*REVIEW*

# Diagnostic and therapeutic biomarkers in pancreaticobiliary malignancy

Domenico Viterbo, Valerie Gausman, Tamas Gonda

Domenico Viterbo, Tamas Gonda, Division of Digestive and Liver Diseases, Columbia University Medical Center, New York, NY 10032, United States

Valerie Gausman, Department of Medicine, Albert Einstein College of Medicine, Bronx, NY 10461, United States

Correspondence to: Tamas Gonda, MD, Assistant Professor of Medicine, Division of Digestive and Liver Diseases, Columbia University Medical Center, 161 Fort Washington Avenue, Suite 862, New York, NY 10032,
United States. tg2214@cumc.columbia.edu
Telephone: +1-212-3051909
Fax: +1-212-3051081

## Abstract

Pancreatic ductal adenocarcinoma (PDAC) and cholangio-carcinoma (CCA) are two malignancies that carry significant morbidity and mortality. The poor prognoses of these cancers are strongly related to lack of effective screening modalities as well as few therapeutic options. In this review, we highlight novel biomarkers that have the potential to be used as diagnostic, prognostic and predictive markers. The focus of this review is biomarkers that can be evaluated on endoscopically-obtained biopsies or brush specimens in the pre-operative setting. We also provide an overview of novel serum based markers in the early diagnosis of both PDAC and CCA. In pancreatic cancer, the emphasis is placed on prognostic and theranostic markers, whereas in CCA the utility of molecular markers in diagnosis and prognosis are highlighted.

Key words: Biological markers; Pancreatic cancer; Cholangiocarcinoma; Diagnostic; Prognostic; Predictive; Brush specimens; Biopsies

Core tip: The poor prognoses of pancreatic ductal adenocarcinoma (PDAC) and cholangiocarcinoma (CCA) are strongly related to lack of effective screening modalities as well as few therapeutic options. Several novel biomarkers have been studied that have shown promise for early diagnosis and targeted therapy of these malignancies. These biomarkers provide a strong background for future clinical studies to screen for PDAC and CCA in the general population as well as to investigate molecularly targeted therapies.

## INTRODUCTION

The focus of this review will serve to summarize diagnostic, prognostic and predictive tumor markers in pancreatic cancer and cholangiocarcinoma (CCA). Despite major advances in the therapies of many solid tumors, survival in pancreatic cancer has not improved[1]. Delayed diagnosis, aggressive biology and marked chemoresistance have all contributed to this disappointing trend. Improving the sensitivity of diagnostic modalities, such as imaging or endoscopic tests and molecular markers, as well as innovation in surgical strategies and novel chemotherapeutic regimens had opened the possibility for significantly changing the status-quo. Although gemcitabine remains the back bone of chemotherapy in this disease, novel regimens have been introduced and some have demonstrated significantly better survival[2,3].

CCA arises from the neoplastic proliferation of cholangiocytes, the epithelial cells in the biliary tree[4]. It is an aggressive malignancy, characterized by early lymph node involvement and distant metastasis, with 5-year survival rates of 5%-10%[5]. The identification of new biomarkers with diagnostic, prognostic or theranostic value is especially important as resection (by surgery or combined with a liver transplant) has shown promising results and novel therapies are emerging[6]. However, the relatively low incidence of CCA, high frequency of co-existing cholestasis or cholangitis, and difficulties with obtaining adequate samples have complicated the search for accurate biomarkers.

## DIAGNOSTIC SERUM MARKERS

### *Pancreatic cancer*

Non-invasive blood-based biomarkers with high diagnostic accuracy would be ideal for the early diagnosis of pancreatic cancer. Current tumor markers [cancer antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA), *etc.*] do not have adequate accuracy. The most commonly used marker, CA 19-9, has been reported to have sensitivity and specificity rates ranging from 60%-90% and 65%-92%. Both tumor size[7], concurrent biliary or pancreatic obstruction and the presence of Lewis antigen has significant impact on CA 19-9 levels, making them even less useful as a diagnostic modality. Therefore novel molecular markers may fill an important void in non-invasive testing for early detection of pancreatic ductal adenocarcinoma (PDAC).

MicroRNAs (miRNAs) are highly stable 18-25 nucleotide single-stranded transcripts that function primarily as negative regulators of gene expression by inhibiting translation of their target messenger RNA. Emerging evidence suggests that initiation and progression of PDAC involves aberrant expression of miRNAs. Nearly 100 miRNAs are differentially expressed in pancreatic cancer. Many of these miRNAs are overexpressed and promote tumorigenesis by targeting tumor suppressor genes[8,9]. miRNAs have recently gained attention as potential diagnostic biomarkers and have been analyzed in human blood, bile, pancreatic juice, pancreatic cysts and stool. Relevant articles pertaining to miRNA and pancreatic cancer are summarized below.

