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**Diagnostic and therapeutic biomarkers in pancreaticobiliary malignancy**

Viterbo D *et al.* Biomarkers in pancreaticobiliary malignancy

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**Abstract**
Pancreatic ductal adenocarcinoma (PDAC) and cholangiocarcinoma (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

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**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.

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**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[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 1summarizes 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 noninvansive 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 patients with 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 primary sclerosing cholangitis (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 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 3summarizes 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 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 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.

**REFERENCES**1 **Yadav D**, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 2013; **144**: 1252-1261 [PMID: 23622135 DOI: 10.1053/j.gastro.2013.01.068]

2 **Von Hoff DD**, Ramanathan RK, Borad MJ, Laheru DA, Smith LS, Wood TE, Korn RL, Desai N, Trieu V, Iglesias JL, Zhang H, Soon-Shiong P, Shi T, Rajeshkumar NV, Maitra A, Hidalgo M. Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. *J Clin Oncol* 2011; **29**: 4548-4554 [PMID: 21969517 DOI: 10.1200/JCO.2011.36.5742]

3 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]

4 **Alvaro D**, Cardinale V. Molecular profiling. In: Herman JM, Pawlik TM, Thomas Jr. CR, editors. Biliary Tract and Gallbladder Cancer: A Multidisciplinary Approach. Second ed. Springer, 2014: 99-117

5 **Reddy SB**, Patel T. Current approaches to the diagnosis and treatment of cholangiocarcinoma. *Curr Gastroenterol Rep* 2006; **8**: 30-37 [PMID: 16510032]

6 **Blechacz B**, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology* 2008; **48**: 308-321 [PMID: 18536057 DOI: 10.1002/hep.22310]

7 **Tian F**, Appert HE, Myles J, Howard JM. Prognostic value of serum CA 19-9 levels in pancreatic adenocarcinoma. *Ann Surg* 1992; **215**: 350-355 [PMID: 1348409]

8 **Bhatti I**, Lee A, James V, Hall RI, Lund JN, Tufarelli C, Lobo DN, Larvin M. Knockdown of microRNA-21 inhibits proliferation and increases cell death by targeting programmed cell death 4 (PDCD4) in pancreatic ductal adenocarcinoma. *J Gastrointest Surg* 2011; **15**: 199-208 [PMID: 21088996 DOI: 10.1007/s11605-010-1381-x]

9 **Park JK**, Lee EJ, Esau C, Schmittgen TD. Antisense inhibition of microRNA-21 or -221 arrests cell cycle, induces apoptosis, and sensitizes the effects of gemcitabine in pancreatic adenocarcinoma. *Pancreas* 2009; **38**: e190-e199 [PMID: 19730150 DOI: 10.1097/MPA.0b013e3181ba82e1]

10 **Bloomston M**, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP, Liu CG, Bhatt D, Taccioli C, Croce CM. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA* 2007; **297**: 1901-1908 [PMID: 17473300 DOI: 10.1001/jama.297.17.1901]

11 **Lee EJ**, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, Morgan DL, Postier RG, Brackett DJ, Schmittgen TD. Expression profiling identifies microRNA signature in pancreatic cancer. *Int J Cancer* 2007; **120**: 1046-1054 [PMID: 17149698 DOI: 10.1002/ijc.22394]

12 **Szafranska AE**, Davison TS, John J, Cannon T, Sipos B, Maghnouj A, Labourier E, Hahn SA. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. *Oncogene* 2007; **26**: 4442-4452 [PMID: 17237814 DOI: 10.1038/sj.onc.1210228]

13 **Sadakari Y**, Ohtsuka T, Ohuchida K, Tsutsumi K, Takahata S, Nakamura M, Mizumoto K, Tanaka M. MicroRNA expression analyses in preoperative pancreatic juice samples of pancreatic ductal adenocarcinoma. *JOP* 2010; **11**: 587-592 [PMID: 21068491]

14 **Xue Y**, Abou Tayoun AN, Abo KM, Pipas JM, Gordon SR, Gardner TB, Barth RJ, Suriawinata AA, Tsongalis GJ. MicroRNAs as diagnostic markers for pancreatic ductal adenocarcinoma and its precursor, pancreatic intraepithelial neoplasm. *Cancer Genet* 2013; **206**: 217-221 [PMID: 23933230 DOI: 10.1016/j.cancergen.2013.05.020]

15 **Ma MZ**, Kong X, Weng MZ, Cheng K, Gong W, Quan ZW, Peng CH. Candidate microRNA biomarkers of pancreatic ductal adenocarcinoma: meta-analysis, experimental validation and clinical significance. *J Exp Clin Cancer Res* 2013; **32**: 71 [PMID: 24289824 DOI: 10.1186/1756-9966-32-71]

16 **Link A**, Becker V, Goel A, Wex T, Malfertheiner P. Feasibility of fecal microRNAs as novel biomarkers for pancreatic cancer. *PLoS One* 2012; **7**: e42933 [PMID: 22905187 DOI: 10.1371/journal.pone.0042933]

17 **Yang JY**, Sun YW, Liu DJ, Zhang JF, Li J, Hua R. MicroRNAs in stool samples as potential screening biomarkers for pancreatic ductal adenocarcinoma cancer. *Am J Cancer Res* 2014; **4**: 663-673 [PMID: 25520858]

