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Molecular profiling and treatment pattern differences between intrahepatic and extrahepatic cholangiocarcinoma

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Abstract

Background: Treatment patterns for intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC) differ, but limited studies exist comparing them. This study examines differences in molecular profiling rates and treatment patterns in these populations, focusing on use of adjuvant, liver-directed, targeted, and investigational therapies.

Methods: This multicenter collaboration included patients with ICC or ECC treated at 1 of 8 participating institutions. Retrospective data were collected on risk factors, pathology, treatments, and survival. Comparative statistical tests were 2-sided.

Results: Among 1039 patients screened, 847 patients met eligibility (ICC = 611, ECC = 236). Patients with ECC were more likely than those with ICC to present with early stage disease (53.8% vs 28.0%), undergo surgical resection (55.1% vs 29.8%), and receive adjuvant chemoradiation (36.5% vs 4.2%) (all $P < .00001$). However, they were less likely to undergo molecular profiling (50.3% vs 64.3%) or receive liver-directed therapy (17.9% vs 35.7%), targeted therapy (4.7% vs 18.9%), and clinical trial therapy (10.6% vs 24.8%) (all $P < .001$). In patients with recurrent ECC after surgery, the molecular profiling rate was 64.5%. Patients with advanced ECC had a shorter median overall survival than those with advanced ICC (11.8 vs 15.1 months; $P < .001$).

Conclusions: Patients with advanced ECC have low rates of molecular profiling, possibly in part because of insufficient tissue. They also have low rates of targeted therapy use and clinical trial enrollment. While these rates are higher in advanced ICC, the prognosis for both subtypes of cholangiocarcinoma remains poor, and a pressing need exists for new effective targeted therapies and broader access to clinical trials.

Cholangiocarcinoma (CCA) is a rare, aggressive cancer of the bile ducts with a poor prognosis. It is anatomically subclassified into intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC). ECC is further subclassified into perihilar CCA, also known as Klatskin tumors, and distal CCA (1,2). This classification has varied over time, with perihilar tumors

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being classified as ICC until the International Classification of Diseases (ICD) coding system (ICD-O-3) was adopted in 2001 (3,4). The reclassification of perihilar tumors as ECC poses challenges in characterizing epidemiologic trends and patterns of treatment utilization in the patients with ICC vs ECC.

ICC and ECC have been suspected to be distinct entities as early as Klatskin's description of the hilar tumor in 1965 (5). This theory has been supported by reports of histologic heterogeneity, including disparate cells of origin (6,7). The recent focus on the molecular characterization of CCA has suggested even more heterogeneity in the pathogenesis of ICC and ECC and has provided insights into therapeutic targets unique to each subtype that may result in more tailored approaches to this disease.

Prior reports comparing the anatomic subtypes of CCA have not yet comprehensively examined differences in treatment utilization patterns in large studies (1,2,6-32). Surgical resection is the cornerstone of management for ICC and ECC, and although liver transplant is uncommonly used in CCA, it is considered more commonly in ECC than ICC. However, only a minority of patients are eligible for these approaches as most patients with CCA present with advanced stage disease. Liver-directed therapy (LDT) is sometimes used for locally advanced unresectable ICC, but it is less commonly used in ECC and usually palliatively in this setting (33-42). Most importantly, details regarding the patterns of use and efficacy of systemic therapies, particularly targeted and investigational therapies, in different subtypes of CCA are limited (43-54).

Single institution retrospective reports often contain comprehensive data but are restricted by small numbers of patients and capture practice patterns individual to the institution. Registry studies, such as those involving the Surveillance, Epidemiology, and End Results Program or National Cancer Database databases, involve large sample sizes but are often constrained by incomplete treatment data and variations in data reporting. We sought to overcome these limitations through a multi-institutional collaboration between high-volume CCA programs and through granular data collection using uniform definitions for data points. We placed a particular focus on patterns of use of systemic therapy in advanced ICC vs ECC, particularly targeted therapy and clinical trial enrollment.

Methods

Data collection

Patients were selected from institutional tumor registries using the ICD codes for CCA (ICD-9-155.1, 156.9, and 156.1 or ICD-10 C22.1, 24.9, 24.8, and 24.0) or from institutional databases of patients with CCA. Eligible patients were aged 18 years or older with a confirmed histologic diagnosis of CCA after June 2, 2009. This date was chosen because the ABC-02 data for gemcitabine and cisplatin were publicly presented and became the standard first-line treatment of advanced CCA on this date. Data were collected on demographics, risk factors, treatments, pathology, tumor genotyping, and survival outcomes through retrospective review of the electronic medical record. Self-reported demographics included age, gender, race (White, Black, Asian, or Other defined as not one of the above or not specific), and ethnicity. Medical oncologists with an expertise in hepatobiliary tumors performed quality control checks on the database. Eight institutions participated in this study: Massachusetts General Cancer Center, University of California San Francisco, MD Anderson Cancer Center, Mayo Clinic, Vanderbilt University, University of Virginia, Beth Israel Deaconess Medical Center, and St. Vincent's Medical Center. The data were collected in accordance with individual institutional review board–approved protocols and in accordance with the Declaration of Helsinki.

Molecular profiling

Tumor tissue underwent molecular profiling as a routine part of clinical care using FoundationOne (55) or institutional platforms (Supplementary Table 1, available online).

