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ORIGINAL ARTICLE

Claudin 18.2: a promising actionable target in biliary tract cancers

V. Angerilli[1,2†], D. Sacchi[2†], M. Rizzato[3†], J. Gasparello[1], C. Ceccon[2], M. Sabbadin[2], M. Niero[2], F. Bergamo[3], U. Cillo[4], C. Franzina[5], C. Luchini[5], A. P. Dei Tos[1], S. Lonardi[3‡] & M. Fassan[1,6*‡]

[1]Department of Medicine (DIMED), University of Padua, Padua; [2]Surgical Pathology Unit, Azienda ULSS2, Marca Trevigiana, Treviso; [3]Medical Oncology 1, Veneto Institute of Oncology IOV — IRCCS, Padua; [4]Department of Surgical, Oncological, and Gastroenterological Sciences, University of Padua, Padua; [5]Department of Diagnostics and Public Health, Section of Pathology, University and Hospital Trust of Verona, Verona; [6]Veneto Institute of Oncology (IOV-IRCCS), Padua, Italy

Available online xxx

Background and purpose: Anti-claudin 18.2 (anti-CLDN18.2) therapy has been approved for patients with CLDN18-positive gastric and gastroesophageal junction adenocarcinomas. The current study aims at evaluating the expression of CLDN18 in a large cohort of pathologically characterized biliary tract cancers (BTCs).

Materials and methods: A series of 237 BTCs were collected and reviewed under the BITCOIN protocol. All samples were assessed for CLDN18 status using immunohistochemistry (clone 43-14A). Tumor positivity for CLDN18 was determined if $\geq$75% of tumor cells exhibited moderate-to-strong membranous staining.

Results: CLDN18 expression was found in 29.5% of BTCs (70/237), with the highest rates in gallbladder carcinoma (GBC; 62.5%; 20/32) and extrahepatic cholangiocarcinoma (eCCA; 53.4%; 31/58), compared with intrahepatic cholangiocarcinoma (iCCA; 12.9%; 19/147) ($P < 0.0001$). CLDN18 positivity was detected in 5.5% of cases (13/237), most common in GBC (15.6%; 5/32), followed by eCCAs (8.6%; 5/58) and iCCAs (2.0%; 3/147) ($P = 0.0045$). Most CLDN18-positive samples (10/13) exhibited a heterogenous staining pattern. In iCCAs, large duct subtypes had higher CLDN18 expression [33.3% (10/30) versus 7.7% (9/117), $P = 0.0002$] and positivity [6.7% (2/30) versus 0.9% (1/117), $P = 0.106$] than small duct iCCAs. No significant differences were observed across GBC and eCCA histotypes, and CLDN18 was not associated with *IDH1* or *FGFR2* status in iCCAs.

Conclusions: This study demonstrates that CLDN18 expression is present in a subset of BTCs, with significantly higher positivity rates in GBCs and eCCAs compared with iCCAs. In iCCAs, CLDN18 expression was more frequent in the large duct subtype but was not associated with *IDH1* or *FGFR2* status. These findings suggest that CLDN18 could be a potential therapeutic target in BTCs, warranting further prospective studies to evaluate its clinical significance and impact on patient outcomes.

Key words: claudin 18.2, CLDN18.2, biliary tract cancer, cholangiocarcinoma, gallbladder carcinoma, immunohistochemistry

INTRODUCTION

Biliary tract cancers (BTCs) encompass a range of malignancies originating from the epithelial cells of the bile ducts. This includes cholangiocarcinoma (CCA), which develops in the intrahepatic, perihilar, or distal regions of the biliary tree, as well as gallbladder carcinoma (GBC), each following its own distinct epidemiological and molecular pathological pathways.[1] The overall incidence of BTCs is currently increasing in Western countries, particularly that of intrahepatic CCA (iCCA), and their prognosis remains poor.[1]

Surgical resection is the primary treatment offering potential cure for BTCs.[2] Only a small percentage of patients have tumors that can be surgically removed, however, and the majority present with advanced-stage, unresectable disease.[3,4] Recent genomic profiling studies have enhanced our understanding of the complex and heterogeneous molecular landscape of BTCs, uncovering several genetic alterations.[5,6] These include alterations that are rarely seen in other solid tumors, such as *IDH1* mutations and a wide range of *FGFR2* rearrangements.[5,6] Notably, level I actionable genetic alterations—those enabling treatment with already approved therapies or therapies in advanced stages of clinical development—are identified in up to 40% of CCAs.[7]

*Correspondence to: Prof. Matteo Fassan, Department of Medicine (DIMED), University of Padua, via Gabelli 61, 35121 Padua, Italy. Tel: +39-049-8217931
E-mail: matteo.fassan@unipd.it (M. Fassan).

†Equally contributed as first authors.
‡Equally contributed as senior authors.