Much of the research effort in this field was initially devoted to the characterization of miRNAs in pancreatic cancer. Bloomston *et al*[10] was one of the first to compare the global miRNA expression pattern of resected pancreatic cancer with healthy pancreatic tissue and chronic pancreatitis. He identified miRNAs miR-21, miR-155, miR-221 and miR-196a as key oncogenic miRNAs that correlated with aggressive tumors. In a similar fashion, miRNAs-221, -376a, -301, miR-93, -196a, -196b, -203, -205, -210, -221, -222 and -224 were found to be overexpressed in pancreatic cancer[11,12]. A supportive study by Sadakari *et al*[13] showed the relative expression levels of microRNA-21 and microRNA-155 in pancreatic juice was significantly higher when compared to chronic pancreatitis. Elevated levels of miR-196a and miR-10b were subsequently discovered in pancreatic intraepithelial neoplasia (*PanIN*) lesions suggesting these molecular compounds may be important for early carcinogenesis[14]. The prognostic significance of miRNA in pancreatic cancer was demonstrated by one study which associated elevated levels of miR-21 and miR-31 and low levels of miR-375 with poor clinical outcomes after surgical resection[15].

Circulating miRNAs in whole blood have been investigated in patients with pancreatic cancer. Whole blood miRNA analysis is an attractive screening test because of its easy clinical application and minimal patient involvement. Table 1 summarizes the largest and most recent studies to analyze the utility of novel serum-based miRNAs in the diagnosis of PDAC.

Given the overall stability of miRNA and the large abundance of hepatobiliary juice in stool, analysis for miRNA biomarker expression in feces offers another noninvasive screening option to evaluate for pancreatic cancer. Fecal miRNA expression profiling by Link *et al*[16] showed that dysregulated miRNAs can be found in stool. They report miRNAs-196a, -216a, -143 and -155 are differentially expressed in patients with PDAC when compared to controls. The purpose of this study was to evaluate the feasibility of stool miRNAs as novel biomarker for PDAC screening[17].

### *CCA*

Acquisition of tumor tissue for histology or biomarker testing can be difficult and requires even more invasive and potentially risky procedures than diagnostic studies for PC. The most frequently used serologic markers of CCA are CA19-9 and possibly CEA. CEA has a sensitivity/specificity of 33%-84%/50%-87.8%[18-20]. CA 19-9 not only has a wide variation of sensitivity/specificity: 38%-93%/67%-98%[18-22], but can also be undetectable in 7% of the general population due to absence of the Lewis antigen[23]. Although CA 19-9 may have a role in the diagnostic algorithm, especially in

**Table 1  Diagnostic serum markers for pancreatic ductal adenocarcinoma and cholangiocarcinoma**

| | Diagnostic markers | Countries | CCA or PDAC patients, *n* | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|
| Pancreatic cancer | miRNA-10b, -30c, -106b, -155, and -212[120] | United States | 77 | 73-100 | 83-100 |
| | Index 1: (miR-145, miR-150, miR-223, miR-636) | Denmark | 409 | Index 1: 77 | Index 1: 66 |
| | Index 2: (miR-26b, miR-34a, miR-122, miR-126, miR-145, miR-150, miR-223, miR-505, miR-636, miR-885.5p)[121] | | | Index 2: 80 | Index 2: 82 |
| | miR-21, miR-210, miR-155, and miR-196[122] | United States | 49 | 64 | 89 |
| CCA | CYFRA 21-1[18,24,30] | United Kingdom, Italy | 30 | 17-76 | 79-95 |
| | MMP-7[24,123,124] | Thailand, Italy | 120 | 53-78 | 72.5-92 |
| | Combo (CEA, CA 19-9, MMP-7, CYFRA 21-1)[24] | Italy | 24 | 92 | 96 |
| | Combo (CYFRA 21-1, CA 19-9)[30] | United Kingdom | 6 | 45 | 96 |
| | IL-6[35,125-127] | United States, Thailand | 207 | 71.1-100 | 90-92 |
| | SSP411[37] | China | 35 | 90 | 83.3 |
| | miR-21[128] | United States | 23 | 95 | 100 |
| | miR-150[129] | China | 15 | 80.6 | 58.1 |
| | [1]miR-192[130] | Japan | 51 | 74 | 72 |
| | MUC5AC[131,132] | Thailand | 348 | 62.6-71 | 90-96.9 |
| | Combo (AFP, CEA, CA 19-9, CA 125)[133] | China | 30 | 90 | 90 |

All markers identified with ELISA, except for [1]Western blot. All cases of cholangiocarcinoma are histologically-proven. Control patients for CCA include those with benign liver diseases, HCC and healthy controls. Combo: Combination; PDAC: Pancreatic ductal adenocarcinoma; CCA: Cholangiocarcinoma; CYFRA 21-1: Cytokeratin 19 fragment; MMP-7: Matrix metalloproteinase-7; CEA: Carcinoembryonic antigen; CA 19-9: Cancer antigen 19-9; IL-6: Interleukin-6; SSP411: Sperm-specific protein 411; MUC5AC: Mucin 5AC; AFP: Alpha-fetoprotein; ELISA: Enzyme-linked immunosorbent assay; HCC: Hepatocellular carcinoma.

patients with primary sclerosing cholangitis (PSC) in the absence of concurrent cholangitis or pancreatitis, the low accuracy of the test limits its role in screening and early diagnosis. Thus, novel biomarkers with potential diagnostic utility have been studied (Table 1).