Statistical analysis

A set of prespecified definitions were used across institutions for uniform data collection as per Supplementary Table 2 (available online). Data were extracted by medical trainees or trained research assistants, and attending medical oncologists adjudicated ambiguous cases. Fisher exact, χ^2, and Wilcoxon rank sum tests were used for comparisons of independent variables. Statistical analyses were performed using Graph Pad Prism version 9 and Medcalc version 19.7, and multivariate analyses were performed with STATA17. A 2-sided test with a P value less than .05 was considered statistically significant. Recurrence-free survival (RFS), progression-free survival (PFS), and overall survival (OS) were calculated by Kaplan-Meier analysis, and log-rank sum tests were used to calculate P values between the cohorts. Patients who did not meet a particular endpoint at the time of last follow-up were censored for that endpoint.

Results

Baseline characteristics and clinical presentation

Among the 1039 patients screened, 847 were eligible, including 611 (72.1%) patients with ICC and 236 (27.9%) with ECC (Table 1). The median age for patients with ICC vs ECC was similar (63.3 vs 65.4 years, respectively; $P = .23$). Patients with ICC were more likely to be female (51.1% vs 41.9%; $P = .02$), and those with ECC were more likely to be male (58.1% vs 48.9%; $P = .02$). Patients with ICC more commonly had a history of chronic hepatitis B infection than patients with ECC (6.3% vs 0.8%; $P = .02$). No difference was seen in the frequency of other risk factors between the 2 groups.

Stage at diagnosis and patterns of metastasis were also statistically significantly different between the ICC and ECC cohorts. Patients with ECC were more likely to present with early stage disease amenable to surgery or transplant (53.8% vs 28.0%; $P < .00001$), and those with ICC were more likely to present with metastatic disease (49.3% vs 23.3%; $P < .00001$). Patterns of metastasis also differed between the patients. ICC, when compared with ECC, was more likely to metastasize to the liver (92.3% vs 58.5%; $P < .00001$), lung (26.6% vs 9.8%; $P = .02$), and lymph nodes (51.8% vs 26.8%; $P = .003$) and less likely to metastasize to the peritoneum (31.7% vs 12.2%; $P = .001$). Multifocal liver involvement of ICC vs liver metastasis could not be distinguished.

Genomics

Genomic profiling data were available for 64.3% (n = 349/543) of patients with advanced ICC and 50.3% (n = 92/183) of patients with advanced ECC ($P < .001$) (Figure 1). Molecular alterations more commonly seen in ICC included *FGFR2* fusions (21.0% vs 0%; $P < .00001$), any *FGFR* alteration (20.6% vs 6.0%; $P = .002$), and *IDH1* mutations (20.3% vs 0%; $P < .00001$). Genomic alterations that were statistically significantly more likely to occur in patients with ECC included mutations in *KRAS* (37.1% vs 12.1%; $P < .00001$), *TP53* (34.4% vs 20.2%; $P = .004$), *SMAD4* (8.2% vs 2.0%; $P = .003$), and *APC* (8.2% vs 1.8%; $P = .001$) (Table 2).

Table 1. Patient characteristics and presentation in intrahepatic cholangiocarcinoma vs extrahepatic cholangiocarcinoma

Characteristics	Diagnosis (n = 847[a])		
	Intrahepatic cholangiocarcinoma (n = 611)	Extrahepatic cholangiocarcinoma (n = 236)	P
Age			
≤ 50 years old	95 (15.5%)	29 (12.3%)	.23
> 50 years old	516 (84.5%)	207 (87.7%)	
Median (range)	63.3 (22.5-92.5)	65.4 (22.0-93.9)	
Gender			
Male	299 (48.9%)	137 (58.1%)	.02
Female	312 (51.1%)	99 (41.9%)	
Ethnicity			
Hispanic or Latino	19/242 (7.9%)	13/101 (12.9%)	.15
Non-Hispanic or Latino	223/242 (92.1%)	88/101 (87.1%)	
Unknown	369 (60.4%)	135 (57.2%)	
Race			
Asian	39/493 (7.9%)	11/184 (6.0%)	
Black	25/493 (5.1%)	9/184 (4.9%)	
Other	10/493 (2.0%)	2/184 (1.1%)	
White	419/493 (85.0%)	162/184 (88.0%)	.18
ECOG PS at Diagnosis			
0	65/175 (37.1%)	21/52 (40.4%)	.89
1	94/175 (53.7%)	27/52 (51.9%)	
2	16/175 (9.1%)	4/52 (7.7%)	
Risk Factors			
Primary Sclerosing Cholangitis	24/597 (4.0%)	8/236 (3.4%)	.67
Diabetes	66/402 (16.4%)	35/189 (18.5%)	.53
Cirrhosis	43/400 (10.8%)	13/189 (6.9%)	.13
Median BMI, kg/m^2	27.3 (15.4-59.2)	26.8 (17.6-65.8)	NE
Mean BMI, kg/m^2	28.21	27.64	.28
BMI ≥ 30, kg/m^2	159/477 (33.3%)	50/190 (26.3%)	.08
HBV	17/272 (6.3%)	1/121 (0.8%)	.02
HCV	29/486 (6.0%)	6/198 (3.0%)	.11
Presentation			
Resected, resectable, or transplanted	171 (28.0%)	127 (53.8%)	<.00001
Locally advanced	139 (22.7%)	54 (22.9%)	.97
Primary metastatic	301 (49.3%)	55 (23.3%)	<.00001
Metastatic Sites (Patients with Primary Metastatic Disease)			
Liver	205/222 (92.3%)	24/41 (58.5%)	<.00001
Lymph Node	115/222 (51.8%)	11/41 (26.8%)	.003
Lung	59/222 (26.6%)	4/41 (9.8%)	.02
Bone	33/222 (14.9%)	2/41 (4.9%)	.08
Peritoneum	27/222 (12.2%)	13/41 (31.7%)	.001
Other	12/222 (5.4%)	4/41 (9.8%)	.28
Bilirubin			
Median (range)	0.6 (0.0-31.9)	1.9 (0.2-35.0)	<.00001
Bilirubin > 2 mg/dL	61/460 (13.3%)	83/172 (48.3%)	<.00001
CA19-9, U/mL			
Median (range)	84 (0-364, 190)	179.5 (0-22, 432)	.11