18 **Uenishi T**, Yamazaki O, Tanaka H, Takemura S, Yamamoto T, Tanaka S, Nishiguchi S, Kubo S. Serum cytokeratin 19 fragment (CYFRA21-1) as a prognostic factor in intrahepatic cholangiocarcinoma. *Ann Surg Oncol* 2008; **15**: 583-589 [PMID: 17955299 DOI: 10.1245/s10434-007-9650-y]

19 **Björnsson E**, Kilander A, Olsson R. CA 19-9 and CEA are unreliable markers for cholangiocarcinoma in patients with primary sclerosing cholangitis. *Liver* 1999; **19**: 501-508 [PMID: 10661684]

20 **Qin XL**, Wang ZR, Shi JS, Lu M, Wang L, He QR. Utility of serum CA19-9 in diagnosis of cholangiocarcinoma: in comparison with CEA. *World J Gastroenterol* 2004; **10**: 427-432 [PMID: 14760772]

21 **Charatcharoenwitthaya P**, Enders FB, Halling KC, Lindor KD. Utility of serum tumor markers, imaging, and biliary cytology for detecting cholangiocarcinoma in primary sclerosing cholangitis. *Hepatology* 2008; **48**: 1106-1117 [PMID: 18785620 DOI: 10.1002/hep.22441]

22 **Patel AH**, Harnois DM, Klee GG, LaRusso NF, Gores GJ. The utility of CA 19-9 in the diagnoses of cholangiocarcinoma in patients without primary sclerosing cholangitis. *Am J Gastroenterol* 2000; **95**: 204-207 [PMID: 10638584 DOI: 10.1111/j.1572-0241.2000.01685.x]

23 **Nehls O**, Gregor M, Klump B. Serum and bile markers for cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 139-154 [PMID: 15192787 DOI: 10.1055/s-2004-828891]

24 **Lumachi F**, Lo Re G, Tozzoli R, D'Aurizio F, Facomer F, Chiara GB, Basso SM. Measurement of serum carcinoembryonic antigen, carbohydrate antigen 19-9, cytokeratin-19 fragment and matrix metalloproteinase-7 for detecting cholangiocarcinoma: a preliminary case-control study. *Anticancer Res* 2014; **34**: 6663-6667 [PMID: 25368272]

25 **Takada M**, Masuda N, Matsuura E, Kusunoki Y, Matui K, Nakagawa K, Yana T, Tuyuguchi I, Oohata I, Fukuoka M. Measurement of cytokeratin 19 fragments as a marker of lung cancer by CYFRA 21-1 enzyme immunoassay. *Br J Cancer* 1995; **71**: 160-165 [PMID: 7529525]

26 **Nakata B**, Chung YS, Kato Y, Ogawa M, Ogawa Y, Inui A, Maeda K, Sawada T, Sowa M. Clinical significance of serum CYFRA 21-1 in gastric cancer. *Br J Cancer* 1996; **73**: 1529-1532 [PMID: 8664124]

27 **Nakata B**, Takashima T, Ogawa Y, Ishikawa T, Hirakawa K. Serum CYFRA 21-1 (cytokeratin-19 fragments) is a useful tumour marker for detecting disease relapse and assessing treatment efficacy in breast cancer. *Br J Cancer* 2004; **91**: 873-878 [PMID: 15280913 DOI: 10.1038/sj.bjc.6602074]

28 **Andreadis C**, Touloupidis S, Galaktidou G, Kortsaris AH, Boutis A, Mouratidou D. Serum CYFRA 21-1 in patients with invasive bladder cancer and its relevance as a tumor marker during chemotherapy. *J Urol* 2005; **174**: 1771-1775; discussion 1771-1775; [PMID: 16217281 DOI: 10.1097/01.ju.0000176742.53556.25]

29 **Yuan CC**, Huang TS, Ng HT, Liu RS, Hung MW, Tsai LC. Elevated cytokeratin-19 expression associated with apoptotic resistance and malignant progression of human cervical carcinoma. *Apoptosis* 1998; **3**: 161-169 [PMID: 14646497]

30 **Chapman MH**, Sandanayake NS, Andreola F, Dhar DK, Webster GJ, Dooley JS, Pereira SP. Circulating CYFRA 21-1 is a Specific Diagnostic and Prognostic Biomarker in Biliary Tract Cancer. *J Clin Exp Hepatol* 2011; **1**: 6-12 [PMID: 22228935 DOI: 10.1016/S0973-6883(11)60110-2]

31 **Adachi Y**, Itoh F, Yamamoto H, Matsuno K, Arimura Y, Kusano M, Endoh T, Hinoda Y, Oohara M, Hosokawa M, Imai K. Matrix metalloproteinase matrilysin (MMP-7) participates in the progression of human gastric and esophageal cancers. *Int J Oncol* 1998; **13**: 1031-1035 [PMID: 9772296]

32 **Adachi Y**, Yamamoto H, Itoh F, Hinoda Y, Okada Y, Imai K. Contribution of matrilysin (MMP-7) to the metastatic pathway of human colorectal cancers. *Gut* 1999; **45**: 252-258 [PMID: 10403738]

33 **Crawford HC**, Scoggins CR, Washington MK, Matrisian LM, Leach SD. Matrix metalloproteinase-7 is expressed by pancreatic cancer precursors and regulates acinar-to-ductal metaplasia in exocrine pancreas. *J Clin Invest* 2002; **109**: 1437-1444 [PMID: 12045257 DOI: 10.1172/JCI15051]

