.1]	0.0007	0.63	0.43
CEA	48 (40%)	3.6 [1.8-11.0]	4.3 [1.8-11.1]	3.4 [1.9-10.9]	0.98	0.12	0.08
CA19-9	48 (40%)	144 [21-944]	223 [14-3204]	97 [29-433]	0.56	0.21	0.12

Figure 1: 18-month overall survival experience of the study cohort (n=80).

The curve represents a crude Kaplan-Meier survivor function.



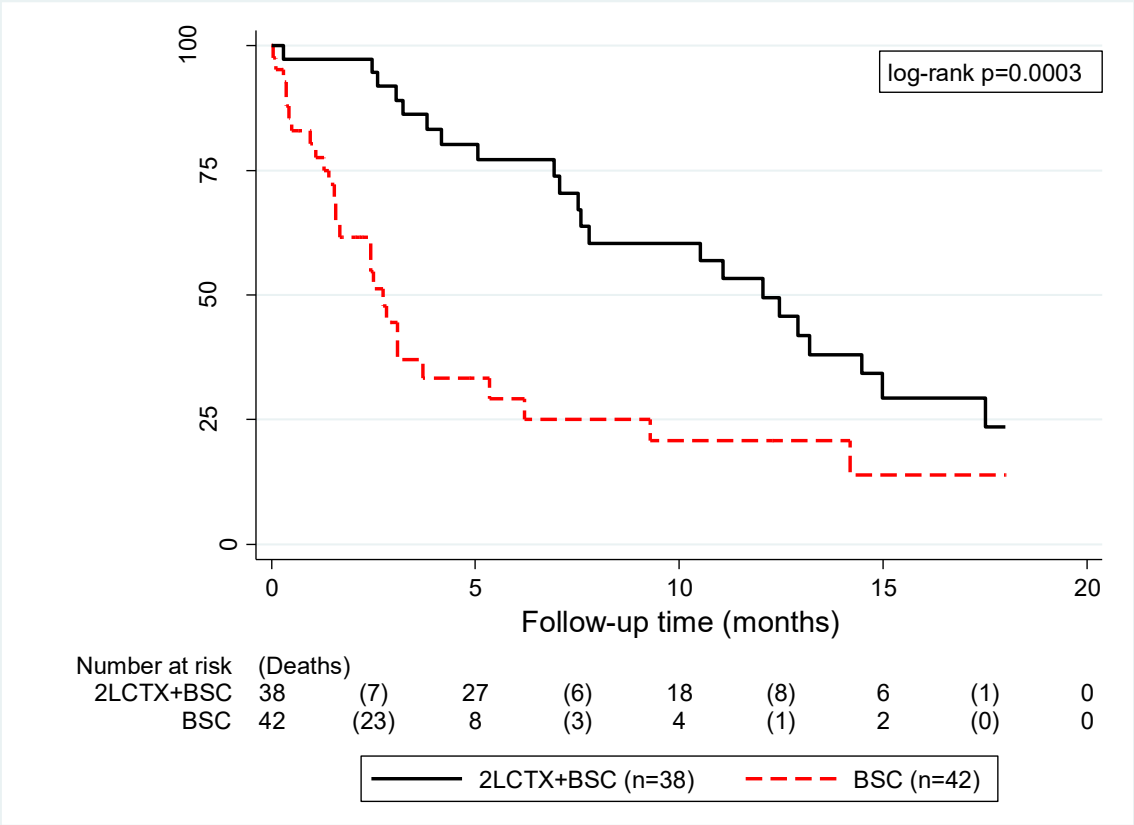
Crude analysis of overall survival according to 2nd-line treatment group

After progression or discontinuation of 1st-line chemotherapy, 42 patients (53%) were treated with best-supportive-care (BSC), and 38 patients (48%) were treated with BSC and 2nd-line chemotherapy (2LCTX). In terms of 2LCTX, most patients received fluoropyrimidine monotherapy (n=26 (68%)). Eight (21%) and 4 (11%) patients were treated with fluoropyrimidine-based combination chemotherapy or other regimens, respectively (**Table 8**). Median OS was 12.1 months in the 2LCTX+BSC group, and 2.7 months in the BSC group, respectively. Three-, 6-, 12- and 18-months OS were 92%, 77%, 53%, and 23% in the 2LCTX+BSC group, and 44%, 29%, 21%, and 14% in the BSC group (log-rank p=0.0003, **Figure 2**). In univariable Cox regression, 2LCTX+BSC was associated with a

0.4-fold lower relative risk of death-from-any-cause than BSC alone (Hazard ratio (HR)=0.36, 95%CI: 0.20-0.64, p=0.001).

Figure 2: Unadjusted Kaplan-meier curves of overall survival according to treatment assignment to 2LCTX+BSC versus BSC alone.