[a] Denominators denote number of patients with known status of variable. BMI = body mass index; ECOG PS = Eastern Cooperative Oncology Group Performance Status; HBV = hepatitis B virus; HCV = hepatitis C virus; NE = not evaluated.

We explored whether tissue availability explained the low rate of genomic profiling seen in patients with ECC. Molecular testing in patients with ECC is often complicated by nondiagnostic biopsies and inadequate tissue specimens because of limitations of brushings and fine needle aspirates (5). Thus, we evaluated patients who had tumor recurrence after resection where tissue specimens were expected to be of sufficient quantity and quality. Of the 76 patients with ECC with tumor recurrence after surgical resection, 49 (64.5%) had molecular profiling, similar to the overall profiling rate in patients with ICC. Data on attempted but failed genotyping efforts from patients with advanced ECC were not available.

Treatment patterns

Differences in presentation and genomics also led to notable differences in treatment patterns. Patients with ECC more commonly underwent surgical resection or transplant than those with ICC (55.1% vs 29.8%; $P < .00001$) (Table 3). Resected ICCs were statistically significantly larger than ECCs (tumor size ≥ 5 cm in 58.0% vs 5.3%; $P < .00001$), but no difference was seen between the groups in rates of margin or node positivity or lymphovascular invasion. Despite this, patients with ECC were statistically significantly more likely to receive some form of adjuvant therapy (58.3% vs 29.7%; $P < .00001$).

Differences between the ICC and ECC cohorts were also seen in utilization of locoregional therapy and systemic therapy for unresectable and metastatic disease. Patients with advanced ICC more commonly received LDT (35.7% vs 17.9%; $P = .00001$), particularly radioembolization (22.2% vs 0%; $P < .001$), though patients with ECC were more likely to receive radiation to the liver (90.3% vs 56.8%; $P = .0004$) (Table 4). This may reflect the preponderance

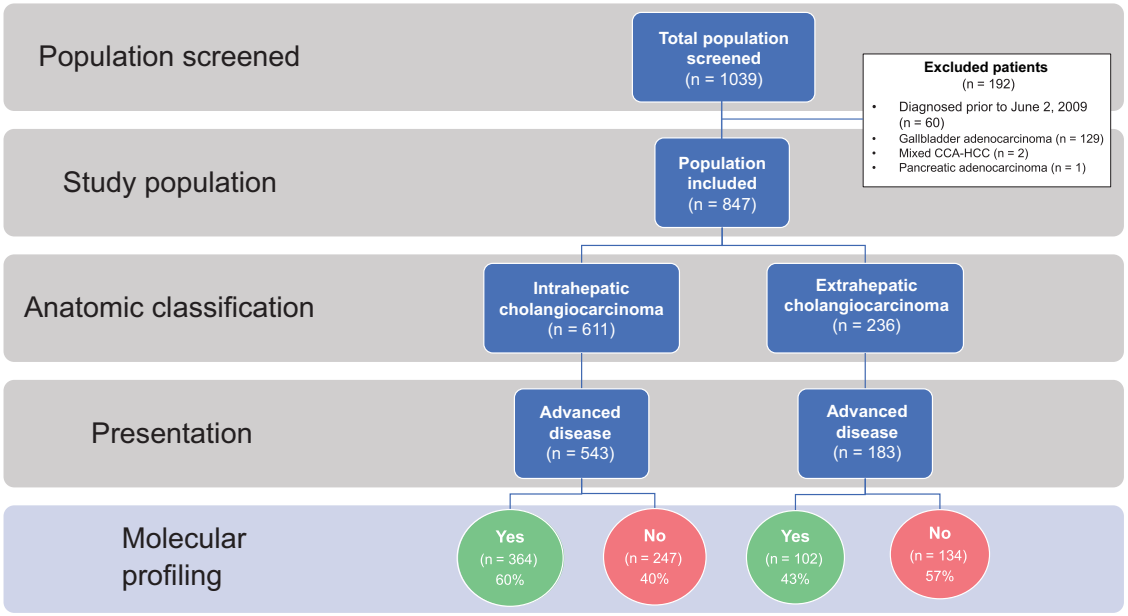

Figure 1. Study consort diagram. Of 1039 patients screened, 847 patients with cholangiocarcinoma (CCA) met eligibility for inclusion in the study, including 611 (72.1%) patients with intrahepatic cholangiocarcinoma (ICC) and 236 (27.9%) with extrahepatic cholangiocarcinoma (ECC). Molecular profiling was performed during routine clinical care in 64.3% of patients with advanced ICC and 50.3% of patients with advanced ECC. CCA-HCC = mixed cholangiocarcinoma-hepatocellular carcinoma.