34 **Matsumoto K**, Fujii H, Michalopoulos G, Fung JJ, Demetris AJ. Human biliary epithelial cells secrete and respond to cytokines and hepatocyte growth factors in vitro: interleukin-6, hepatocyte growth factor and epidermal growth factor promote DNA synthesis in vitro. *Hepatology* 1994; **20**: 376-382 [PMID: 8045498]

35 **Goydos JS**, Brumfield AM, Frezza E, Booth A, Lotze MT, Carty SE. Marked elevation of serum interleukin-6 in patients with cholangiocarcinoma: validation of utility as a clinical marker. *Ann Surg* 1998; **227**: 398-404 [PMID: 9527063]

36 **Bonney GK**, Craven RA, Prasad R, Melcher AF, Selby PJ, Banks RE. Circulating markers of biliary malignancy: opportunities in proteomics? *Lancet Oncol* 2008; **9**: 149-158 [PMID: 18237849 DOI: 10.1016/S1470-2045(08)70027-5]

37 **Shen J**, Wang W, Wu J, Feng B, Chen W, Wang M, Tang J, Wang F, Cheng F, Pu L, Tang Q, Wang X, Li X. Comparative proteomic profiling of human bile reveals SSP411 as a novel biomarker of cholangiocarcinoma. *PLoS One* 2012; **7**: e47476 [PMID: 23118872 DOI: 10.1371/journal.pone.0047476]

38 **Mitchell PS**, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008; **105**: 10513-10518 [PMID: 18663219 DOI: 10.1073/pnas.0804549105]

39 **Lu J**, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature* 2005; **435**: 834-838 [PMID: 15944708 DOI: 10.1038/nature03702]

40 **Meng F**, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, Jiang J, Schmittgen TD, Patel T. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology* 2006; **130**: 2113-2129 [PMID: 16762633 DOI: 10.1053/j.gastro.2006.02.057]

41 **Chen L**, Yan HX, Yang W, Hu L, Yu LX, Liu Q, Li L, Huang DD, Ding J, Shen F, Zhou WP, Wu MC, Wang HY. The role of microRNA expression pattern in human intrahepatic cholangiocarcinoma. *J Hepatol* 2009; **50**: 358-369 [PMID: 19070389 DOI: 10.1016/j.jhep.2008.09.015]

42 **Plieskatt JL**, Rinaldi G, Feng Y, Peng J, Yonglitthipagon P, Easley S, Laha T, Pairojkul C, Bhudhisawasdi V, Sripa B, Brindley PJ, Mulvenna JP, Bethony JM. Distinct miRNA signatures associate with subtypes of cholangiocarcinoma from infection with the tumourigenic liver fluke Opisthorchis viverrini. *J Hepatol* 2014; **61**: 850-858 [PMID: 25017828 DOI: 10.1016/j.jhep.2014.05.035]

43 **Plieskatt J**, Rinaldi G, Feng Y, Peng J, Easley S, Jia X, Potriquet J, Pairojkul C, Bhudhisawasdi V, Sripa B, Brindley PJ, Bethony J, Mulvenna J. A microRNA profile associated with Opisthorchis viverrini-induced cholangiocarcinoma in tissue and plasma. *BMC Cancer* 2015; **15**: 309 [PMID: 25903557 DOI: 10.1186/s12885-015-1270-5]

44 **Karakatsanis A**, Papaconstantinou I, Gazouli M, Lyberopoulou A, Polymeneas G, Voros D. Expression of microRNAs, miR-21, miR-31, miR-122, miR-145, miR-146a, miR-200c, miR-221, miR-222, and miR-223 in patients with hepatocellular carcinoma or intrahepatic cholangiocarcinoma and its prognostic significance. *Mol Carcinog* 2013; **52**: 297-303 [PMID: 22213236 DOI: 10.1002/mc.21864]

45 **Liu CZ**, Liu W, Zheng Y, Su JM, Li JJ, Yu L, He XD, Chen SS. PTEN and PDCD4 are bona fide targets of microRNA-21 in human cholangiocarcinoma. *Chin Med Sci J* 2012; **27**: 65-72 [PMID: 22770403]

46 **Chusorn P**, Namwat N, Loilome W, Techasen A, Pairojkul C, Khuntikeo N, Dechakhamphu A, Talabnin C, Chan-On W, Ong CK, Teh BT, Yongvanit P. Overexpression of microRNA-21 regulating PDCD4 during tumorigenesis of liver fluke-associated cholangiocarcinoma contributes to tumor growth and metastasis. *Tumour Biol* 2013; **34**: 1579-1588 [PMID: 23417858 DOI: 10.1007/s13277-013-0688-0]

47 **Wu J**, Li G, Wang Z, Yao Y, Chen R, Pu X, Wang J. Circulating MicroRNA-21 Is a Potential Diagnostic Biomarker in Gastric Cancer. *Dis Markers* 2015; **2015**: 435656 [PMID: 26063956 DOI: 10.1155/2015/435656]

48 **Yan LX**, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, Zeng YX, Shao JY. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. *RNA* 2008; **14**: 2348-2360 [PMID: 18812439 DOI: 10.1261/rna.1034808]