Abbreviations: 2LCTX – 2nd-line chemotherapy, BSC – best supportive care.



Derivation of the IPTW

Importantly, patients in the 2LCTX+BSC had a significantly higher prevalence of favorable prognostic factors (**Table 8**). For example, the median Karnofsky Index was 90% in 2LCTX+BSC, and 80% in BSC (rank-sum p=0.0001; standardized mean difference (SMD)=1.21, with SMDs>0.20 indicating a potentially important imbalance between study groups). Further, patients in the 2LCTX+BSC group had, among others, lower CRP levels (SMD=0.45), lower alkalic phosphatase levels (SMD=0.34) and lower bilirubin levels (SMD=0.49) that patients in the BSC group, and all these variables were associated with a more favorable overall survival experience (**Table 9**). Because this is a major source of bias

for the 2LCTX+BSC vs. BSC comparison, we constructed a propensity score (PS) to predict probabilities of treatment assignment conditional on covariates at baseline. We constructed the PS using a multivariable logistic regression model, in which we included a broad set of variables irrespective of their association with OS (**Table 10**). The distribution of the PS (**Figure 3A**) covered the whole probability range from 0 to 1, and was then transformed into the IPTW according to the inverse of the probability of receiving the treatment that the patient actually received (**Figure 3B**). Re-weighing of the data with the IPTW removed most imbalances of baseline covariates between the two treatment groups (**Table 8**). For example, IPTW-weighing reduced the SMDs for they key prognostic variables (1) Karnofsky Index from 1.21 to 0.20, (2) alkalic phosphatase from 0.34 to 0.14, and (3) bilirubin from 0.49 to 0.07, respectively. Although IPTW-weighing did not fully reduce imbalances below the pre-specified SMD threshold of 0.20 for a small number of variables such as haemoglobin, we considered these balance diagnostics to be indicative of an adequate propensity score model.

Table 9: Univariable predictors of time-to-death in the overall study population. Estimates were derived from univariable Cox regression models. Abbreviations: 95%CI – 95% confidence interval, p – Wald-test p-value, BMI – body mass index, ECOG – Eastern Cooperative Oncology Group performance status, CUP-CCC – cancer of unknown primary with cholangiocellular differentiation, CTX – chemotherapy, AST – Aspartate aminotransferase, ALT – Alanine aminotransferase, CEA – carcinoembryonic antigen, CA 19-9 – Cancer antigen 19-9.

Table 8: Univariable predictors of time-to-death in the overall study population.

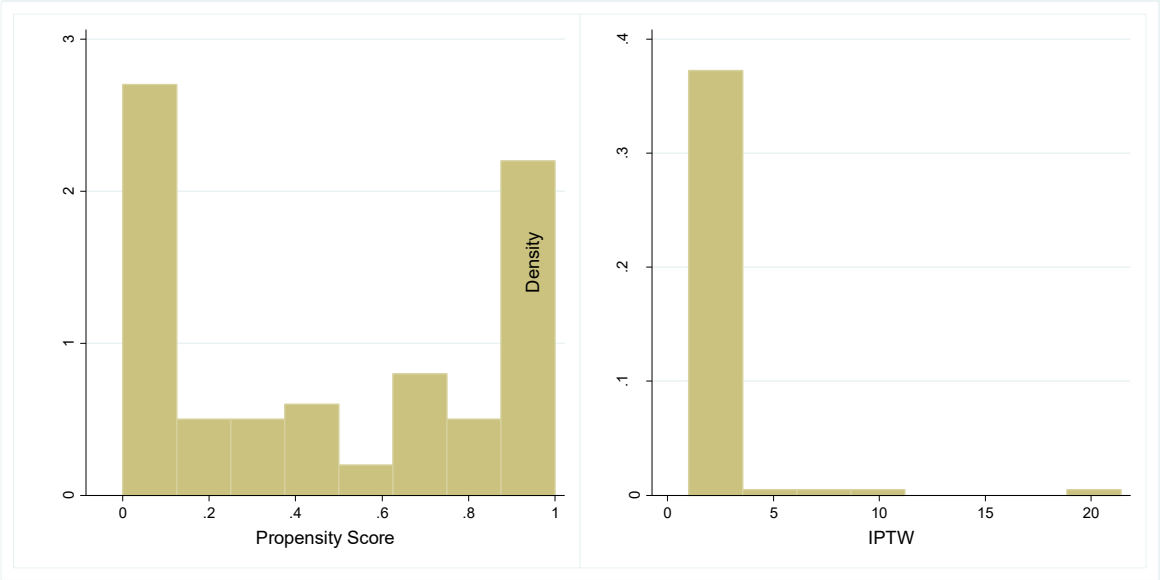
Variable	Hazard Ratio	95%CI	p
Demographics			
Age (per 5 years increase)	0.93	0.80-1.07	0.314
Female Gender	1.24	0.70-2.18	0.463
BMI (per 5 kg/m² increase)	0.76	0.54-1.06	0.100
History of smoking	1.15	0.61-2.15	0.669
Charleson Comorbidity Index (per 1 point increase)	1.04	0.91-1.19	0.534
Synchronous aBTC	1.95	1.08-3.53	0.028