Claudin 18.2 (CLDN18.2), a transmembrane protein isoform of claudin-18 (CLDN18), plays a role in tight junctions, maintaining epithelial barrier function and cell polarity.[8] During malignant transformation, the loss of cell polarity exposes the CLDN18.2 epitope, enhancing its accessibility for therapeutic targeting. In normal tissue, CLDN18.2 expression is strictly limited to the gastric mucosa and often remains preserved during malignant transformation in gastric cancer. Frequent ectopic activation, however, has been found in various cancer types.[9]

Based on the promising results of the two recent phase III randomized trials SPOTLIGHT[10] and GLOW,[11] the anti-CLDN18.2 zolbetuximab has been proposed as a new standard of care in patients with human epidermal growth factor receptor 2 (HER2)-negative, locally advanced, and metastatic gastric and gastroesophageal junction adenocarcinomas having at least 75% of tumor cells with moderate-to-strong membranous CLDN18 expression by means of immunohistochemistry (IHC). Additional trials are currently investigating possible synergies between CLDN18.2 targeting and immunotherapy.[12]

Preliminary data suggest potential therapeutic relevance of CLDN18.2 in BTCs[13]; however, only one recent study has assessed CLDN18 expression in a large cohort of iCCAs and extrahepatic CCA (eCCAs).[14] The current study aims at evaluating CLDN18 expression and positivity in a large cohort of pathologically characterized BTCs, including GBCs.

MATERIAL AND METHODS

Study population and histopathologic revision

A series of 237 formalin-fixed paraffin-embedded samples of BTCs (30 biopsy and 207 surgical resection specimens) were collected and reviewed under the BITCOIN protocol, approved by the local Ethic Committee (EM 2024-34, 05/15/2024).[15] The original slides were re-evaluated by two expert pathologists (MF and DS) to confirm the primary tumor site and assess pathological characteristics, including subtype, grading and association with pre-invasive lesions, according to WHO 2019 criteria.[16]

Immunohistochemistry

All samples were assessed for CLDN18 status using IHC (clone 43-14A; Roche Ventana, Oro Valley, AZ). The assessment of CLDN18 expression was conducted manually, with cellular positivity defined based on previously published criteria: strong or intermediate circumferential staining, or only strong partial staining.[17] Presence of CLDN18 expression and tumor positivity for CLDN18 were assessed. CLDN18 expression was defined as the presence of CLDN18 cellular positivity regardless of the percentage of tumor cells. Tumor positivity for CLDN18 was determined if $\geq$75% of tumor cells exhibited moderate-to-strong membranous staining, following the evaluation methods used in the SPOTLIGHT and GLOW trials.[10,11,18]

The presence and pattern of intratumoral heterogeneity was assessed only in cases with CLDN18 positivity, as weak staining makes it difficult to assess staining patterns. The absence of intratumoral heterogeneity (i.e. homogenous pattern of expression of CLDN18) was defined as CLDN18 being expressed in >90% of the area with a moderate-to-strong membranous staining. The heterogeneous pattern was further classified into three subtypes, as previously described[19,20]: superficial, invasive-front and random. The superficial pattern was defined by expression predominantly located in the mucosa. The invasive-front pattern showed prominent expression in the deep invasive areas of the tumor. The random pattern was characterized by patchy expression with varying intensities distributed evenly. The type of pattern of intratumoral heterogeneity of CLDN18 expression was assessed only surgical specimens.

Molecular analysis

iCCAs were characterized for *IDH1* mutations and *FGFR2* fusions in a previous study under the BITCOIN protocol[15] by targeted DNA and RNA next generation sequencing [FoundationOne®CDx (F1CDx; Foundation Medicine, Cambridge, MA) and Archer® FusionPlex® Oncology Research Panel (ArcherDX, Boulder, CO)]. eCCAs and GBCs were not characterized for genetic alterations.

Statistical analysis

Two-tailed Fisher's exact test and chi-square test were applied for categorical variables. *P* value <0.05 was considered statistically significant. Statistical analysis was carried out using the STATA software (Stata Corporation, College Station, TX).

RESULTS

Clinicopathologic and molecular characteristics

A total of 237 patients with a confirmed diagnosis of BTCs—comprising 207 patients who underwent surgical resection and 30 who had a biopsy—were included in the study for IHC analysis. The majority were patients >65 years old (52.3%) and the male-to-female ratio was 1.01. BTCs were subdivided as follows according to anatomic location: 147 iCCAs, 58 eCCAs and 32 GBCs. Of the 58 eCCAs, 32 were peri-hilar CCAs (pCCAs) and 26 were distal CCA (dCCA). Nearly half of the cases were moderately differentiated (G2; 47.7%). As for histotypes, most iCCAs were of the small duct subtype (79.6%), while most eCCAs/GBC were of pancreatobiliary type (83.3%). *IDH1* mutations and *FGFR2* fusions were identified in 19.7% and 10.9% of the 147 cases of iCCAs, respectively; genetic alterations in eCCA and GBC were not evaluated. Associated pre-invasive lesions were documented in 13 cases (1 iCCA; 5 eCCA; 7 GBCs), namely biliary intraepithelial neoplasia (BilIN; $n = 11$); intracholecystic papillary neoplasm (ICPN; $n = 1$) and intraductal papillary neoplasm of the bile duct (IPNB; $n = 1$). Table 1 summarizes the clinico-pathologic and molecular features of the 237 BTCs included in the study.