In malignant epithelial cells, activated proteases release cytokeratin-19 fragments (CYFRA 21-1) into the bloodstream[24]. CYFRA 21-1 levels have previously been shown to be a sensitive biomarker in non-small-cell lung cancer[25], gastric cancer[26], breast cancer[27], bladder cancer[28] and cervical carcinoma[29]. Several studies have shown elevated CYFRA 21-1 expression in CCA, but sensitivity varied depending on the cut-off value[18,24,30]. High matrix metalloproteinase-7 (MMP-7) expression has been found to be associated with cancer invasion in esophageal[31], colon[32] and pancreatic[33] cancers. The elevation of CYFRA 21-1 and MMP-7 in various malignancies can preclude their use as CCA-specific diagnostic biomarkers. Thus, combinations of serum markers can be used to improve sensitivity without compromising specificity. Using CYFRA 21-1 and MMP-7 in a multi-marker panel along with CEA and CA 19-9 demonstrated the highest diagnostic accuracy of 93.9%[24].

Interleukin-6 (IL-6) has been shown to be a growth factor for bile duct epithelium[34] and has demonstrated sensitivity as high as 100% in diagnosing CCA[35]. However, IL-6 is also elevated in many patients with hepatocellular carcinoma, benign biliary disease, and metastatic lesions, limiting its specificity[36]. This reinforces the need for more serum-based CCA-specific proteins that are not normally expressed in healthy liver tissue nor elevated in other malignancies. Sperm-specific protein 411 (SSP411) is one such protein which is elevated in the bile of CCA patients and recently found to successfully distinguish CCA from choledocholithiasis as a single serum-based biomarker[37].

miRNAs are usually stable in the circulation when bound to proteins. When miRNAs are dysregulated in cancers, they enter the circulation in free form and can be detected as potential diagnostic markers[38]. The utility of miRNAs lies in their tissue-specific patterns of expression. miRNAs commonly upregulated in other epithelial cancers (miR-192, 194 and 215 in colon, liver, pancreas and stomach cancer[39]) are not altered in CCA, while CCA-specific miRNA expression profiles exist (miR-125a, -31, and -95 are downregulated, while multiple miRNAs are upregulated as compared to nonmalignant cholangiocytes)[40,41]. The role of miRNAs in tumor invasion in CCA is supported by similar miRNA profiles between tumor tissue and adjacent non-tumor tissue as compared to normal tissue[42,43]. The most commonly overexpressed miRNA in CCA is miR-21[44-46]. However, it is also up-regulated in a variety of other cancers (gastric[47], breast[48] and colon[49]), suggesting that the most effective use of miRNAs is likely as multi-marker panels specific for CCA. MicroRNA biomarker discovery has extended from serum and plasma samples to the utilization of bile vesicles, which have demonstrated high accuracy in PSC patients[50].

The presence of circulating tumor cells (CTCs) in other solid cancers (including breast[51], prostate[52], colon[53] and pancreatic[54]) is associated with more aggressive disease and increased metastasis. Similarly,

CTCs in CCA were found to be prognostic of poor overall survival[55,56]. Using a cut-off of 2 CTCs/7.5 mL of peripheral blood, the sensitivity of CTCs for CCA diagnosis is only 17%-23%[55,56]. Despite their poor diagnostic utility, CTCs are potentially useful in detection and monitoring treatment of metastatic spread in real time.

## DIAGNOSTIC BRUSH OR BIOPSY-BASED MARKERS

### Pancreatic cancer
The diagnostic approach to pancreatic masses is dominated by endoscopic ultrasound-fine needle aspiration (EUS-FNA) and histologic or cytologic analysis. EUS-FNA is highly sensitive and specific for solid pancreatic lesions, with sensitivities as high as 85%-95% and specificities of 90%-95%[57].