ECOG 1-2	2.59	1.33-5.02	0.005
Tumor location	/	/	/
---Gallbladder	Ref.	Ref.	Ref.
---Intrahepatic	0.89	0.45-1.75	0.741
---Perihilar/Klatskin	0.69	0.28-1.73	0.435
---Distal/Ampulla	0.27	0.10-0.72	0.008
---CUP-CCC*	NE	NE	NE
Tumor grade	/	/	/
---G3	1.62	0.90-2.90	0.107
1st line CTX data	/	/	/
---Cisplatin/Gemcitabine	Ref.	Ref.	Ref.
---Gemcitabine mono	0.56	0.29-1.05	0.072
---Other CTX regimens	0.44	0.19-1.02	0.056
Laboratory parameters	/	/	/
Haemoglobin (per 1g/dL increase)	0.67	0.55-0.82	<0.0001
Leukocyte count (per 1G/L increase)	1.16	1.09-1.23	<0.0001
Neutrophil count (per 1G/L increase)	1.06	1.03-1.09	<0.0001
Lymphocyte count (per 1G/L increase)	0.93	0.90-0.97	<0.0001
Platelet count (per 50G/L increase)	0.95	0.87-1.04	0.260
Neutrophil-Lymphocyte ratio (NLR, per 1 unit increase)	1.17	1.11-1.24	<0.0001
C-reactive protein (per doubling)	1.64	1.35-1.99	<0.0001
Bilirubin (per doubling)	1.47	1.24-1.75	<0.0001
Gamma-GT (per doubling)	1.29	1.08-1.53	0.004
Alkalic Phosphatase (per doubling)	1.92	1.46-2.52	<0.0001
Creatinine (per 1mg/dL increase)	0.49	0.16-1.49	0.211
AST (per doubling)	1.57	1.17-2.11	0.003
ALT (per doubling)	1.36	1.05-1.75	0.020
Albumin (per 1g/dL increase)	0.38	0.21-0.67	0.001
CEA (per doubling)	1.15	0.99-1.34	0.072
CA199 (per doubling)	1.21	1.08-1.36	0.001

Table 10. Propensity score model for treatment assignment to 2LCTX+BSC versus BSC alone. This model is a multivariable logistic regression model which uses data that were multiply imputed with a chained equations algorithm. Abbreviations: 2LCTX – 2nd-line chemotherapy, BSC – best supportive care, 95%CI – 95% confidence interval, p – Wald-test p-value, aBTC – Advanced biliary tract cancer, ECOG – Eastern Cooperative Oncology Group performance status, CTX – chemotherapy, AST – Aspartate aminotransferase, ALT – Alanine aminotransferase, CEA – carcinoembryonic antigen, CA 19-9 – Cancer antigen 19-9.

Table 9: Propensity score model for treatment assignment to 2LCTX+BSC versus BSC alone.			
Variable	**Odds Ratio**	**95%CI**	**p**
Demographics			
Age (per 1 year increase)	0.86	0.70-1.06	0.151
Male Gender	3.55	0.27-46.52	0.334
History of smoking	0.15	0.01-1.98	0.150
Charleson Comorbidity Index (per 1 point increase)	1.02	0.65-1.60	0.919
Metachronous aBTC	11.17	0.66-189.07	0.093
ECOG 1-2	0.18	0.01-3.88	0.271
Tumor parameters	/	/	/
---Gallbladder	Ref.	Ref.	Ref.
---Intrahepatic	3.22	0.24-43.73	0.379
---Perihilar/Klatskin	248.16	0.18-338153.2	0.130
---Others	31.39	0.89-1106.49	0.058
Tumor grade G3	0.23	0.01-5.16	0.346
1st line CTX data	/	/	/
---Cisplatin/Gemcitabine	Ref.	Ref.	Ref.
---Gemcitabine mono	0.38	0.02-6.06	0.490
---Other CTX regimens	0.36	0.01-27.07	0.640
Objective response in 1st line CTX	0.01	0.00-0.74	0.038
CTX cycles in 1st line (per 1 cycle increase)	1.39	0.76-2.55	
Laboratory parameters	/	/	/
Haemoglobin (per 1g/dL increase)	1.50	0.52-4.40	0.451
Neutrophil count (per 1G/L increase)	0.84	0.45-1.56	0.568
Platelet count (per 1G/L increase)	1.01	1.00-1.01	0.067
C-reactive protein (per 1mg/dL increase)	1.01	0.96-1.07	0.631
Bilirubin (per 1mg/dL increase)	0.75	0.38-1.47	0.403
Gamma-GT (per 1unit/L increase)	1.00	1.00-1.01	0.414
Alkalic Phosphatase (per 1unit/L increase)	0.99	0.99-1.00	0.290
Albumin (per 1g/dL increase)	2.18	0.34-14.19	0.412
CA199 (per 1 unit increase)	1.00	1.00-1.00	0.422

Figure 3: Histograms of the propensity score and the inverse-probability-of-treatment-weights (IPTW).