Table 1. Clinico-pathologic features of 237 biliary tract cancers included in the study

Sex	M	119 (50.2%)
	F	118 (49.8%)
Age, years	<65	111 (47.7%)
	≥65	126 (52.3%)
Anatomic location	iCCA	147 (62.0%)
	eCCA	58 (24.5%)
	— pCCA	32
	— dCCA	26
	GBC	32 (14.5%)
Grading	G1	21 (8.9%)
	G2	113 (47.7%)
	G3	103 (43.5%)
Association with pre-invasive lesions	Yes	13 (5.2%)
	— BilIN	11
	— IPNB	1
	— ICPN	1
	No	224 (94.8%)
iCCA ($n = 147$)		
Histotype	Small duct	117 (79.6%)
	Large duct	30 (20.4%)
IDH1 mutation	Present	29 (19.7%)
	Absent	118 (80.3%)
FGFR2 fusion	Present	16 (10.9%)
	Absent	131 (89.1%)
eCCA/GBC ($n = 90$)		
Histotype	Pancreato-biliary	75 (83.3%)
	Intestinal	6 (6.7%)
	Clear cell	3 (3.3%)
	Adenosquamous	3 (3.3%)
	Mucinous	2 (2.2%)
	Lymphoepithelial	1 (1.1%)

BilIN, biliary intraepithelial neoplasia; dCCA, distal cholangiocarcinoma; eCCA, extrahepatic cholangiocarcinoma; F, female; GBC, gallbladder carcinoma; iCCA, intrahepatic cholangiocarcinoma; ICPN, intracholecistic papillary neoplasm; IPNB, intraductal papillary neoplasm of the bile duct; M, male; pCCA, perihilar cholangiocarcinoma.

CLDN18 expression and positivity according to BTC subtype

Overall, CLDN18 expression was observed in 70/237 BTCs (29.5%) samples, while CLDN18 positivity (i.e. moderate-to-strong membranous expression in ≥75% of the tumor cells) was found in 13/237 BTCs (5.5%). CLDN18 expression was significantly more common in GBCs (62.5%; 20/32) and eCCAs (53.4%; 31/58) compared with iCCAs (12.9%; 19/147), with a P value <0.0001. Similarly, CLDN18 positivity rates were highest in GBCs (15.6%; 5/32), followed by eCCAs (8.6%; 5/58) and iCCAs (2.0%; 3/147), with a P value of 0.00249.

Within eCCAs there were no significant differences in CLDN18 expression or positivity rates between pCCAs and dCCAs: CLDN18 expression was observed in 18 of 32 (56.3%) pCCAs and in 13 of 26 (50%) dCCAs, while CLDN18 positivity was observed in 2 cases of pCCAs (6.3%) and 3 cases of dCCAs (11.5%). Table 2 summarizes the prevalence of CLDN18 expression and positivity in BTCs according to anatomic location.

CLDN18 expression and positivity according to histopathologic and molecular features

Table 3 summarizes the prevalence of CLDN18 expression and positivity in BTCs according to histopathologic and molecular features.

Table 2. Prevalence of CLDN18 expression and positivity in biliary tract cancers according to anatomic location

	CLDN18 expression	*P* value	CLDN18 positivity	*P*-value
iCCA	19/147 (12.9%)	<0.0001	3/147 (2.0%)	0.00249
eCCA	31/58 (53.4%)		5/58 (8.6%)	
GBC	20/32 (62.5%)		5/32 (15.6%)	
BTC	70/237 (29.5%)	—	13/237 (5.5%)	—

BTC, biliary tract cancer; CLDN18, claudin-18; eCCA, extrahepatic cholangiocarcinoma; GBC, gallbladder carcinoma; iCCA, intrahepatic cholangiocarcinoma.

CLDN18 expression was more prevalent in well-differentiated (G1) BTCs, followed by moderately differentiated (G2) and poorly differentiated (G3) BTCs (61.9%, 31.0%, and 21.4%, respectively; $P = 0.0009$). Although a similar trend was noted for CLDN18 positivity, it was not statistically significant.