49 **Yamada A**, Horimatsu T, Okugawa Y, Nishida N, Honjo H, Ida H, Kou T, Kusaka T, Sasaki Y, Yagi M, Higurashi T, Yukawa N, Amanuma Y, Kikuchi O, Muto M, Ueno Y, Nakajima A, Chiba T, Boland CR, Goel A. Serum miR-21, miR-29a, and miR-125b Are Promising Biomarkers for the Early Detection of Colorectal Neoplasia. *Clin Cancer Res* 2015; **21**: 4234-4242 [PMID: 26038573 DOI: 10.1158/1078-0432.CCR-14-2793]

50 **Li L**, Masica D, Ishida M, Tomuleasa C, Umegaki S, Kalloo AN, Georgiades C, Singh VK, Khashab M, Amateau S, Li Z, Okolo P, Lennon AM, Saxena P, Geschwind JF, Schlachter T, Hong K, Pawlik TM, Canto M, Law J, Sharaiha R, Weiss CR, Thuluvath P, Goggins M, Shin EJ, Peng H, Kumbhari V, Hutfless S, Zhou L, Mezey E, Meltzer SJ, Karchin R, Selaru FM. Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis. *Hepatology* 2014; **60**: 896-907 [PMID: 24497320 DOI: 10.1002/hep.27050]

51 **Cristofanilli M**, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW, Hayes DF. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 2004; **351**: 781-791 [PMID: 15317891 DOI: 10.1056/NEJMoa040766]

52 **de Bono JS**, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, Doyle GV, Terstappen LW, Pienta KJ, Raghavan D. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin Cancer Res* 2008; **14**: 6302-6309 [PMID: 18829513 DOI: 10.1158/1078-0432.CCR-08-0872]

53 **Cohen SJ**, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse M, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW, Meropol NJ. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 3213-3221 [PMID: 18591556 DOI: 10.1200/JCO.2007.15.8923]

54 **Bidard FC**, Huguet F, Louvet C, Mineur L, Bouché O, Chibaudel B, Artru P, Desseigne F, Bachet JB, Mathiot C, Pierga JY, Hammel P. Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial. *Ann Oncol* 2013; **24**: 2057-2061 [PMID: 23676420 DOI: 10.1093/annonc/mdt176]

55 **Yang JD**, Campion MB, Liu MC, Chaiteerakij R, Giama NH, Ahmed Mohammed H, Zhang X, Hu C, Campion VL, Jen J, Venkatesh SK, Halling KC, Kipp BR, Roberts LR. Circulating tumor cells are associated with poor overall survival in patients with cholangiocarcinoma. *Hepatology* 2015 [PMID: 26096702 DOI: 10.1002/hep.27944]

56 **Al Ustwani O**, Iancu D, Yacoub R, Iyer R. Detection of circulating tumor cells in cancers of biliary origin. *J Gastrointest Oncol* 2012; **3**: 97-104 [PMID: 22811877 DOI: 10.3978/j.issn.2078-6891.2011.047]

57 **Chen J**, Yang R, Lu Y, Xia Y, Zhou H. Diagnostic accuracy of endoscopic ultrasound-guided fine-needle aspiration for solid pancreatic lesion: a systematic review. *J Cancer Res Clin Oncol* 2012; **138**: 1433-1441 [PMID: 22752601 DOI: 10.1007/s00432-012-1268-1]

58 **Okasha HH**, Ashry M, Imam HM, Ezzat R, Naguib M, Farag AH, Gemeie EH, Khattab HM. Role of endoscopic ultrasound-guided fine needle aspiration and ultrasound-guided fine-needle aspiration in diagnosis of cystic pancreatic lesions. *Endosc Ultrasound* 2015; **4**: 132-136 [PMID: 26020048 DOI: 10.4103/2303-9027.156742]

59 **van der Waaij LA**, van Dullemen HM, Porte RJ. Cyst fluid analysis in the differential diagnosis of pancreatic cystic lesions: a pooled analysis. *Gastrointest Endosc* 2005; **62**: 383-389 [PMID: 16111956]

60 **Brugge WR**, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, del Castillo CF, Warshaw AL. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. *Gastroenterology* 2004; **126**: 1330-1336 [PMID: 15131794]

61 **Winner M**, Sethi A, Poneros JM, Stavropoulos SN, Francisco P, Lightdale CJ, Allendorf JD, Stevens PD, Gonda TA. The role of molecular analysis in the diagnosis and surveillance of pancreatic cystic neoplasms. *JOP* 2015; **16**: 143-149 [PMID: 25791547]

62 **Khalid A**, Zahid M, Finkelstein SD, LeBlanc JK, Kaushik N, Ahmad N, Brugge WR, Edmundowicz SA, Hawes RH, McGrath KM. Pancreatic cyst fluid DNA analysis in evaluating pancreatic cysts: a report of the PANDA study. *Gastrointest Endosc* 2009; **69**: 1095-1102 [PMID: 19152896 DOI: 10.1016/j.gie.2008.07.033]

63 **Shen J**, Brugge WR, Dimaio CJ, Pitman MB. Molecular analysis of pancreatic cyst fluid: a comparative analysis with current practice of diagnosis. *Cancer* 2009; **117**: 217-227 [PMID: 19415731 DOI: 10.1002/cncy.20027]

64 **Singhi AD**, Nikiforova MN, Fasanella KE, McGrath KM, Pai RK, Ohori NP, Bartholow TL, Brand RE, Chennat JS, Lu X, Papachristou GI, Slivka A, Zeh HJ, Zureikat AH, Lee KK, Tsung A, Mantha GS, Khalid A. Preoperative GNAS and KRAS testing in the diagnosis of pancreatic mucinous cysts. *Clin Cancer Res* 2014; **20**: 4381-4389 [PMID: 24938521 DOI: 10.1158/1078-0432.CCR-14-0513]