Table 2. Most common molecular alterations in intrahepatic cholangiocarcinoma vs extrahepatic cholangiocarcinoma

	Prevalence % (alteration present/known status)		
Molecular aberration[a]	Intrahepatic cholangiocarcinoma	Extrahepatic cholangiocarcinoma	*P*
FGFR2 Fusion	21.0 (48/229)	0 (0/55)	<.00001
Any FGFR alteration	20.6 (64/311)	6.0 (5/83)	.002
IDH1	20.3 (70/344)	0 (0/96)	<.001
CDKN2A	7.2 (25/348)	5.1 (5/99)	.45
HER2 Amplification	6.8 (17/249)	1.4 (1/73)	.07
HER2 Mutation	6.1 (20/329)	2.2 (2/89)	.15
ATM	5.7 (13/221)	6.6 (4/61)	.84
ARID1A	5.0 (11/222)	3.2 (2/63)	.55
PIK3CA	4.9 (17/348)	2.0 (2/99)	.21
cMET	4.6 (15/329)	1.1 (1/88)	.14
MAP2K1 or MAP3K1	3.2 (11/342)	5.3 (5/95)	.35
SMAD4	2.0 (7/342)	8.2 (8/98)	.003
APC	1.8 (6/342)	8.2 (8/97)	.001
TP53	20.2 (69/342)	34.4 (33/96)	.004
KRAS	12.1 (42/346)	37.1 (36/97)	<.00001

[a] Mutations with ≥5% frequency in either ICC or ECC were included for analysis. ICC = intrahepatic cholangiocarcinoma; ECC = extrahepatic cholangiocarcinoma; FGFR = fibroblast growth factor receptor; IDH1 = isocitrate dehydrogenase 1; CDKN2A = cyclin dependent kinase inhibitor 2A; HER2 = human epidermal growth factor receptor 2; ATM = ataxia-telangiectasia mutated; ARID1A = AT-rich interactive domain-containing protein 1A; PIK3CA = phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; cMET = mesenchymal epithelial transition factor; MAPK = mitogen-activated protein kinase kinase; SMAD4 = SMA- and MAD-related protein 4; APC = adenomatous polyposis coli; TP53 = tumor protein 53; KRAS = Kirsten rat sarcoma viral oncogene homolog.

of data for intra-arterial therapies in ICC (34,38), whereas LDT data in ECC are mainly limited to scant reports on external beam radiation therapy and ablation for the management of malignant biliary tract obstruction (33,40,42,56). Similarly, patients with ICC more commonly received 3 or more lines of palliative chemotherapy (24.2% vs 14.7%; $P = .03$) and triplet first-line chemotherapy (9.0% vs 2.6%; $P = .02$), whereas patients with ECC were more likely to receive singlet first-line chemotherapy (21.6% vs 10.6%; $P = .002$). Patients with ECC were also more likely to receive 5-fluorouracil–based first-line palliative chemotherapy (24.1% vs 9.3%; $P = .00003$), whereas patients with ICC were more likely to receive gemcitabine-based first-line therapy (86.1% vs 69.8%; $P = .00006$).

The most clinically significant differences between the ICC and ECC cohorts were in utilization patterns for clinical trials and targeted therapies. Patients with advanced ICC were statistically significantly more likely to receive targeted therapy (18.9% vs 4.7%; $P < .00001$) and/or participate in a clinical trial (24.8% vs 10.6%; $P = .00009$) (Table 4). Of the trials where the investigational agent was known, the majority was targeted therapy–based in

Table 3. Treatment differences in early stage intrahepatic cholangiocarcinoma vs extrahepatic cholangiocarcinoma

Characteristics	Diagnosis (n = 847[a])		P
	Intrahepatic cholangiocarcinoma (n = 611)	Extrahepatic cholangiocarcinoma (n = 236)	
Underwent surgery or transplant, yes	182 (29.8%)	130 (55.1%)	<.00001
	Of those who underwent surgery		
Tumor Resection Pathology			
Median tumor size of resected patients, cm	5.5 (0.7-20.0)	2.5 (0.1-7.2)	NE
Tumor size ≥ 5 cm	91/157 (58.0%)	6/114 (5.3%)	<.00001
Node status N0	36/60 (60.0%)	52/105 (49.5%)	.19
Node status N1	24/60 (40.0%)	51/105 (48.6%)	.29
Node status N2	0/60 (0%)	2/105 (1.9%)	.53
Margin status R0	136/158 (86.1%)	100/122 (82.0%)	.35
Margin status R1	17/158 (10.8%)	20/122 (16.4%)	.17
Margin status R2	5/158 (3.2%)	2/122 (1.6%)	.42
LVI present	61/104 (58.7%)	69/101 (68.3%)	.15
Adjuvant Therapy			
Adjuvant therapy status known	165/182 (90.7%)	115/130 (88.5%)	NE
Treated with adjuvant therapy	49/165 (29.7%)	67/115 (58.3%)	<.00001
Adjuvant chemotherapy only	44/165 (26.7%)	59/115 (51.3%)	<.00001
Adjuvant radiation only	12/165 (7.3%)	50/115 (43.5%)	<.00001
Adjuvant chemoradiation	7/165 (4.2%)	42/115 (36.5%)	<.00001