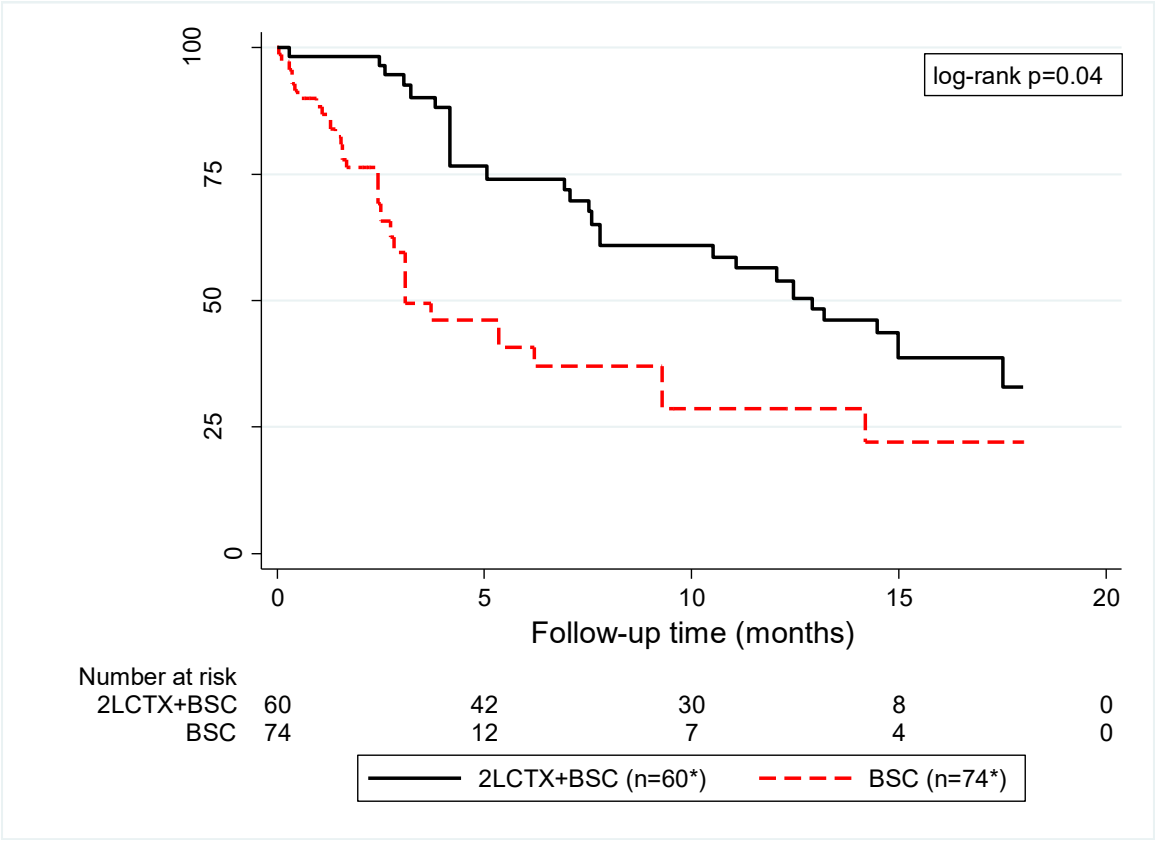


IPTW-weighted analysis of overall survival according to treatment group

After IPTW weighting of the data, median OS was 12.5 months in the 2LCTX+BSC group, and 3.1 months in the BSC group, respectively. Three-, 6-, 12- and 18-months OS were 95%, 74%, 57%, and 33% in the 2LCTX+BSC group, and 60%, 41%, 29%, and 22% in the BSC group (log-rank p=0.04, **Figure 4**). In IPTW-weighted Cox regression, 2LCTX+BSC was associated with a 0.4-fold lower relative risk of death-from-any-cause than BSC alone (Hazard ratio (HR)=0.42, 95%CI: 0.18-0.96, p=0.04). To further increase the efficiency of this estimate, we performed a multivariable adjustment for ECOG performance status, C-reactive protein and bilirubin. Here, the association between 2LCTX and favorable survival outcome prevailed (Adjusted HR=0.38, 95%CI: 0.18-0.81, p=0.01). In a sensitivity analysis using the "trimmed" IPTW, a highly comparable IPTW-adjusted relative risk estimate was observed (IPTW-adjusted HR for 2LCTX-BSC vs. BSC=0.46 (95%CI: 0.23-0.94, p=0.03)).