The two areas where reliance on cytology is not supported by sufficient diagnostic accuracy are cystic neoplasms and inflammatory masses that may mask an underlying neoplasm. EUS-FNA is critical for the evaluation of pancreatic cystic lesions. It is beyond the scope and focus of this review to provide a summary of the data available on the accuracy of cyst fluid based cancer markers and molecular markers. Overall, these markers generally perform well in distinguishing mucinous type lesions from non-mucinous lesions but have thus far shown limited accuracy in identifying high-risk lesions (high grade dysplasia or carcinoma) from lower risk lesions[58-60]. Molecular analysis for DNA disruptions, Kras mutation and miRNAs has enhanced the diagnostic capability of EUS-FNA analysis of pancreatic cysts[61-64]. Similarly, molecular markers are promising in the relatively infrequent setting when a pancreatic mass is noted concurrent with inflammation (either with autoimmune pancreatitis or in the setting of chronic pancreatitis). For example, the presence of *Kras* mutations in FNA specimens has been shown to be a highly sensitive marker[65].

### CCA
Despite advances in sampling techniques and visualization of the bile duct, obtaining representative tissue from the bile duct remains difficult. A single biliary stricture that occurs without associated suspicion of PSC has a different risk of being malignant than biliary strictures, even dominant strictures, identified in a patient with known PSC. Therefore, we consider the diagnostic tests used in these conditions separately.

**PSC associated strictures:** PSC is a chronic liver disease characterized by cholestasis, inflammation, multifocal biliary strictures and a 7%-12% lifetime risk of CCA[66,67]. A minority of CCA patients are surgical candidates and resection carries a 5-year survival rate of only 18%-32.5%[68-71]. The specialized protocol for PSC-associated CCA developed at the Mayo Clinic

(neoadjuvant radio- and chemo-therapy with liver transplantation), has the highest 5-year survival rate of 79%[72]. Inclusion requires early-stage disease, thus excluding the majority of patients diagnosed by standard methods. Because the clinical presentation of CCA can mimic benign dominant biliary strictures, the major challenge lies in identifying potential biomarkers that detect early dysplasia and CCA (Table 2).

Conventional cytology has a low sensitivity due to inadequate cellular yield, but a near 100% specificity. Fluorescent *in situ* hybridization (FISH) trisomy/tetrasomy-positive results have a limited role in the detection of CCA in PSC because they were found to have a similar outcome to FISH negative patients[73]. However, polysomy has been shown to increase the sensitivity of routine cytology. There may be some reduction in specificity with FISH as PSC patients may have benign strictures that manifest with chromosomal abnormalities. The importance of sampling the biliary tree at multiple locations, regardless of the location of the dominant stricture, was demonstrated in a recent study that found that multifocal polysomy carried a greater risk of CCA diagnosis than polysomy detected at a single location[74]. Therefore, FISH should be part of the evaluation of PSC patients presenting with dominant strictures. In one retrospective study of PSC patients with polysomy on initial FISH testing but no signs of CCA, polysomy detected on repeat FISH was associated with increased incidence of CCA compared to patients with non-serial polysomy (polysomy present only on initial FISH)[75]. Repeat sampling without ongoing symptoms or signs remains an area of uncertainty but may be the most effective way to survey patients.

Kirsten rat sarcoma viral oncogene homolog (Kras) is a GTPase downstream of the epidermal growth factor receptor (EGFR) receptor that activates proteins involved in cell growth and proliferation. The high specificity of Kras analysis in biliary strictures can be useful, but the low sensitivity precludes it from diagnostic use as a sole biomarker. When used in combination with cytology, sensitivity increased to 100%[76].

**Indeterminate biliary strictures:** In certain series, up to a quarter of patients who undergo surgical resection for suspected CCA-related strictures turn out have benign etiology[77]. The utility of a highly sensitive modality beyond cytology or histology may therefore reduce the number of unnecessary surgeries. Thus far, assessment of polysomy by FISH has shown the greatest accuracy in brush cytology specimens. Some studies have found that the inclusion of the 9p21/p16 deletion in FISH analysis of indeterminate strictures increased the sensitivity of FISH-polysomy for pancreatobiliary tract cancers from 58% to 89% and from 70% to 76%[73,78,79].