In the iCCA subgroup, large duct iCCAs showed significantly higher CLDN18 expression compared with small duct iCCAs [33.3% (10/30) versus 7.7% (9/117), $P = 0.0002$]. Likewise, CLDN18 positivity was higher in large duct iCCAs than in small duct iCCAs, but this difference did not reach statistical significance [6.7% (2/30) versus 0.9% (1/117), $P = 0.106$]. No association was found between CLDN18 expression and *FGFR2*/*IDH1* status in iCCA; genetic alterations in eCCA and GBC were not evaluated. No variation in CLDN18 expression or positivity was observed across histotypes in eCCAs and GBCs.

Of the 13 identified pre-invasive lesions, only one BilIN lacked CLDN18 expression, while all others expressed CLDN18, encompassing BilIN, ICPN and IPNB. Additionally, all pre-invasive lesions identified were associated with CLDN18-expressing tumors, meaning CLDN18 expression was observed in both the pre-invasive lesion and the corresponding adenocarcinoma in all but one case.

Figure 1 shows representative cases of CLDN18-positive BTC samples. Figure 2 shows distinguishing features of CLDN18 expression in BTCs.

CLDN18 positive expression heterogeneity

Among the 13 CLDN18-positive BTCs, most cases (8/13; 61.5%) exhibited a heterogeneous expression pattern, while the remaining (5/13; 38.5%) displayed a homogeneous expression pattern. Intratumoral heterogeneity of CLDN18 expression was observed exclusively in eCCAs ($n = 5$) and GBCs ($n = 3$). All three heterogeneity patterns were identified: superficial in two cases, invasive-front in two cases and random in three cases. In one case, the pattern could not be determined due to the limitation of the biopsy specimen.

DISCUSSION

CLDN18.2 overexpression has been demonstrated an attractive target alteration with the recent approval of the anti-CLDN18.2 zolbetuximab for advanced gastric cancer.[10] This study investigates CLDN18.2 as a potential druggable biomarker in BTCs, employing the same antibody used in the pivotal SPOTLIGHT trial. Key aspects evaluated include

Table 3. Prevalence of CLDN18 expression and positivity in biliary tract cancers according to histopathologic and molecular features

		CLD18 expression		*P* value	CLD18 positivity		*P* value
		Yes	No		Yes	No	
Grading	G1	13 (61.9%)	8 (38.1%)	0.0009	2 (9.5%)	19 (90.5%)	0.218
	G2	35 (31.0%)	78 (69.0%)		8 (7.1%)	105 (92.9%)	
	G3	22 (21.4%)	81 (78.6%)		3 (2.9%)	100 (97.1%)	
iCCA ($n = 147$)							
Histotype	Small duct	9 (7.7%)	108 (92.3%)	0.0002	1 (0.9%)	116 (9.1%)	0.106
	Large duct	10 (33.3%)	20 (66.6%)		2 (6.7%)	28 (93.3%)	
IDH1 mutations	Present	1 (3.6%)	28 (96.4%)	0.1235	0 (0%)	29 (100%)	1
	Absent	18 (18%)	100 (84.7%)		3 (2.5%)	115 (97.5%)	
FGFR2 fusions	Present	1 (6.3%)	15 (93.7%)	0.6948	1 (6.3%)	15 (93.7%)	0.2072
	Absent	18 (13.7%)	113 (86.3%)		2 (1.5%)	129 (98.5%)	
eCCA/GBC ($n = 90$)							
Histotype	Pancreato-biliary	43 (57.3%)	32 (42.7%)	0.7753	8 (10.7%)	67 (89.3%)	0.6707
	Other	8 (53.3%)	7 (46.7%)		2 (13.3%)	13 (86.7%)	

CLDN18, claudin18; eCCA, extrahepatic cholangiocarcinoma; GBC, gallbladder carcinoma; iCCA, intrahepatic cholangiocarcinoma.

expression levels, positivity rates, intratumoral heterogeneity and its presence in pre-invasive lesions.

In the present study, we found that almost one-third of BTCs express CLDN18 (29.5%) and a notable subset exhibit CLDN18 positivity (5.5%), as defined by the SPOTLIGHT and GLOW trials[10,11] (i.e. moderate-to-strong membranous staining in ≥75% of tumor cells). Rates of CLDN18 expression and positivity, however, were not equally distributed among BTC subtypes and are significantly higher in GBCs and eCCAs. This is of great therapeutic interest, because GBCs and eCCAs currently have fewer therapeutic options than iCCAs. In fact, *IDH1* mutations and *FGFR2* fusions are present in ∼10%-20% and 10%-15% of iCCA patients; but their prevalence is much lower in eCCAs and GBC,[7] thus excluding important target therapies like anti-IDH1 ivosidenib, pemigatinib and futibatinib, both targeting *FGFR2* fusions, from the treatment of the majority of eCCAs and GBC patients. Undoubtedly, this subgroup of BTC patients is now treated in first line with immunotherapy plus doublet chemotherapy (durvalumab or pembrolizumab plus cisplatin and gemcitabine), but the benefit is marginal.[21,22] Therefore, to identify a not negligible, molecular defined subgroup of CLDN18-positive patients (in our study 15.6% of GBC and 8.6% in eCCAs) to investigate target-directed therapies like zolbetuximab (but CLDN18.2-directed antibody drug conjugate and CAR-T are under development) may be of pivotal importance.