Figure 4: IPTW-weighted Kaplan-Meier curves of overall survival according to treatment assignment to 2LCTX+BSC versus BSC alone.

Abbreviations: IPTW – Inverse probability of treatment weight, 2LCTX – 2nd-line chemotherapy, BSC – best supportive care.



Exploring potential time-dependencies of 2LCTX benefit

We observed strong evidence for a violation of the proportional hazards association (Schoenfeld test p≤0.005 for both unadjusted and IPTW analyses, respectively). Indeed, non-proportional analysis of mortality hazards using flexible parametric modeling showed the rate of death was much higher during the first few months of follow-up in the BSC only group. However, the rate of death increased over time in the 2LCTX+BSC group, which ultimately lead to a crossing of the two death rates at around 8 months of follow-up (**Figure 5**). This non-proportionality was confirmed in IPTW-weighted Cox regression, where we observed a time-dependent association between 2LCTX and OS benefit (Hazard ratio for interaction between 2LCTX and linear follow-up in months=1.23, 95%CI: 0.99-1.51,

p=0.06). Consistent with a weakening "effect" of 2LCTX over time, the IPTW-weighted HRs for 2LCTX+BSC vs. BSC alone were 0.27 (p=0.007), 0.34 (p=0.01) and 0.42 (p=0.04) for prediction horizons of 6 months, 12 months, and 18 months of follow-up, respectively. Finally, to explicitly allow for these time-dependencies, we fitted a fully parametric, IPTW-weighted survival model with restricted cubic splines on the log(cumulative hazard) scale (3 degrees of freedom for the time-invariant and 2 degrees of freedom for the time-dependent "effect" of 2LCTX on OS). In this model, the association between 2LCTX and a lower relative risk prevailed (HR=0.24, 95%CI: 0.08-0.69, p=0.008, **Figure 6**).

Figure 5: Hazards of death-from-any-cause according to treatment assignment to 2LCTX+BSC vs. BSC alone.

Hazard curves were predicted from a flexible parametric survival model (log(cumulative hazard) scale) with 3 degrees of freedom for the time-invariant treatment variable and 2 degrees of freedom for the time-varying treatment variable. Abbreviations: 2LCTX – 2nd-line chemotherapy, BSC – best supportive care.