## PROGNOSTIC MARKERS
General prognostic markers, not specific to a defined

**Table 2  Tissue-based diagnostic biomarkers for cholangiocarcinoma**

| | Diagnostic marker | Countries | Total patients, *n* | CCA patients, *n* | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|---|
| PSC-associated strictures | Brush Cytology[21,76,134-140] | United States, The Netherlands, Sweden, Norway | 828 | 138 | 7-73 | 89-100 |
| | FISH polysomy[21,73,136,137,140] | United States | 387 | 89 | 22-50 | 88-100 |
| | FISH polysomy or trisomy[21,73,136,140] | United States | 373 | 75 | 60-88 | 57-87 |
| | Kras[141,142] | Norway, United States | 180 | 74 | 29-47 | 96-100 |
| | p53[142] | Norway | 48 | 33 | 31 | 100 |
| | Cytology + CA19-9[135] | United States | 333 | 44 | 87.5 | 97.3 |
| | Cytology + DNA aneuploidy + CA19-9 + CEA[138] | Sweden | 20 | 7 | 88-100 | 80-85 |
| | Cytology + p53 + KRAS[76] | The Netherlands | 23 | 10 | 100 | 79 |
| | FISH + KRAS[141] | United States | 14 | 14 | 50-68 | 96 |
| Indeterminate strictures | Brush Cytology[75,78,79,136,140,143-150] | United States, Germany, France, Italy | 640 | 199 | 5.8-80 | 92-100 |
| | FISH Polysomy[75,78,79,136,137,140] | United States, Italy | 386 | 165 | 31-80 | 97-100.0 |
| | FISH Polysomy or trisomy[75,136,140] | United States | 147 | 88 | 49-64 | 79.6-100 |
| | Biopsy[144,145,147,150-153] | United States, Japan, France | 347 | 65 | 30-88 | 97-100 |
| | FNA[145,154] | United States | 133 | 30 | 25-61.6 | 100.0 |
| | Cytology + biopsy[144,150] | France, Austria | 258 | 28 | 63-86 | 97-100 |
| | Cytology + FNA + biopsy[145] | United States | 133 | 30 | 47-52 | 100 |
| | Cytology + KRAS[155] | Belgium | 142 | 12 | 55 | 100 |

Some studies included all biliary tract cancers (cholangiocarcinoma, gallbladder, pancreatic and ampullary), but sensitivity and specificity values were similar, so data is merged. Endobiliary sampling technique for cytology and FISH: ERCP or PTC brushing; Biopsy technique: Standard forceps, mini-forceps or transpapillary biopsy. FISH: Fluorescent *in situ* hybridization; PTC: Percutaneous transhepatic cholangiography; CCA: Cholangiocarcinoma; PSC: Primary sclerosing cholangitis; p53: Tumor protein p53; KRAS: Kirsten rat sarcoma viral oncogene homolog; CEA: Carcinoembryonic antigen; CA 19-9: Cancer antigen 19-9; FNA: Fine-needle aspiration.

therapeutic regimen, can be useful in distinguishing which patients are at higher risk of a poor outcome and should therefore be managed more aggressively. Table 3 summarizes the dysregulation of certain markers in PDAC and CCA and their effect on overall survival and/or rate of metastasis.

### Pancreatic cancer

**Secreted protein acidic and rich in cysteine:** Secreted protein acidic and rich in cysteine (SPARC) is a matricellular glycoprotein with important implications in pancreatic cancer. SPARC undergoes epigenetic silencing in pancreatic adenocarcinoma, but is often strongly expressed at the interface between the tumor and stroma by stromal fibroblasts[80]. Supporting data suggest this interaction is important for tumor progression, metastasis and protects against chemotherapeutic agents. Stromal SPARC expression is observed in all disease stages suggesting early expression is critical for tumor progression[81].

Numerous studies have identified stromal SPARC as a negative prognostic marker in pancreatic cancer[81]. Strong stromal SPARC expression in patients with well to moderately differentiated cancer who underwent surgical resection was associated with decreased overall survival when compared to patients with no SPARC expression[81,82]. Furthermore, patients with diffuse stromal SPARC expression extending beyond the peri-tumoral region had a significantly worse prognosis[83]. Interestingly, many report cytoplasmic SPARC expression

by malignant pancreatic cells to have no prognostication value[81]. Others have revealed both stromal and cytoplasmic SPARC expression is associated with decreased overall survival in patients who were treated with gemcitabine[84]. Similarly, elevated SPARC mRNA expression in pancreatic cancer is also associated with worse patient outcome[85].

**Human equilibrative nucleoside transporter 1:** Human equilibrative nucleoside transporter 1 (hENT1) plays a major role in the internalization of (transportation of) gemcitabine by cancer cells. Among patients who did not receive gemcitabine in one study, hENT1 levels did not have any prognostic or predictive value[86]. Conversely, another study showed high hENT1 expression was a poor prognostic factor for early disease recurrence in the absence of gemcitabine therapy[87].

**miRNAs:** A large supportive study analyzing miRNA levels in PDAC revealed high expression of miR-21 and miR-31 with low expression of miR-375 were associated with poor overall survival following surgical resection[15].