65 **Bournet B**, Souque A, Senesse P, Assenat E, Barthet M, Lesavre N, Aubert A, O'Toole D, Hammel P, Levy P, Ruszniewski P, Bouisson M, Escourrou J, Cordelier P, Buscail L. Endoscopic ultrasound-guided fine-needle aspiration biopsy coupled with KRAS mutation assay to distinguish pancreatic cancer from pseudotumoral chronic pancreatitis. *Endoscopy* 2009; **41**: 552-557 [PMID: 19533561 DOI: 10.1055/s-0029-1214717]

66 **Bergquist A**, Ekbom A, Olsson R, Kornfeldt D, Lööf L, Danielsson A, Hultcrantz R, Lindgren S, Prytz H, Sandberg-Gertzén H, Almer S, Granath F, Broomé U. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. *J Hepatol* 2002; **36**: 321-327 [PMID: 11867174]

67 **Burak K**, Angulo P, Pasha TM, Egan K, Petz J, Lindor KD. Incidence and risk factors for cholangiocarcinoma in primary sclerosing cholangitis. *Am J Gastroenterol* 2004; **99**: 523-526 [PMID: 15056096 DOI: 10.1111/j.1572-0241.2004.04067.x]

68 **Rea DJ**, Munoz-Juarez M, Farnell MB, Donohue JH, Que FG, Crownhart B, Larson D, Nagorney DM. Major hepatic resection for hilar cholangiocarcinoma: analysis of 46 patients. *Arch Surg* 2004; **139**: 514-523; discussion 523-525 [PMID: 15136352 DOI: 10.1001/archsurg.139.5.514]

69 **Jang JY**, Kim SW, Park DJ, Ahn YJ, Yoon YS, Choi MG, Suh KS, Lee KU, Park YH. Actual long-term outcome of extrahepatic bile duct cancer after surgical resection. *Ann Surg* 2005; **241**: 77-84 [PMID: 15621994]

70 **Jarnagin WR**, Fong Y, DeMatteo RP, Gonen M, Burke EC, Bodniewicz BS J, Youssef BA M, Klimstra D, Blumgart LH. Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 2001; **234**: 507-517; discussion 517-519 [PMID: 11573044]

71 **DeOliveira ML**, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, Choti MA, Yeo CJ, Schulick RD. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007; **245**: 755-762 [PMID: 17457168 DOI: 10.1097/01.sla.0000251366.62632.d3]

72 **Rosen CB**, Heimbach JK, Gores GJ. Liver transplantation for cholangiocarcinoma. *Transpl Int* 2010; **23**: 692-697 [PMID: 20497401 DOI: 10.1111/j.1432-2277.2010.01108.x]

73 **Bangarulingam SY**, Bjornsson E, Enders F, Barr Fritcher EG, Gores G, Halling KC, Lindor KD. Long-term outcomes of positive fluorescence in situ hybridization tests in primary sclerosing cholangitis. *Hepatology* 2010; **51**: 174-180 [PMID: 19877179 DOI: 10.1002/hep.23277]

74 **Eaton JE**, Barr Fritcher EG, Gores GJ, Atkinson EJ, Tabibian JH, Topazian MD, Gossard AA, Halling KC, Kipp BR, Lazaridis KN. Biliary multifocal chromosomal polysomy and cholangiocarcinoma in primary sclerosing cholangitis. *Am J Gastroenterol* 2015; **110**: 299-309 [PMID: 25623660 DOI: 10.1038/ajg.2014.433]

75 **Barr Fritcher EG**, Kipp BR, Voss JS, Clayton AC, Lindor KD, Halling KC, Gores GJ. Primary sclerosing cholangitis patients with serial polysomy fluorescence in situ hybridization results are at increased risk of cholangiocarcinoma. *Am J Gastroenterol* 2011; **106**: 2023-2028 [PMID: 21844920 DOI: 10.1038/ajg.2011.272]

76 **Ponsioen CY**, Vrouenraets SM, van Milligen de Wit AW, Sturm P, Tascilar M, Offerhaus GJ, Prins M, Huibregtse K, Tytgat GN. Value of brush cytology for dominant strictures in primary sclerosing cholangitis. *Endoscopy* 1999; **31**: 305-309 [PMID: 10376457 DOI: 10.1055/s-1999-18]

77 **Clayton RA**, Clarke DL, Currie EJ, Madhavan KK, Parks RW, Garden OJ. Incidence of benign pathology in patients undergoing hepatic resection for suspected malignancy. *Surgeon* 2003; **1**: 32-38 [PMID: 15568422]

78 **Gonda TA**, Glick MP, Sethi A, Poneros JM, Palmas W, Iqbal S, Gonzalez S, Nandula SV, Emond JC, Brown RS, Murty VV, Stevens PD. Polysomy and p16 deletion by fluorescence in situ hybridization in the diagnosis of indeterminate biliary strictures. *Gastrointest Endosc* 2012; **75**: 74-79 [PMID: 22100297 DOI: 10.1016/j.gie.2011.08.022]

79 **Boldorini R**, Paganotti A, Andorno S, Orlando S, Mercalli F, Orsello M, Ballarè M, Magnani C, Sartori M. A multistep cytological approach for patients with jaundice and biliary strictures of indeterminate origin. *J Clin Pathol* 2015; **68**: 283-287 [PMID: 25681513 DOI: 10.1136/jclinpath-2014-202731]