Differences in CLDN18 expression were also evident within the iCCA subgroup, with CLDN18 positivity more frequently observed in the large duct-type than in the small duct-type. This can be attributed to the fact that large duct-type iCCA originates in larger intrahepatic bile ducts, which are functionally closer to the perihilar region where eCCA arises.[23-25] As a result, large duct-type iCCA shares notable similarities with eCCA in morphology, molecular profile and prognosis.[23-25]

In our study, we found no overlap between CLDN18 positivity and *IDH1* mutations, with only minimal overlap with *FGFR2* fusions in iCCAs. If a similar pattern is observed in eCCAs and GBCs, for which we had no available data, it would be clinically significant, as CLDN18.2 inhibition could offer new treatment options for a specific subset of patients, without replacing existing targeted therapies for those who may not respond to them.

Clinically, this is significant because, if CLDN18.2 inhibition proves effective in BTCs and this pattern is also observed in eCCAs and GBCs, it could offer new treatment options for a specific patient subset, without replacing existing targeted therapies for those who may not respond to them.

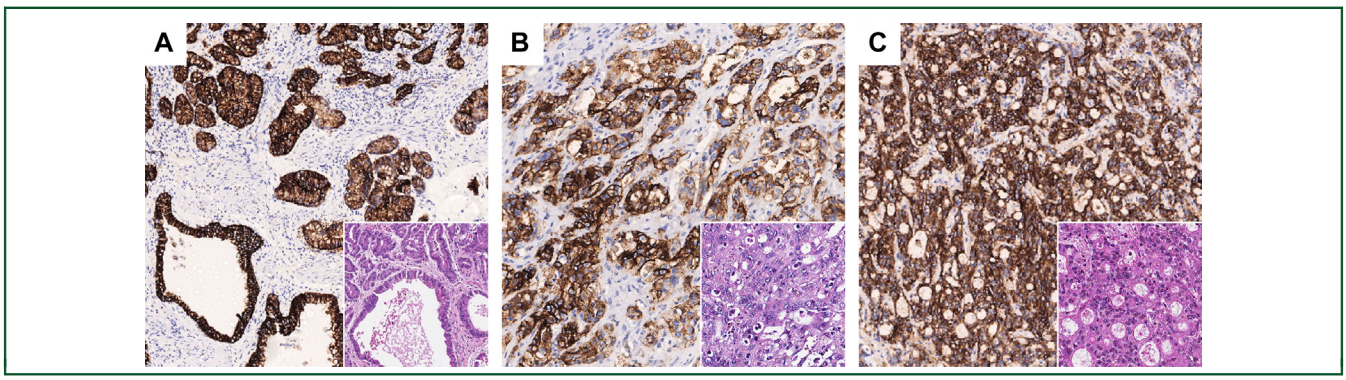

Figure 1. Representative examples of CLDN18-positive (A) gallbladder carcinoma, (B) extrahepatic cholangiocarcinoma, and (C) intrahepatic cholangiocarcinoma, small duct type.
Original magnifications ×20 and ×10. CLDN18, claudin18.