### CCA

**miRNAs:** Recent studies have been successful in establishing miRNA signatures that can discriminate between CCA and normal tissue as well as provide prognostic clues[41,88]. As various miRNA expression patterns correlate with overall survival and rate of metastasis, the identification of accurate and predictive

**Table 3 Prognostic markers in pancreatic ductal adenocarcinoma and cholangiocarcinoma**

| | Marker | Country | Total patients, *n* | Marker positive PDAC | Type of dysregulation | Prognostic value, OS months | HR or *P* value for OS |
|---|---|---|---|---|---|---|---|
| Pancreatic cancer | SPARC | United States[81] | 299 | 200 | Up-regulated | +SPARC: 15 -SPARC: 30 | 1.89 |
| | | Germany[83] | 58 | 58 | Up-regulated | +SPARC: 7.6 -SPARC: 10.2 | 2.23 |
| | | Germany[84] | 160 | 95 | Up-regulated | +SPARC: 17.9 -SPARC: 30.2 | *P* = 0.006 |
| | | Japan[85] | 104 | 104 | Up-regulated | Decreased survival | 2.92 |
| | | Sweden[82] | 88 | 68 | Up-regulated | +SPARC: 11.5 -SPARC: 25.3 | 2.12 |
| | Marker | Country | Total Patients, *n* | CCA patients, *n* | Type of dysregulation | Prognostic value | HR (95%CI) or *P* value for OS |
| CCA | [1]miR-192[130] | Japan | 83 | 51 | Up-regulated | Increased LN mets; shorter survival | 2.076 (1.004-4.291) *P* < 0.05 mets |
| | miR-675-5p[88] | China | 72 | 63 | Up-regulated | Shorter survival | 2.562 (1.295-4.929) |
| | miR-652-3p, miR-338-3p[88] | China | 72 | 63 | Down-regulated | Increased survival | 0.477 (0.247-0.922); 0.498 (0.257-0.966) |
| | miR-151-3p and miR-126[156] | United States | 32 | 32 | Up-regulated and down-regulated, respectively | Increased survival | 0.201 (0.043-0.928) |
| | [1]miR-21[46] | Thailand, China | 41 | 32 | Up-regulated | Increased LN mets; shorter survival | *P* < 0.05 OS *P* = 0.037 mets |
| | miR-214[157] | China | 14 | 14 | Down-regulated | Increased mets | *P* < 0.05 mets |
| | miR-373[90] | China | 48 | 48 | Down-regulated | Shorter survival | *P* < 0.05 OS |
| | Group 1: miR-21, miR-31, miR-223 Group 2: miR-122, miR-145, miR-146a, miR-200c, miR-221, and miR-222[44] | Greece | 179 | 21 | Group 1: Up-regulated Group 2: Down-regulated | None | - |
| | CYFRA 21-1[18,30] | United Kingdom, Japan | 195 | 137 | Up-regulated | Shorter survival | *P* = 0.001[30] *P* < 0.01[18] |
| | EGFR[93,94] | Japan | 373 | 338 | Up-regulated | Shorter survival | 5.655 (2.72–11.74)[93] 2.67 (1.52-4.69)[94] |

[1]Liver fluke-associated CCA. PDAC: Pancreatic ductal adenocarcinoma; CCA: Cholangiocarcinoma; mets: Metastases; OS: Overall survival; SPARC: Secreted protein acidic and rich in cysteine; CYFRA 21-1: Cytokeratin 19 fragment; EGFR: Epidermal growth factor receptor; LN: Lymph node.

multi-marker panels can identify patients in need of more aggressive management earlier (Table 3). However, the majority of these studies analyzed histologic samples from tumor resections, and therefore their utility from samples obtained at time of ERCP has not yet been demonstrated[44,88-92].

**EGFR and CYFRA 21-1:** Over-expression of EGFR[93,94] and CYFRA 21-1 values above 2.7-3 ng/mL[18,30] were each prognostic of decreased overall survival.

## THERANOSTIC MARKERS

The goal of theranostic markers is to predict response to a specific therapy. In many other cancers, the role of targeted therapy has changed the approach to treatment. Various genetic mutations have been identified in PDAC and CCA (Table 4) that can be used to guide a personalized approach to therapy.

### Pancreatic cancer

**SPARC**: One the most interesting clinical features of SPARC is its potential role as a predictive marker for response to therapy with nab-paclitaxel. Von hoff *et al*[2] identified stromal SPARC to be an important therapeutic marker in patients treated with combination nab-paclitaxel and gemcitabine chemotherapy. Specifically, patients with high SPARC expression treated with combination therapy had increased overall survival when compared to combination therapy in patients with low SPARC or absence of SPARC. This finding is thought to be due to nab-paclitaxel targeting stromal SPARC and is thought to facilitate delivery of gemcitabine by depleting tumor stroma. Contradictory results by Sinn *et al*[84] revealed high stromal SPARC expression in patients with pancreatic cancer treated solely with gemcitabine resulted in decreased overall survival. Such studies suggest the theranostic impact of SPARC is restricted to patients who receive therapy with nab-paclitaxel.