[a] Denominators denote number of patients with known status of variable. NE = not evaluated; LVI = lymphovascular invasion.

both groups (64.6% in patients with ICC, 53.9% ECC), followed by immunotherapy-based (21.2% ICC, 38.5% ECC) (Supplementary Figure 1, available online). Patients with ICC who received targeted therapy most commonly received fibroblast growth factor receptor (FGFR)-, isocitrate dehydrogenase 1 (IDH1)-, and epidermal growth factor receptor (EGFR)–targeted therapy (31.2%, 12.5%, and 17.9%, respectively), whereas patients with ECC most commonly received EGFR–targeted (25.0%), followed by human epidermal growth factor receptor 2 (HER2)-, FGFR-, phosphatidylinositol-3 kinase (PI3K)-, and other mitogen-activated protein kinase (MAPK) alteration-targeted agents in 12.5% of patients each for each target (Figure 2). To investigate this further, we again studied the 76 patients with ECC whose disease recurred after surgical resection, of which only 7 (9.2%) enrolled on a clinical trial and only 2 (2.6%) received targeted therapy on or off trial. Of the patients who underwent genomic sequencing but did not receive targeted therapy, a majority (87.0%) of patients did not have actionable targets. Importantly, 35.0% of the patients with advanced ECC who did not receive targeted therapy or enroll into a clinical trial did receive second-line therapy or beyond, suggesting viable treatment options were needed.

Outcomes on systemic therapy and prognosis

Direct comparisons of ICC and ECC with regard to clinical outcomes are limited (14,57). We compared outcomes on systemic therapy between the cohorts and found median OS from the time of diagnosis of advanced disease was longer in patients with ICC compared with ECC (15.1 vs 11.8 months; $P < .001$) (Figure 3). This difference was maintained when adjusting for several demographic, clinical, and molecular parameters (Supplementary Table 3, available online). No statistically significant difference in RFS after surgery was seen between the groups.

Discussion

The advent of molecular profiling has renewed interest in understanding ICC and ECC as distinct entities beyond genomic signatures. This study is the first multicenter collaboration to examine the differences in treatment patterns between patients with ICC and ECC in a large, modern cohort of patients in granular detail. We identified several key findings: 1) patients with resected or transplanted ECC were more likely to receive adjuvant therapy, 2) patients with advanced ECC were less likely to receive palliative systemic chemotherapy, 3) patients with advanced ECC less commonly enrolled in a clinical trial or received targeted therapy, and 4) patients with advanced ECC had statistically significantly shorter survival than those with advanced ICC.

Many prior attempts at comparing management approaches in ICC and ECC have focused on the perioperative setting (2,28,47,58,59), however, the earlier stage at presentation for many patients with ECC introduces inherent bias (2,5). We confirmed this pattern of earlier stage presentation in ECC and also uncovered statistically significantly different rates of adjuvant therapy use in ECC and ICC. Patients with ECC were more likely to receive adjuvant therapy, although there were no statistically significant differences between the groups in pathology-based prognostic factors other than tumor size, which was greater in the ICC group. Historically, much of the data in support of adjuvant therapy in CCA was in patients with ECC (44,47,60-65), particularly findings from the Southwest Oncology Group (SWOG) S0809 study, which supported adjuvant chemoradiotherapy in patients with ECC or gallbladder carcinoma (66). In 2019, the phase III BILCAP trial also demonstrated a trend toward improved OS with adjuvant capecitabine in patients with ICC (hazard ratio [HR] = 0.65, 95% confidence interval [CI] = 0.35 to 1.18; $P = .47$) (60). Thus, rates of adjuvant therapy use in patients with ICC and with ECC may become more comparable over time. Notably, in spite of, or perhaps because of, the aforementioned differences in

Table 4. Treatment differences in patients with unresectable or metastatic intrahepatic cholangiocarcinoma vs extrahepatic cholangiocarcinoma