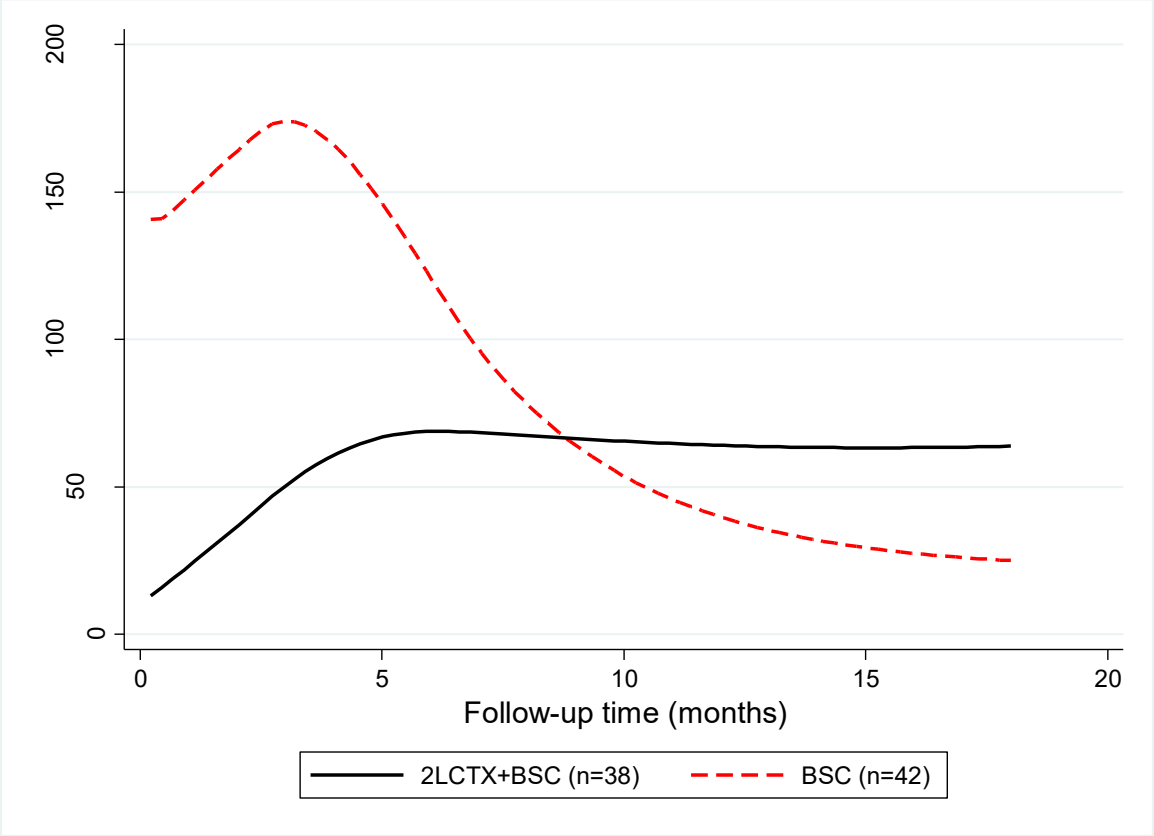
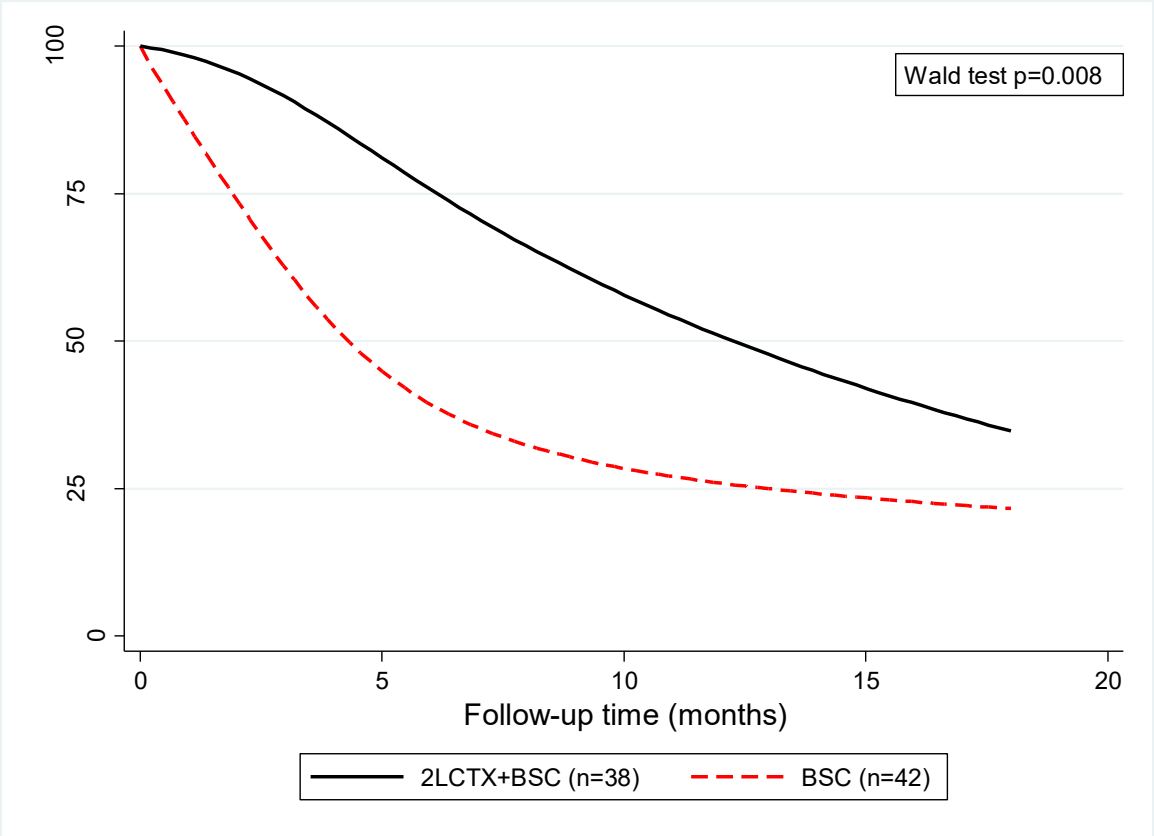


Figure 6: Predicted probability of overall survival according to treatment assignment to 2LCTX+BSC vs. BSC alone.

Survival curves were predicted from a flexible parametric survival model (log(cumulative hazard) scale) with 3 degrees of freedom for the time-invariant treatment variable and 2 degrees of freedom for the time-varying treatment variable. Abbreviations: 2LCTX – 2nd-line chemotherapy, BSC – best supportive care.