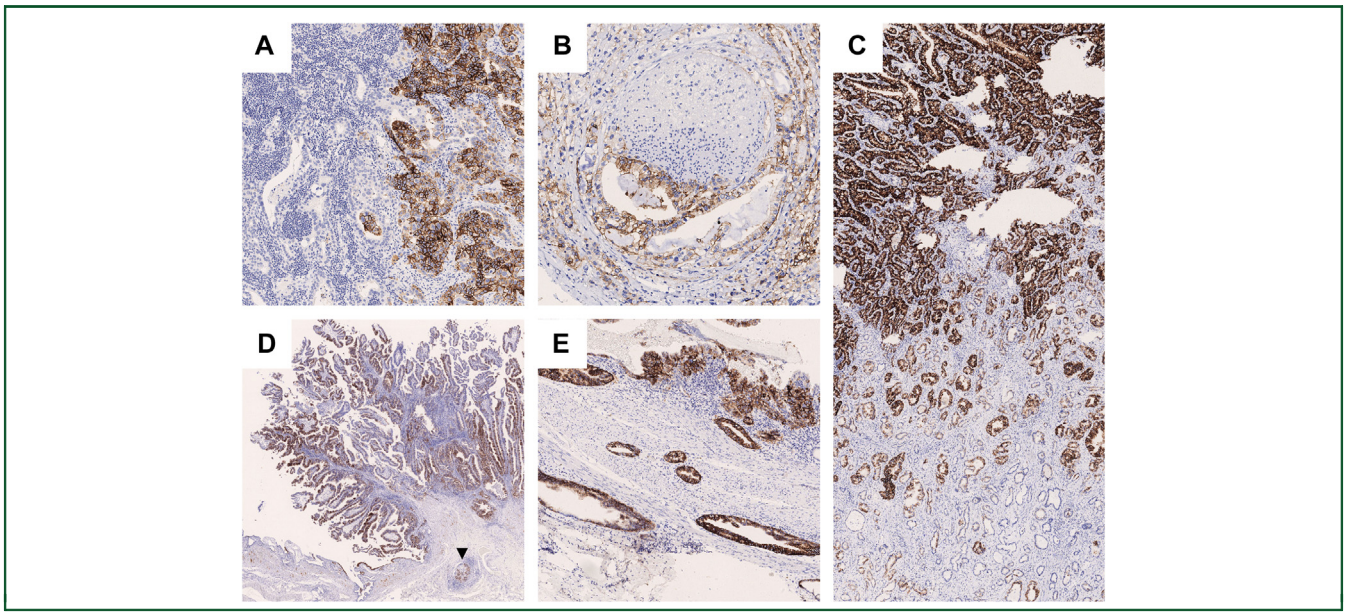

Figure 2. (A) Extrahepatic cholangiocarcinoma displaying intratumoral heterogeneity for CLDN18 expression (random pattern); (B) extrahepatic cholangiocarcinoma with perineural invasion by CLDN18-positive neoplastic glands; (C) extrahepatic cholangiocarcinoma with superficial positivity and decreasing gradient of CLDN18 expression from the surface (i.e. superficial pattern of intratumoral heterogeneity for CLDN18); (D) intracholecystic papillary neoplasm with invasion (black arrow) expressing CLDN18 in both pre-invasive lesion and adenocarcinoma focus; (E) biliary intraepithelial neoplasia (BilIN) of the gallbladder with associated adenocarcinoma showing CLDN18 positivity in both pre-invasive and invasive components. Original magnifications ×20 and ×10.
CLDN18, claudin18.

To the best of our knowledge, only one recent study by Kinzler et al.[14] evaluated CLDN18 status in a large cohort of CCAs ($n = 160$) using the VENTANA CLDN18 (43-14A) assay, identifying CLDN18 positivity in 13.1% of patients. The findings showed a higher prevalence in eCCAs compared with iCCAs and in large duct iCCAs compared with small duct iCCAs, consistent with our observations. The higher prevalence of CLDN18 in their study, compared with our results, might be due to the use of tissue microarrays, which could impact the reproducibility of the findings. Also, no data were provided in CLDN18 expression in GBC, which was the subgroup with the highest positivity rate in our study. Data on CLDN18 expression in CCA using different antibodies from those in the current study are scarce and inconsistent.[13,26]

The potential introduction of CLDN18 in the diagnostic workup for BTCs poses several challenges.[27] First, obtaining adequate biopsy samples in terms of neoplastic cell content for biomarker testing from the biliary tree is technically challenging, particularly in pCCA and dCCA, often resulting in insufficient neoplastic content. Even when a biopsy yields a sufficient tumor sample, comprehensive genomic profiling, along with MMR and HER2 IHC testing, is required,[7,28] potentially leading to depletion of the sample.

Second, we demonstrated that CLDN18 is a heterogeneous biomarker in the BTC setting as well, with most positive samples exhibiting a heterogeneous staining pattern. Thus, small biopsy samples or biliary brushings (which are often the only available samples for eCCAs) may not accurately represent CLDN18 status. A previous study[29] suggested that at least six tissue fragments should be considered the standard of sampling for proper immunohistochemical profiling in gastroesophageal cancer, but additional research is required to define the optimal sampling standards for CCA.

Last, CLDN18 is frequently expressed by pancreatic ductal adenocarcinoma and, as shown by our results, by pancreatobiliary pre-invasive lesions (i.e. IPNB, ICPN, intraductal papillary mucinous neoplasms of the bile duct, BilIN and pancreatic intraepithelial neoplasm).[13,30] This underscores the importance of having dedicated and experienced pathologists for CLDN18 evaluation, as distinguishing pre-invasive lesions from adenocarcinomas (particularly in biopsy samples) and differentiating CCA from pancreatic adenocarcinoma can be challenging for non-experts.