Characteristics	Diagnosis (n = 847[a])		P
	Intrahepatic cholangiocarcinoma (n = 611)	Extrahepatic cholangiocarcinoma (n = 236)	
Total No. of patients with advanced disease			
Locally advanced, primary metastatic, recurrent metastatic	543 (88.9%)	183 (77.5%)	.00002
Liver-directed therapy (LDT) in patients with advanced disease			
Known status of LDT	493/543 (90.8%)	173/183 (94.5%)	.11
Received LDT	176/493 (35.7%)	31/173 (17.9%)	.00001
	% of those receiving LDT		
Liver radiation	100 (56.8%)	28 (90.3%)	.0004
Extrahepatic radiation	58 (33.0%)	12 (38.7%)	.05
Ablation	19 (10.8%)	4 (12.9%)	.73
Radioembolization	39 (22.2%)	0 (0%)	.002
Chemoembolization	22 (12.5%)	1 (3.2%)	.13
Bland embolization	4 (2.3%)	1 (3.2%)	.75
Irreversible electroporation	8 (4.6%)	0 (0%)	.61
Palliative Systemic Chemotherapy in Patients with Advanced Disease			
Known status of palliative systemic chemotherapy	589/611 (96.4%)	219/236 (92.8%)	.0249
Treated with palliative systemic chemotherapy	388/589 (65.9%)	116/219 (53.0%)	.0008
Median No. of lines of systemic therapy	1 (0-7)	1 (0-5)	NE
Mean No. of lines of systemic therapy	1.6	1.1	<.0001
1 line of systemic therapy	154 (39.7%)	41 (35.3%)	.40
2 lines of systemic therapy	97 (25.0%)	23 (19.8%)	.25
≥3 lines of systemic therapy	94 (24.2%)	17 (14.7%)	.03
Singlet first line palliative therapy	41 (10.6%)	25 (21.6%)	.002
Doublet first line palliative therapy	310 (79.9%)	84 (72.4%)	.09
Triplet first line palliative therapy	35 (9.0%)	3 (2.6%)	.02
Fluoropyrimidine-based first line palliative therapy	36 (9.3%)	28 (24.1%)	.00003
Gemcitabine-based first line palliative therapy	334 (86.1%)	81 (69.8%)	.00006
Fluoropyrimidine and Gemcitabine-based first line palliative therapy	5 (1.3%)	1 (0.9%)	.71
Other	11 (2.8%)	5 (4.3%)	.43
Targeted Therapy in Patients with Advanced Disease			
Known status of targeted therapy	520/543 (95.8%)	170/183 (92.9%)	NE
Received targeted therapy	98 (18.9%)	8 (4.7%)	<.00001
Clinical Trial in Patients with Advanced Disease			
Known status of clinical trial	520/543 (95.8%)	170/183 (92.9%)	
Enrolled in a clinical trial	129 (24.8%)	18 (10.6%)	.00009
Recurrence Free Survival, mo			
All resected patients	13.9	15.6	.31
First Progression Free Survival, mo			
All patients who received first line Gemcitabine + Cisplatin	4.53	6.0	.38
Second Progression Free Survival, mo			
All patients who received second line mFOLFOX	2.0	3.3	.26
Median Overall Survival, mo			
All patients	21.4	18.5	.80
From diagnosis of advanced disease	15.1	11.8	<.001

[a] Denominators denote number of patients with known status of variable. NE = not evaluated.

receipt of adjuvant treatment, there was no statistically significant difference in median RFS between the groups. Further investigation into novel perioperative approaches, including biomarkers predictive of benefit, holds the potential to improve outcomes for patients with CCA and save some patients unnecessary treatment.

Most patients with CCA present with advanced disease (2,22,28,29,51), and thus we performed a focused analysis on the use of systemic therapies. Patients with ECC were statistically significantly less likely than those with ICC to receive any palliative chemotherapy and less likely to receive multiple lines of therapy. Several prior studies have reported similar observations (67-69). A study by the Dutch Pancreatic Cancer Group demonstrated that although the use of palliative chemotherapy in patients with distal CCA improved over time, only 32.9% of patients received at least 1 line of palliative chemotherapy by 2016 (67). The limited use of systemic therapy in patients with ECC may in part be related to 1) worse nutritional and performance status due to patterns of metastasis (ie, high rates of multi-organ and peritoneal metastasis) (69-71) and 2) local tumor-related complications such as biliary obstruction leading to elevated liver function tests and recurrent cholangitis (2,5,35,70,72,73).

The rate of incorporation of targeted therapies and clinical trials into treatment was encouraging in our population, but both were lowest for the patients with ECC. This higher trial accrual rate in ICC vs ECC appears to be a widespread problem. In the European MOSCATO-01 trial of molecularly matched targeted therapies where 4.0% of the 1035-patient study population had