Exploring potential predictive markers for 2LCTX benefit

Potential effect modifications between 2LCTX benefit and selected baseline covariables were examined by fitting an interaction between treatment assignment and the covariate of interest within IPTW-weighted Cox models (**Table 11**). In this analysis, "effect" estimates of 2LCTX were highly similar between patients who (1) did and did not respond to 1st line chemotherapy, and (2) had bilirubin levels ≤ and > the 50th percentile of its distribution. Patients with elevated CRP, ECOG performance status of 1-2, and synchronous aBTC had numerically but not statistically significantly more favorable hazard ratios for 2LCTX benefit.

Table 11. Subgroup analyses of 2LCTX benefit in aBTC. Hazard ratios and interaction p-values were derived from IPTW-weighted Cox regression models of overall survival. Abbreviations: 2LCTX – 2nd-line chemotherapy, aBTC – Advanced biliary tract cancer, 95%CI – 95% confidence interval, ECOG – Eastern Cooperative Oncology Group performance status, CTX – chemotherapy

Table 10: Subgroup analyses of 2LCTX benefit in aBTC.

	Hazard ratio	95%CI	Interaction p-value
ECOG: 0	0.71	0.20-2.48	0.515
ECOG: 1-2	0.34	0.07-1.64	
Synchronous aBTC	0.28	0.05-1.44	0.408
Metachronous aBTC	0.64	0.25-1.64	
No response during 1stline CTX	0.37	0.14-1.01	0.961
Response during 1stline CTX	0.36	0.05-2.36	
Bilirubin ≤ 50th percentile	0.54	0.17-1.71	0.901
Bilirubin > 50th percentile	0.59	0.22-1.64	
C-reactive protein ≤ 50th percentile	0.55	0.17-1.81	0.355
C-reactive protein > 50th percentile	0.27	0.12-0.62	

Discussion

Randomized data on the benefit of 2LCTX in addition to BSC alone in patients with aBTC are lacking. In this study, we performed a propensity-score weighted analysis of 18-month observational data from 80 aBTC patients to obtain estimates on the association between 2LCTX and OS. We found that patients receiving 2LCTX+BSC had a significantly better OS experience than patients with BSC alone. However, this "univariable" finding was highly confounded by the non-random selection of patients with favorable covariables into the 2LCTX+BSC group. To account for this bias, we re-weighted the data according to probabilities of assignment to 2LCTX conditional on covariates. Upon this adjustment, the favorable association between 2LCTX and 18-month OS became slightly weaker but prevailed. Additional multivariable adjustment for key prognostic variables such as performance status and serum bilirubin levels did not materially alter this estimate. Importantly, we found evidence for a time-dependency of 2LCTX, indicating that the benefit of this intervention slightly weakened over time. Nonetheless, the overall benefit of 2LCTX prevailed also after fully taking into account this time-dependency using flexible parametric models. In subgroup analyses, we did not identify a patient population with a particularly strong or weak benefit from 2LCTX. Within the limitations of an observational study, these data support the concept that 2LCTX+BSC delays death in patients with aBTC.

A fundamental question for oncologists treating patients with aBTC after failure of first-line chemotherapy is whether 2LCTX improves survival over BSC alone. This question has not been systematically addressed before. In the absence of these data, several retrospective single-arm studies have reported favorable OS outcomes in aBTC patients treated with systemic chemotherapy in the second-line setting. For example, Kim and colleagues recently observed a median OS of 6.5 months in 321 patients treated with fluoropyrimidine-based mono- or polychemotherapy.(126) Other median OS estimates in patients undergoing 2LCTX for aBTC ranged from approximately 7 months in the large cooperative studies by Brieau et al. (6.5 months), Fornaro et al (6.6 months) and Walter et al. (7.5 months)(118, 122, 128) and the meta-analysis by Lamarca et al. (7.2 months),(132) to 13.8 months in a single-center chart review study by Rogers and colleagues.(124) The median OS of 12 months observed in this study for patients who received 2LCTX compares well with these previous reports. Together, these data provide preliminary support for the clinically-

plausible concept that aBTC patients may benefit from 2LCTX in addition to BSC. Moreover, theoretical support for this concept comes from Hagen-Pouiseuille's law, which states that only a small decrease in biliary lumen due to local tumor progression may dramatically reduce biliary flow.(116) The resulting biliary stenosis is an established risk factor for life-threatening infection, and often leads to discontinuation of chemotherapy and morbidity subsequent to repeated interventional procedures for restoring biliary patency. Thus, 2LCTX may not only delay the adverse effects of metastatic tumor spread and progression at distant organs, but may also prolong the time-to-biliary-stenosis and its adverse effects on morbidity and survival.(154)