80 **Sato N**, Fukushima N, Maehara N, Matsubayashi H, Koopmann J, Su GH, Hruban RH, Goggins M. SPARC/osteonectin is a frequent target for aberrant methylation in pancreatic adenocarcinoma and a mediator of tumor-stromal interactions. *Oncogene* 2003; **22**: 5021-5030 [PMID: 12902985 DOI: 10.1038/sj.onc.1206807]

81 **Infante JR**, Matsubayashi H, Sato N, Tonascia J, Klein AP, Riall TA, Yeo C, Iacobuzio-Donahue C, Goggins M. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. *J Clin Oncol* 2007; **25**: 319-325 [PMID: 17235047 DOI: 10.1200/JCO.2006.07.8824]

82 **Gundewar C**, Sasor A, Hilmersson KS, Andersson R, Ansari D. The role of SPARC expression in pancreatic cancer progression and patient survival. *Scand J Gastroenterol* 2015; **50**: 1170-1174 [PMID: 25765175 DOI: 10.3109/00365521.2015.1024281]

83 **Mantoni TS**, Schendel RR, Rödel F, Niedobitek G, Al-Assar O, Masamune A, Brunner TB. Stromal SPARC expression and patient survival after chemoradiation for non-resectable pancreatic adenocarcinoma. *Cancer Biol Ther* 2008; **7**: 1806-1815 [PMID: 18787407]

84 **Sinn M**, Sinn BV, Striefler JK, Lindner JL, Stieler JM, Lohneis P, Bischoff S, Bläker H, Pelzer U, Bahra M, Dietel M, Dörken B, Oettle H, Riess H, Denkert C. SPARC expression in resected pancreatic cancer patients treated with gemcitabine: results from the CONKO-001 study. *Ann Oncol* 2014; **25**: 1025-1032 [PMID: 24562449 DOI: 10.1093/annonc/mdu084]

85 **Miyoshi K**, Sato N, Ohuchida K, Mizumoto K, Tanaka M. SPARC mRNA expression as a prognostic marker for pancreatic adenocarcinoma patients. *Anticancer Res* 2010; **30**: 867-871 [PMID: 20393008]

86 **Maréchal R**, Bachet JB, Mackey JR, Dalban C, Demetter P, Graham K, Couvelard A, Svrcek M, Bardier-Dupas A, Hammel P, Sauvanet A, Louvet C, Paye F, Rougier P, Penna C, André T, Dumontet C, Cass CE, Jordheim LP, Matera EL, Closset J, Salmon I, Devière J, Emile JF, Van Laethem JL. Levels of gemcitabine transport and metabolism proteins predict survival times of patients treated with gemcitabine for pancreatic adenocarcinoma. *Gastroenterology* 2012; **143**: 664-674.e1-6 [PMID: 22705007 DOI: 10.1053/j.gastro.2012.06.006]

87 **Fisher SB**, Patel SH, Bagci P, Kooby DA, El-Rayes BF, Staley CA, Adsay NV, Maithel SK. An analysis of human equilibrative nucleoside transporter-1, ribonucleoside reductase subunit M1, ribonucleoside reductase subunit M2, and excision repair cross-complementing gene-1 expression in patients with resected pancreas adenocarcinoma: implications for adjuvant treatment. *Cancer* 2013; **119**: 445-453 [PMID: 22569992 DOI: 10.1002/cncr.27619]

88 **Zhang MY**, Li SH, Huang GL, Lin GH, Shuang ZY, Lao XM, Xu L, Lin XJ, Wang HY, Li SP. Identification of a novel microRNA signature associated with intrahepatic cholangiocarcinoma (ICC) patient prognosis. *BMC Cancer* 2015; **15**: 64 [PMID: 25880914 DOI: 10.1186/s12885-015-1067-6]

89 **Namwat N**, Chusorn P, Loilome W, Techasen A, Puetkasichonpasutha J, Pairojkul C, Khuntikeo N, Yongvanit P. Expression profiles of oncomir miR-21 and tumor suppressor let-7a in the progression of opisthorchiasis-associated cholangiocarcinoma. *Asian Pac J Cancer Prev* 2012; **13** Suppl: 65-69 [PMID: 23480766]

90 **Chen YJ**, Luo J, Yang GY, Yang K, Wen SQ, Zou SQ. Mutual regulation between microRNA-373 and methyl-CpG-binding domain protein 2 in hilar cholangiocarcinoma. *World J Gastroenterol* 2012; **18**: 3849-3861 [PMID: 22876037 DOI: 10.3748/wjg.v18.i29.3849]

91 **Zhang J**, Han C, Wu T. MicroRNA-26a promotes cholangiocarcinoma growth by activating β-catenin. *Gastroenterology* 2012; **143**: 246-256.e8 [PMID: 22484120 DOI: 10.1053/j.gastro.2012.03.045]

92 **Olaru AV**, Ghiaur G, Yamanaka S, Luvsanjav D, An F, Popescu I, Alexandrescu S, Allen S, Pawlik TM, Torbenson M, Georgiades C, Roberts LR, Gores GJ, Ferguson-Smith A, Almeida MI, Calin GA, Mezey E, Selaru FM. MicroRNA down-regulated in human cholangiocarcinoma control cell cycle through multiple targets involved in the G1/S checkpoint. *Hepatology* 2011; **54**: 2089-2098 [PMID: 21809359 DOI: 10.1002/hep.24591]