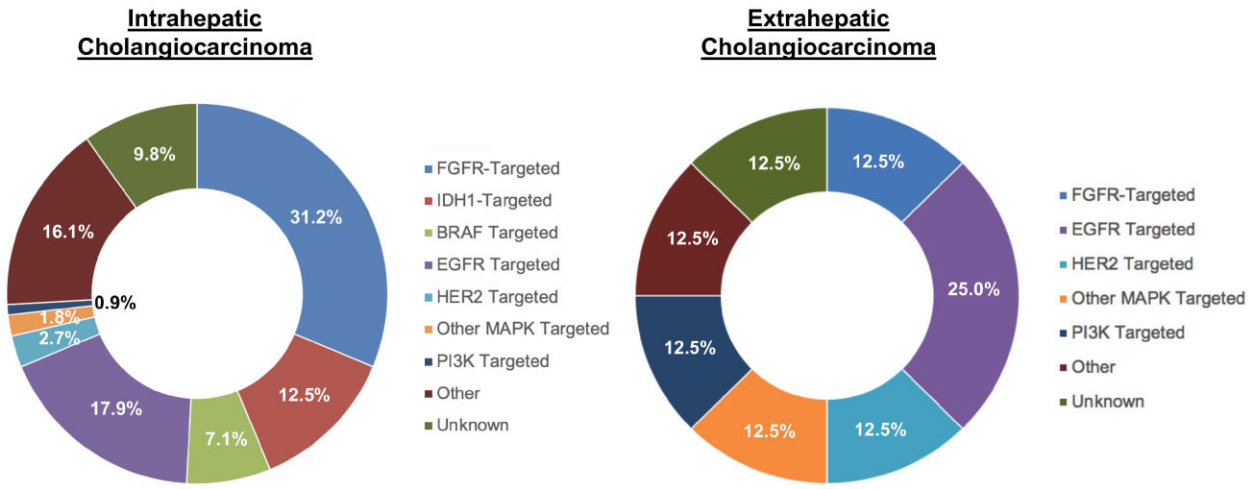

Figure 2. Profile of targeted therapy received in intrahepatic cholangiocarcinoma vs extrahepatic cholangiocarcinoma. Spectrum of targeted therapies received by patients with intrahepatic cholangiocarcinoma (ICC) and patients with extrahepatic cholangiocarcinoma (ECC) during the course of their clinical care. Patients with ICC primarily received FGFR-, EGFR- and IDH1-targeted therapies, and patients with ECC predominantly received EGFR-targeted therapy, followed equally by FGFR-, HER2-, PI3K-, IDH1-, BRAF-, and MAPK-targeted therapies. BRAF = v-raf murine sarcoma viral oncogene homolog B1; EGFR = epidermal growth factor receptor; FGFR = fibroblast growth factor receptor; IDH1 = isocitrate dehydrogenase 1; MAPK = mitogen-activated protein kinase; PI3K = phosphatidylinositol-3 kinase.

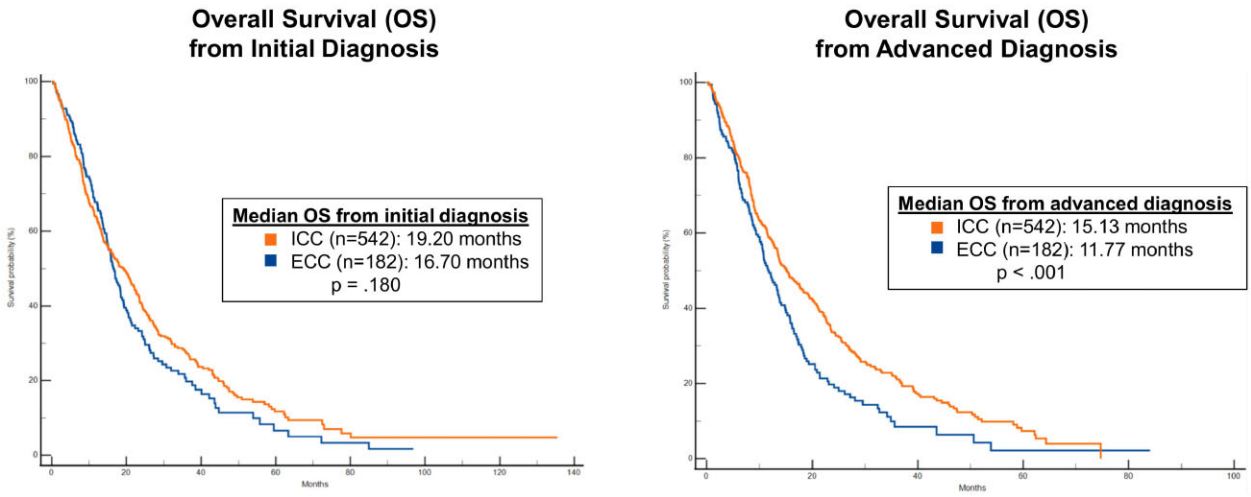

Figure 3. Overall survival in intrahepatic cholangiocarcinoma vs extrahepatic cholangiocarcinoma. Overall survival (OS) in patients with intrahepatic cholangiocarcinoma (ICC) compared with patients with extrahepatic cholangiocarcinoma (ECC) from the time of diagnosis and from the time of diagnosis of advanced disease. Patients with ICC lived longer with advanced disease than patients with ECC.

biliary tract cancer (BTC), a majority (67.0%) of them had ICC (54). Further, in a single-institution study, of the 40 patients with BTC enrolled on a phase I trial, only 4 (10.0%) had ECC as compared with 30 (75.0%) patients with ICC (49). Low rates of trial enrollment among patients with ECC may be related to the aforementioned barriers to receiving systemic therapy and also to lower rates of molecular profiling, a lack of actionable genomic alterations, and poor access to suitable trials for rare targets or biomarker-agnostic trials.