**hENT1:** A great deal of enthusiasm surrounds hENT1 because of its potential to remodel chemotherapy regimens in pancreatic cancer. There is overwhelming data to support its use as a first line test in pancreatic cancer. hENT1 plays a major role in the internalization

**Table 4 Theranostic markers in pancreatic ductal adenocarcinoma and cholangiocarcinoma**

| | Marker (drug) | Countries | Patients with + marker | Staining | Median survival (mo) | HR |
|---|---|---|---|---|---|---|
| Pancreatic cancer | *SPARC*[2] (nab-paclitaxel/ gemcitabine) | United States | 67 | 36 | +SPARC: 17.8 -SPARC: 8.1 | *P* = 0.0431 |
| | *hENT1* (Gemncitabine) | Canada[96] | 21 | Low hENT1: 12 High hENT1: 9 | Low: 4 High: 13 | 1 |
| | | Italy[158] | 83 | Low hENT1: 27 Inter: 28 High hENT1: 26 | Low: 8.5 Inter:15.7 High: 25.7 | 4.21 |
| | | United States[97] | 91 | Low hENT1: 39 High hENT1: 34 | 2 | 0.51 |
| | | Belgium[98] | 45 | Low hENT1: 26 High hENT1: 19 | Low: 13.3 High: 18.7 | 4.31 (HR for death) *P* = 0.0001 (OS) |
| | | Japan[159] | 40 | Low hENT1: 26 High hENT1: 14 | Low: 8 High:25 | *P* = 0.011 (OS) |
| | | Japan[160] | 55 | Low hENT1: 16 High hENT1: 39 | Low: 11.8 High: 24.9 | 3.15 (OS) |
| | | Belgium France[86] | 243 | Low hENT1: 142 High hENT1: 92 | 2 | 0.34 |
| | | Worldwide multicenter[99] | 177 | Low hENT1: 118 High hENT1: 59 | Low: 6.1 High: 5.2 | 1.147 |
| | | England[161] | 176 | Low hENT1: 77 High hENT1: 99 | Low: 17.1 High: 26.2 | 0.6 |
| | Marker | Countries | CCA patients | % mutated | Type of mutation | Potential theranostic value |
| CCA | EGFR[94,105,109,112] | United States, South Korea, Japan, Italy | 400 | 1-81 | G719S kinase activation | EGFR inhibitors |
| | VEGF[108,162] | South Korea | 272 | 41.7-56.8 | Up-regulation | Anti-VEGF therapies |
| | Kras[109,111,142,163-166] | United States, Germany, China, Norway, Japan | 197 | 7.4-45 | Substitution | U0126 (MEK inhibitor) |
| | BRAF[109,110,164,167] | United States, Germany, China | 222 | 0-22 | Activating missense | BRAF inhibitors |
| | ErbB2 (HER2/neu)[94,112] | South Korea, Italy | 284 | 4-5.1 | Up-regulation | Anti-ErbB2 therapies |
| | IDH1/2[109,114,115,168] | United States | 576 | 10-22.3[1] | Gain of function | α-KG-mimics reverse methylation |
| | miR-21, miR-200b[40]; miR-29b, miR-205, miR-221[117] | United States, Japan | 1 | 1 | Up-regulated | Increased sensitivity to gemcitabine |
| | miR-494[92] | United States | 43 | 1 | Down-regulated | Up-regulation decreases tumor growth |
| | Panel: CDO1, DCLK1, ZSCAN18 and SFRP1[169] | Norway | 39 | 87 | Promoter methylation | Anti-methylation therapy |
| | Panel: CDO1, CNRIP1, SEPT9, and VIM[170] | | 30 | 85 | | |
| | SFRP1[169,171-173] | Norway, United Kingdom, South Korea, Thailand | 255 | 59-83.6 | Promoter methylation | Tumor suppression with gene therapy (RNAi) |

[1]Not reported; [2]Results graphed. OS: Overall survival; PDAC: Pancreatic ductal adenocarcinoma; CCA: Cholangiocarcinoma; Kras: Kirsten rat sarcoma viral oncogene homolog; MEK: Mitogen-activated protein kinase/ERK kinase; EGFR: Epidermal growth factor receptor; ErbB2: Erythroblastosis oncogene B 2; VEGF: Vascular endothelial growth factor; CDO1: Cysteine dioxygenase type 1; DCLK1: Doublecortin-like kinase 1; ZSCAN18: Zinc finger and SCAN domain containing 18; SFRP1: Secreted frizzled-related protein 1; CNRIP1: Cannabinoid receptor interacting protein 1; SEPT9: Septin 9; VIM: Vimentin; IDH1/2: Isocitrate dehydrogenase 1/2; α-KG: Alpha-ketoglutarate.

of gemcitabine by pancreatic cancer cells[95] and is an important prognostic and predictive biomarker for gemcitabine efficacy in patients with pancreatic cancer. Its value as a biomarker is supported by an abundance of clinical studies. Acceptance of its clinical use is limited by a lack of large prospective validation studies. Supportive data is reviewed in this review.