However, previous retrospective single-arm analyses of highly-selected patients can obviously not answer the underlying systematic question on whether 2LCTX has any benefit at all over BSC alone in a general population of aBTC patients. This is also highlighted by a recent systematic review, which concludes that there is currently insufficient evidence to recommend 2LCTX in aBTC.(132) In this study, we aimed to address this important clinical question using an inverse-probability-of-treatment-weighted comparative effectiveness analysis of observational data. This approach was necessary to account for the large amount of selection bias likely affecting such analyses.(155) Given the statistical assumptions underlying this propensity-score-based approach are met, such an analysis generates a synthetic pseudo-population whose treatment assignment is independent of covariates, hence mimicking randomization.(150) In our study, "naïve" analysis of OS outcomes was consistent with a potentially large benefit of 2LCTX+BSC over BSC alone, both from an absolute (OS estimates) and relative (hazard ratios) perspective. IPTW weighing was performed according to best practice recommendations,(145) and removed most covariate imbalances between these two study groups. The beneficial association between 2LCTX and favorable OS prevailed upon IPTW weighting of time-to-death-data both with respect to magnitude and strength of association. Interestingly, we found that the beneficial "effect" of 2LCTX slightly weakened over time. Indeed, the survival curves of the two treatment groups approached over time. We took this time-dependency into account by specifically modeling non-proportionality of hazards within flexible parametric models,(152) and the beneficial association of 2LCTX with OS also prevailed in this analysis. Synoptically, this suggests that 2LCTX in this setting is an archetypical palliative treatment which delays death but not necessarily leads to a higher proportion of long-term survivors. Physicians should take this into account when discussing second-line options with their patients.

An important aspect of clinical cancer research is to identify predictive markers for treatment response.(156) In the second-line aBTC setting, such markers may inform clinical decision making by stratifying patients according to potentially high or low likelihoods of benefiting from 2LCTX and thus facilitate the decision in favor or against 2LCTX.(157) We have performed such an analysis by fitting interactions between selected baseline variables and OS benefit from 2LCTX. Importantly, patients who did and did not respond to 1st-line chemotherapy appeared to have a similar benefit from 2LCTX. Moreover, this also applied to patients with bilirubin levels below and above the 50th percentile of this covariate's distribution. This allows us to carefully speculate that neither lack of objective response during 1st line chemotherapy nor moderate biliary stenosis as indicated by elevated bilirubin levels should preclude oncologists from considering 2LCTX. Although not reaching statistical significance, we observed numerically higher relative risk reductions of mortality with 2LCTX in patients with elevated C-reactive protein. This generates the hypothesis that patients with more "inflamed" biliary tract cancers may particularly benefit from 2LCTX. However, we urge readers to interpret these subgroup findings with the necessary caution until validated in other cohorts.

Finally, we want to mention six limitations of this study. First, due to the heterogeneity of 2LCTX regimens in our population and the relatively low number of patients treated with some of these individual 2LCTX regimens, we cannot provide robust estimates on the most optimal chemotherapy regimen in this setting. Second, the potentially large magnitude of "effect" of 2LCTX in our study may not exclusively be attributable to a "true" benefit from 2LCTX, but may also be due to from residual confounding not removed by the IPTW. Importantly, the validity of an IPTW analysis depends on the difficult-to-test assumption that the propensity score model is correctly specified and does not omit unmeasured confounders.(145) We have addressed this issue by balance diagnostics after IPTW weighting and careful multivariable adjustment. Third, we included a diverse spectrum of BTC histologies from intrahepatic cholangicarcinoma to cancer-of-unknown-primary (CUP-CCC) with cholangiocellular differentiation. Although these subentities are all classified as biliary tract cancers, translational studies have shown that they can substantially differ with respect to molecular features.(158) These differences may have an impact of 2LCTX benefit, but also here, low numbers of patients within some histologic subentities precluded an important subanalysis of 2LCTX between these subentities. Fourth, our dataset does not yet include data on interventional procedures such as biliary stenting, which may modify the

survival experience of this patient population.(154) Fifth, in the absence of validated cut-offs, we empirically dichotomized our patients at the 50th percentile of continuous variables for predictive biomarker analysis. Higher or lower cut-offs may have yielded different subgroup estimates of 2LCTX benefit, but we refrained from examining other cut-offs in order not to further inflate the type I error rate. Finally, quality-of-life is a paramount issue for patients suffering from a lifetime-limiting disease such as aBTC after first-line chemotherapy.(115) However, due to the retrospective nature of this study, data on quality-of-life were not available to us.

Conclusion

Within the limitations of an observational cohort study, these data support the concept that 2LCTX+BSC is associated with an overall survival benefit over BSC alone in patients with aBTC after failure of first-line chemotherapy. This benefit slightly weakens over time, but appears to be consistent across several subgroups defined by clinical and laboratory variables such as performance status, treatment response during 1st-line chemotherapy, and bilirubin levels. Until randomized evidence becomes available in the future, our findings provide guidance to oncologists and their aBTC patients for treatment decision making in the second-line setting. Future studies should address the benefit of 2LCTX in aBTC within a randomized setting, and identify those patient subgroups with the highest benefit from 2LCTX.

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