This study has several limitations, including (i) its retrospective design, (ii) a small sample size for certain subtypes, such as GBC, (iii) the lack of clinical correlations, (iv) the under-representation of biopsy samples, (v) the lack of molecular data on eCCA and GBC and (vi) the use of the VENTANA CLDN18 (43-14A) assay, which detects both CLDN18.1 and CLDN18.2 isoforms. Nevertheless, it was the same antibody used in the SPOTPLGHT Trial, and, more importantly in the setting of gastroesophageal and pancreatic adenocarcinoma, CLDN18 positivity is considered equivalent to CLDN18.2 positivity, as the expression of the isoform CLDN18.1 was shown to be insignificant from gene expression data from The Cancer Genome Atlas.[31] Data on the expression of the specific isoform CLDN18.2 in BTCs, however, are not available yet.

In conclusion, this is the largest study investigating CLDN18 expression among BTCs and the first study to assess CLDN18 expression in GBC. The present study shows that a subset of BTC patients exhibit CLDN18 positivity and that

CLDN18 positivity rates are significantly higher in GBCs and eCCA. Additional prospective studies with larger, more diverse cohorts, and with clinical correlations, are necessary to confirm its role as a therapeutic target and to explore the impact of CLDN18 expression on patient outcomes.

FUNDING

The study was funded by European Union - Next Generation EU, Mission 4 Component 1 CUP: C53D23002200006 to MF and CL.

DISCLOSURE

CL received funds from Merck Sharp & Dohme (MSD) and Medica s.r.l. (consulting, speakers bureau). APDT has reported advisory boards for GlaxoSmithKline (GSK), Boehringer and Novartis Oncology, and grants for traveling from PharmaMar. FB received personal honoraria as invited speaker from Eli-Lilly, MSD, EISAI, Bristol Myers Squibb (BMS), AstraZeneca, Pierre Fabre; participation in advisory board for Servier, AAA Novartis. SL held consulting or advisory role from Amgen, Astellas, AstraZeneca, Bayer, BMS, Daiichi-Sankyo, GSK, Incyte, Lilly, Merck Serono, MSD, Servier, Takeda, Rottapharm, BeiGene; speakers' bureau: Amgen, AstraZeneca, BMS, Incyte, GSK, Lilly, Merck Serono, MSD, Pierre-Fabre, Roche, Servier; research funding (to institution): Amgen, Astellas, AstraZeneca, Bayer, BMS, Daichii Sankyo, Hutchinson, Incyte, Merck Serono, Mirati, MSD, Pfizer, Roche, Servier. MF reported the following: Amgen, Astellas, AstraZeneca, BMS, Diapath, Eli Lilly, GSK, Incyte, IQVIA, Janssen Pharma, MSD, Novartis, Pierre Fabre, Pfizer, Sanofi, Roche.