Clinical studies have demonstrated response to gemcitabine parallels hENT1 expression. Namely, patients with tumors that test positive for hENT1 have longer median survival with gemcitabine therapy than those for whom hENT1 was absent. Spratlin *et al*[96] revealed strong hENT1 expression in patients with pancreatic adenocarcinoma was associated with a 3 fold increase

in overall survival after treatment with gemcitabine. Subsequently, a range of studies have reinforced the positive relationship observed with hENT1 expression and gemcitabine efficacy. Interestingly, this positive finding was not observed with other chemotherapy agents[97]. Additionally, several groups have reported a synergistic survival effect with hENT1 and other tumor markers including hCNT3 and deoxycitidine kinase (dCK) in subjects treated with adjuvant gemcitabine after curative resection[98].

Interestingly, Poplin *et al*[99] discovered that hENT1 expression did not predict gemcitabine sensitivity in patients with metastatic pancreatic cancer. This may be due to increased tumor heterogeneity in select patients. Acquired resistance to gemcitabine is bound to happen and may be due to altered gene expression involving important transport proteins including dCK, ribonucleotide reductase M1 (RRM1), RRM2, and hENT1[100]. Additionally, favorable single nucleotide polymorphisms (SNPs) of enzymes involved in the transportation or metabolism of gemcitabine have been identified and may be absent with an unfavorable phenotype[101,102].

Implementing pretreatment analysis for hENT1 expression is feasible, requiring a small tissue sample which can easily be obtained by EUS-FNA. Quantitative mRNA analysis of HENT1 or protein analysis with immunohistochemistry are both useful approaches that are presently limited by a lack of large validation trials.

### CCA

Small-molecule inhibitors have demonstrated a good response rate in lung carcinoma harboring a mutation in the tyrosine kinase domain of the *EGFR* gene[103]. EGFR mutations can be unique to CCA[104] or identical to those in non-small cell lung cancer[105], highlighting the significance of genotyping in guiding therapy. A phase II study of single agent erlotinib in patients with advanced biliary cancer demonstrated disease stabilization in 17%[106]. Upregulation of vascular endothelial growth factor (VEGF) is associated with an EGFR inhibitor-resistant phenotype[107]. Vandetanib, a dual inhibitor of VEGF and EGFR, has shown prolonged time to metastasis in CCA tumors that harbor both mutations[108].

Genes that function downstream of EGFR can also be important therapeutic targets. Kras is one of the most frequently mutated genes in CCA. BRAF mutations are most commonly associated with malignant melanoma, but have also been identified in up to 22% of CCAs[109,110]. Several studies suggest the potential application for targeted therapy with vemurafenib in this population, while avoiding EGFR-inhibitors[109,111]. There are no studies evaluating the response of BRAF-mutated CCA to vemurafenib therapy. However, there is an on-going phase II "basket" study of vemurafenib in non-melanoma solid tumors harboring BRAF mutations that demonstrated stable disease at 8 wk in 4/7 CCA patients, partial response in 2/7 at 24 wk and the remaining 1/7 with disease progression (clinical trial# NCT01524978).

The small minority (4%-5%) of CCA cases that overexpress erythroblastosis oncogene B2 (ErbB2 or HER2)[94,112] may benefit from targeted anti-HER2 therapy. One case study demonstrated a dramatic regression of metastatic CCA in a HER2-positive patient who was started on trastuzumab after failing third-line chemotherapy[113].

A gain-of-function mutation in isocitrate dehydrogenase 1 (IDH1), leading to inhibition of α-ketoglutarate, has been seen in 23% of intrahepatic CCA cases[114], and a minority (0%-7%) of extrahepatic CCA tumors[114-116]. In-vivo studies have suggested that drugs mimicking α-ketoglutarate alone or in combination with inhibitors of mutant IDH1 can reverse the increased histone methylation[116]. Additionally, IDH enzymes are stable therapeutic targets because the mutation appears early in oncogenesis and is maintained throughout progression to high-grade lesions[115].

The increased expression of some miRNAs predicts a favorable response to gemcitabine treatment[40,117]. The potential of miRNAs lies not only in their theranostic utility, but also as therapeutic agents. Treatment of cholangiocytes with miR-494, which is down-regulated in CCA, induced cell-cycle arrest in tumor cells while sparing normal cells[92]. MicroRNA replacement therapy has seen success in phase I clinical trials for ovarian[118] and hepatocellular carcinoma[119] and appears promising as a therapeutic modality in CCA.

Another benefit of these genes and miRNAs as markers is that they can be identified by mutational analysis on DNA or RNA and are commercially available.

## CONCLUSION

Our review focused on PDAC- and CCA-specific biomarkers that may help in the early diagnosis of cancer or guide therapeutic decisions in the case of inoperable malignancy. The general population will benefit from a non-invasive serologic screening test with a high sensitivity, with multi-marker panels appearing advantageous. Despite the more invasive nature of tissue markers, high-risk patients would benefit from their high specificity. Additionally, the utility of predictive biomarkers will soon pave the way for individualized biliary and pancreatic cancer therapeutics.

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