93 **Chang YT**, Chang MC, Huang KW, Tung CC, Hsu C, Wong JM. Clinicopathological and prognostic significances of EGFR, KRAS and BRAF mutations in biliary tract carcinomas in Taiwan. *J Gastroenterol Hepatol* 2014; **29**: 1119-1125 [PMID: 24372748 DOI: 10.1111/jgh.12505]

94 **Yoshikawa D**, Ojima H, Iwasaki M, Hiraoka N, Kosuge T, Kasai S, Hirohashi S, Shibata T. Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma. *Br J Cancer* 2008; **98**: 418-425 [PMID: 18087285 DOI: 10.1038/sj.bjc.6604129]

95 **Mackey JR**, Mani RS, Selner M, Mowles D, Young JD, Belt JA, Crawford CR, Cass CE. Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. *Cancer Res* 1998; **58**: 4349-4357 [PMID: 9766663]

96 **Spratlin J**, Sangha R, Glubrecht D, Dabbagh L, Young JD, Dumontet C, Cass C, Lai R, Mackey JR. The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. *Clin Cancer Res* 2004; **10**: 6956-6961 [PMID: 15501974 DOI: 10.1158/1078-0432.CCR-04-0224]

97 **Farrell JJ**, Elsaleh H, Garcia M, Lai R, Ammar A, Regine WF, Abrams R, Benson AB, Macdonald J, Cass CE, Dicker AP, Mackey JR. Human equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. *Gastroenterology* 2009; **136**: 187-195 [PMID: 18992248 DOI: 10.1053/j.gastro.2008.09.067]

98 **Maréchal R**, Mackey JR, Lai R, Demetter P, Peeters M, Polus M, Cass CE, Young J, Salmon I, Devière J, Van Laethem JL. Human equilibrative nucleoside transporter 1 and human concentrative nucleoside transporter 3 predict survival after adjuvant gemcitabine therapy in resected pancreatic adenocarcinoma. *Clin Cancer Res* 2009; **15**: 2913-2919 [PMID: 19318496 DOI: 10.1158/1078-0432.CCR-08-2080]

99 **Poplin E**, Wasan H, Rolfe L, Raponi M, Ikdahl T, Bondarenko I, Davidenko I, Bondar V, Garin A, Boeck S, Ormanns S, Heinemann V, Bassi C, Evans TR, Andersson R, Hahn H, Picozzi V, Dicker A, Mann E, Voong C, Kaur P, Isaacson J, Allen A. Randomized, multicenter, phase II study of CO-101 versus gemcitabine in patients with metastatic pancreatic ductal adenocarcinoma: including a prospective evaluation of the role of hENT1 in gemcitabine or CO-101 sensitivity. *J Clin Oncol* 2013; **31**: 4453-4461 [PMID: 24220555 DOI: 10.1200/JCO.2013.51.0826]

100 **Nakano Y**, Tanno S, Koizumi K, Nishikawa T, Nakamura K, Minoguchi M, Izawa T, Mizukami Y, Okumura T, Kohgo Y. Gemcitabine chemoresistance and molecular markers associated with gemcitabine transport and metabolism in human pancreatic cancer cells. *Br J Cancer* 2007; **96**: 457-463 [PMID: 17224927 DOI: 10.1038/sj.bjc.6603559]

101 **Tanaka M**, Javle M, Dong X, Eng C, Abbruzzese JL, Li D. Gemcitabine metabolic and transporter gene polymorphisms are associated with drug toxicity and efficacy in patients with locally advanced pancreatic cancer. *Cancer* 2010; **116**: 5325-5335 [PMID: 20665488 DOI: 10.1002/cncr.25282]

102 **Myers SN**, Goyal RK, Roy JD, Fairfull LD, Wilson JW, Ferrell RE. Functional single nucleotide polymorphism haplotypes in the human equilibrative nucleoside transporter 1. *Pharmacogenet Genomics* 2006; **16**: 315-320 [PMID: 16609362 DOI: 10.1097/01.fpc.0000189804.41962.15]

103 **Chou TY**, Chiu CH, Li LH, Hsiao CY, Tzen CY, Chang KT, Chen YM, Perng RP, Tsai SF, Tsai CM. Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with non-small cell lung cancer. *Clin Cancer Res* 2005; **11**: 3750-3757 [PMID: 15897572 DOI: 10.1158/1078-0432.CCR-04-1981]

104 **Leone F**, Cavalloni G, Pignochino Y, Sarotto I, Ferraris R, Piacibello W, Venesio T, Capussotti L, Risio M, Aglietta M. Somatic mutations of epidermal growth factor receptor in bile duct and gallbladder carcinoma. *Clin Cancer Res* 2006; **12**: 1680-1685 [PMID: 16551849 DOI: 10.1158/1078-0432.CCR-05-1692]

105 **Gwak GY**, Yoon JH, Shin CM, Ahn YJ, Chung JK, Kim YA, Kim TY, Lee HS. Detection of response-predicting mutations in the kinase domain of the epidermal growth factor receptor gene in cholangiocarcinomas. *J Cancer Res Clin Oncol* 2005; **131**: 649-652 [PMID: 16032426 DOI: 10.1007/s00432-005-0016-1]