REFERENCES

1. Valle JW, Kelley RK, Nervi B, Oh D-Y, Zhu AX. Biliary tract cancer. *Lancet*. 2021;397(10272):428-444.
2. Vogel A, Bridgewater J, Edeline J, et al. Biliary tract cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2023;34(2):127-140.
3. Tsilimigras DI, Sahara K, Wu L, et al. Very early recurrence after liver resection for intrahepatic cholangiocarcinoma: considering alternative treatment approaches. *JAMA Surg*. 2020;155(9):823-831.
4. Mavros MN, Economopoulos KP, Alexiou VG, Pawlik TM. Treatment and prognosis for patients with intrahepatic cholangiocarcinoma: systematic review and meta-analysis. *JAMA Surg*. 2014;149(6):565-574.
5. Nakamura H, Arai Y, Totoki Y, et al. Genomic spectra of biliary tract cancer. *Nat Genet*. 2015;47(9):1003-1010.
6. Normanno N, Martinelli E, Melisi D, et al. Role of molecular genetics in the clinical management of cholangiocarcinoma. *ESMO Open*. 2022;7(3):100505.
7. Mosele F, Remon J, Mateo J, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2020;31(11):1491-1505.
8. Schlingmann B, Molina SA, Koval M. Claudins: gatekeepers of lung epithelial function. *Semin Cell Dev Biol*. 2015;42:47-57.
9. Sahin U, Koslowski M, Dhaene K, et al. Claudin-18 splice variant 2 is a pan-cancer target suitable for therapeutic antibody development. *Clin Cancer Res*. 2008;14(23):7624-7634.
10. Shitara K, Lordick F, Bang Y-J, et al. Zolbetuximab plus mFOLFOX6 in patients with CLDN18.2-positive, HER2-negative, untreated, locally advanced unresectable or metastatic gastric or gastro-oesophageal junction adenocarcinoma (SPOTLIGHT): a multicentre, randomised, double-blind, phase 3 trial. *Lancet*. 2023;401(10389):1655-1668.
11. Shah MA, Shitara K, Ajani JA, et al. Zolbetuximab plus CAPOX in CLDN18.2-positive gastric or gastroesophageal junction adenocarcinoma: the randomized, phase 3 GLOW trial. *Nat Med*. 2023;29(8):2133-2141.
12. van Laarhoven HWM, Derks S. Claudin-18.2 targeting by zolbetuximab: results of SPOTLIGHT in perspective. *Lancet*. 2023;401(10389):1630-1631.
13. Shinozaki A, Shibahara J, Noda N, et al. Claudin-18 in biliary neoplasms. Its significance in the classification of intrahepatic cholangiocarcinoma. *Virchows Arch*. 2011;459(1):73-80.
14. Kinzler MN, Gretser S, Schulze F, et al. Expression of claudin-18.2 in cholangiocarcinoma: a comprehensive immunohistochemical analysis from a German tertiary centre. *Histopathology*. 2024;86(4):640-646.
15. Rizzato M, Brignola S, Munari G, et al. Prognostic impact of FGFR2/3 alterations in patients with biliary tract cancers receiving systemic chemotherapy: the BITCOIN study. *Eur J Cancer*. 2022;166:165-175.
16. Nagtegaal I, Arends MJ, Odze D, Lam A. WHO Classification of Tumours Editorial Board, *Digestive System Tumours*. 5th ed.1. Lyon, France: World Health Organization, IARC; 2019.
17. Dottermusch M, Krüger S, Behrens H-M, Halske C, Röcken C. Expression of the potential therapeutic target claudin-18.2 is frequently decreased in gastric cancer: results from a large Caucasian cohort study. *Virchows Arch*. 2019;475(5):563-571.
18. Fassan M, Kuwata T, Matkowskyj KA, Röcken C, Rüschoff J. Claudin-18.2 immunohistochemical evaluation in gastric and gastroesophageal junction adenocarcinomas to direct targeted therapy: a practical approach. *Mod Pathol*. 2024;37(11):100589.
19. Choi E, Shin J, Ryu M-H, Kim H-D, Park YS. Heterogeneity of claudin 18.2 expression in metastatic gastric cancer. *Sci Rep*. 2024;14(1):17648.
20. Kim H-D, Choi E, Shin J, et al. Clinicopathologic features and prognostic value of claudin 18.2 overexpression in patients with resectable gastric cancer. *Sci Rep*. 2023;13(1):20047.
21. Kelley RK, Ueno M, Yoo C, et al. Pembrolizumab in combination with gemcitabine and cisplatin compared with gemcitabine and cisplatin alone for patients with advanced biliary tract cancer (KEYNOTE-966): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2023;401(10391):1853-1865.
22. Oh D-Y, He AR, Bouattour M, et al. Durvalumab or placebo plus gemcitabine and cisplatin in participants with advanced biliary tract cancer (TOPAZ-1): updated overall survival from a randomised phase 3 study. *Lancet Gastroenterol Hepatol*. 2024;9(8):694-704.
23. Banales JM, Marin JJG, Lamarca A, et al. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. *Nat Rev Gastroenterol Hepatol*. 2020;17(9):557-588.
24. Aishima S, Oda Y. Pathogenesis and classification of intrahepatic cholangiocarcinoma: different characters of perihilar large duct type versus peripheral small duct type. *J Hepatobiliary Pancreat Sci*. 2015;22(2):94-100.
25. Kendall T, Verheij J, Gaudio E, et al. Anatomical, histomorphological and molecular classification of cholangiocarcinoma. *Liver Int*. 2019;39(suppl 1):7-18.
26. Li W-T, Jeng Y-M, Yang C-Y. Claudin-18 as a marker for identifying the stomach and pancreatobiliary tract as the primary sites of metastatic adenocarcinoma. *Am J Surg Pathol*. 2020;44(12):1643-1648.
27. Bekaii-Saab TS, Bridgewater J, Normanno N. Practical considerations in screening for genetic alterations in cholangiocarcinoma. *Ann Oncol*. 2021;32(9):1111-1126.
28. Fassan M, Angerilli V, Normanno N, et al. Practical guidelines for molecular testing of cholangiocarcinoma in clinical practice: Italian experts' position paper. *Crit Rev Oncol Hematol*. 2024;194:104224.
29. Pellino A, Brignola S, Riello E, et al. Association of CLDN18 protein expression with clinicopathological features and prognosis in advanced gastric and gastroesophageal junction adenocarcinomas. *J Pers Med*. 2021;11(11):1095.

30. Tanaka M, Shibahara J, Fukushima N, et al. Claudin-18 is an early-stage marker of pancreatic carcinogenesis. *J Histochem Cytochem*. 2011;59(10):942-952.

31. Saito T, Matsuda T, Moran D. P-114 Claudin 18 isoform expression in gastric adenocarcinoma and pancreatic adenocarcinoma. *Ann Oncol*. 2021;32:S138.