Several potential avenues exist to directly address these problems. More than half of the patients with advanced ECC did not have molecular profiling performed, limiting the potential for employing targeted therapy and clinical trials. Encouragingly, patients who had tumor recurrence after surgery had rates of profiling on par with those seen in ICC, suggesting adequate quantity and quality of tissue is a relevant challenge. Cholangioscopy-guided forceps biopsy can offer more adequate tissue acquisition than brushings alone (74,75) in those without surgical tissue. Further, the use of circulating tumor DNA for molecular profiling has shown similar capture rates for actionable alterations as tissue genotyping in BTC (76-79), providing a rapid, noninvasive approach to identifying patients for molecularly matched therapy. Additionally, tissue-agnostic basket trials such as the National Cancer Institute Molecular Analysis for Therapy Choice (NCI-MATCH) and American Society of Clinical Oncology (ASCO) Targeting Agent and Profiling Utilization Registry (TAPUR) trials may offer access to trial therapies for uncommon cancers with uncommon targets. Given most CCAs do not harbor targets that are currently actionable, clinical trials of biomarker-agnostic therapies are also key to improving outcomes for these patients. Finally, patients with CCA can benefit from advances in other cancers with overlapping molecular profiles. For example, 37.0% of patients with ECC in our study had *KRAS* alterations. Recent advances in targeting *KRAS* G12C in

lung and colorectal cancer (80-82), and ongoing trials targeting other *KRAS* variants, open the door for CCA-specific investigations.

Patients with advanced ECC had a worse survival than those with ICC in our study, and the contribution of the treatment pattern differences noted herein requires further study. Notably, the difference in survival was not accounted for by controlling for known positive and negative prognostic mutations, such as FGFR2 fusions found at higher frequencies in ICC and associated with improved survival (16,52,83) and TP53 or KRAS mutations found at higher frequencies in ECC and associated with worse survival (16,84). This disparity persisted on multivariate analysis when controlling for differences in utilization of potentially life-prolonging palliative therapy, however, unknown factors such as recurrent jaundice and cholangitic sepsis due to biliary obstruction may have contributed as main causes of morbidity and mortality in patients with ECC (85-89).

A limitation of our study is the patient population was limited to those evaluated primarily by medical oncologists at academic cancer centers, potentially limiting the generalizability of our findings to the broader CCA population. Although these patients may have been more likely to seek out clinical trials or undergo molecular profiling, this should not appreciably impact the differences we noted between patients with different anatomic subtypes of CCA. Additionally, given the retrospective data collection, selection bias and incomplete data availability may have impacted the findings. We aimed to minimize their impact by performing a large study across multiple institutions and limiting our analyses to variables with enough known values to achieve sufficient power to calculate statistically significant differences.

Taken together, our findings suggest a critical need for profiling approaches that perform well in the setting of scant tissue obtained in CCA, education of clinicians and patients regarding the potential of molecular profiling to open up therapeutic avenues, and development of drugs for currently actionable and nonactionable genomic alterations and that are biomarker and molecularly agnostic. Lastly, further investigation into the factors contributing to the difference in survival between patients with ICC and ECC has the potential to inform treatment decision making. Ultimately, our study suggests nuanced differences between ICC and ECC beyond anatomic location that warrant treatment of them as distinct entities.

Data availability

Individual participant data cannot be shared publicly due to our institutions' ethical regulations, aiming to protect the privacy of individuals that participated in the study. All source data is available and will be archived at Massachusetts General Cancer Center. The data sharing of individual patient data from each participating institution will be subject to the policy and procedures of each institution. Data requests will be honored pending approval of the institutional review board of each institution in accordance with appropriate human subjects protections.

Author contributions

Kristen Spencer, DO, MPH (Conceptualization; Methodology; Project administration; Writing—original draft; Writing—review & editing); Robin Kate Kelley, MD (Writing—review & editing); Rachna T. Shroff, MD (Writing—review & editing); Mitesh J. Borad, MD (Writing—review & editing); Kabir Mody, MD (Writing—review & editing) Laura Williams Goff, MD, MSCI, MMHC (Writing—review & editing); Nora Horick, MS (Formal analysis; Writing—review & editing); Stacie Lindsey, BS (Writing—review & editing); Lewis R. Roberts, MB ChB, PhD (Writing—review & editing); Christine VanCott, MD, FACS, CPE (Writing—review & editing); Kylie Boyhen, CCRP (Data curation; Writing—review & editing); A. John Iafrate, MD, PhD (Writing—review & editing); Jochen K. Lennerz, MD, PhD (Writing—review & editing); Andrew X. Zhu, MD, PhD (Writing—review & editing); Mary Linton B. Peters, MD (Writing—review & editing); Marc Roth, MD (Writing—review & editing); Thomas T. DeLeon, MD (Writing—review & editing); Jennifer Stanton, BS (Data curation; Writing—review & editing); Osama E. Rahma, MD (Writing—review & editing); Tri Minh Le, MD (Data curation; Writing—review & editing); Stephanie Reyes, MD (Data curation; Writing—review & editing); Anaemy Danner De Armas, MPH (Data curation; Writing—review & editing); Apurva Jain, BS (Data curation; Writing—review & editing); Karen Zhang, BS (Data curation; Writing—review & editing); Andrea Grace Bocobo, BS (Data curation; Writing—review & editing); Jordan Maurer, BS, MS (Data curation; Formal analysis; Writing—review & editing); Islam Baiev, BS (Data curation; Formal analysis; Writing—review & editing); Leontios Pappas, MD, PhD (Data curation; Formal analysis; Methodology; Writing—review & editing); Milind M. Javle, MD (Writing—review & editing); and Lipika Goyal, MD, MPhil (Conceptualization; Methodology; Writing—review & editing).

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Conflicts of interest

KS reports advisory/consultancy: QED Therapeutics, Helsinn, the Lynx Group, and Caris Life Sciences.

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