106 **Philip PA**, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, Donehower RC, Fitch T, Picus J, Erlichman C. Phase II study of erlotinib in patients with advanced biliary cancer. *J Clin Oncol* 2006; **24**: 3069-3074 [PMID: 16809731 DOI: 10.1200/JCO.2005.05.3579]

107 **Viloria-Petit A**, Crombet T, Jothy S, Hicklin D, Bohlen P, Schlaeppi JM, Rak J, Kerbel RS. Acquired resistance to the antitumor effect of epidermal growth factor receptor-blocking antibodies in vivo: a role for altered tumor angiogenesis. *Cancer Res* 2001; **61**: 5090-5101 [PMID: 11431346]

108 **Yoshikawa D**, Ojima H, Kokubu A, Ochiya T, Kasai S, Hirohashi S, Shibata T. Vandetanib (ZD6474), an inhibitor of VEGFR and EGFR signalling, as a novel molecular-targeted therapy against cholangiocarcinoma. *Br J Cancer* 2009; **100**: 1257-1266 [PMID: 19319137 DOI: 10.1038/sj.bjc.6604988]

109 **Voss JS**, Holtegaard LM, Kerr SE, Fritcher EG, Roberts LR, Gores GJ, Zhang J, Highsmith WE, Halling KC, Kipp BR. Molecular profiling of cholangiocarcinoma shows potential for targeted therapy treatment decisions. *Hum Pathol* 2013; **44**: 1216-1222 [PMID: 23391413 DOI: 10.1016/j.humpath.2012.11.006]

110 **Tannapfel A**, Sommerer F, Benicke M, Katalinic A, Uhlmann D, Witzigmann H, Hauss J, Wittekind C. Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. *Gut* 2003; **52**: 706-712 [PMID: 12692057]

111 **Robertson S**, Hyder O, Dodson R, Nayar SK, Poling J, Beierl K, Eshleman JR, Lin MT, Pawlik TM, Anders RA. The frequency of KRAS and BRAF mutations in intrahepatic cholangiocarcinomas and their correlation with clinical outcome. *Hum Pathol* 2013; **44**: 2768-2773 [PMID: 24139215 DOI: 10.1016/j.humpath.2013.07.026]

112 **Altimari A**, Fiorentino M, Gabusi E, Gruppioni E, Corti B, D'Errico A, Grigioni WF. Investigation of ErbB1 and ErbB2 expression for therapeutic targeting in primary liver tumours. *Dig Liver Dis* 2003; **35**: 332-338 [PMID: 12846405]

113 **Law LY**. Dramatic response to trastuzumab and paclitaxel in a patient with human epidermal growth factor receptor 2-positive metastatic cholangiocarcinoma. *J Clin Oncol* 2012; **30**: e271-e273 [PMID: 22851567 DOI: 10.1200/JCO.2012.42.3061]

114 **Borger DR**, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, Schenkein DP, Hezel AF, Ancukiewicz M, Liebman HM, Kwak EL, Clark JW, Ryan DP, Deshpande V, Dias-Santagata D, Ellisen LW, Zhu AX, Iafrate AJ. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. *Oncologist* 2012; **17**: 72-79 [PMID: 22180306 DOI: 10.1634/theoncologist.2011-0386]

115 **Kipp BR**, Voss JS, Kerr SE, Barr Fritcher EG, Graham RP, Zhang L, Highsmith WE, Zhang J, Roberts LR, Gores GJ, Halling KC. Isocitrate dehydrogenase 1 and 2 mutations in cholangiocarcinoma. *Hum Pathol* 2012; **43**: 1552-1558 [PMID: 22503487 DOI: 10.1016/j.humpath.2011.12.007]

116 **Xu W**, Yang H, Liu Y, Yang Y, Wang P, Kim SH, Ito S, Yang C, Wang P, Xiao MT, Liu LX, Jiang WQ, Liu J, Zhang JY, Wang B, Frye S, Zhang Y, Xu YH, Lei QY, Guan KL, Zhao SM, Xiong Y. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. *Cancer Cell* 2011; **19**: 17-30 [PMID: 21251613 DOI: 10.1016/j.ccr.2010.12.014]

117 **Okamoto K**, Miyoshi K, Murawaki Y. miR-29b, miR-205 and miR-221 enhance chemosensitivity to gemcitabine in HuH28 human cholangiocarcinoma cells. *PLoS One* 2013; **8**: e77623 [PMID: 24147037 DOI: 10.1371/journal.pone.0077623]

118 **Banno K**, Yanokura M, Iida M, Adachi M, Nakamura K, Nogami Y, Umene K, Masuda K, Kisu I, Nomura H, Kataoka F, Tominaga E, Aoki D. Application of microRNA in diagnosis and treatment of ovarian cancer. *Biomed Res Int* 2014; **2014**: 232817 [PMID: 24822185 DOI: 10.1155/2014/232817]

119 **Nagaraj AB**, Joseph P, DiFeo A. miRNAs as prognostic and therapeutic tools in epithelial ovarian cancer. *Biomark Med* 2015; **9**: 241-257 [PMID: 25731210 DOI: 10.2217/bmm.14.108]

120 **Cote GA**, Gore AJ, McElyea SD, Heathers LE, Xu H, Sherman S, Korc M. A pilot study to develop a diagnostic test for pancreatic ductal adenocarcinoma based on differential expression of select miRNA in plasma and bile. *Am J Gastroenterol* 2014; **109**: 1942-1952 [PMID: 25350767 DOI: 10.1038/ajg.2014.331]

121 **Schultz NA**, Dehlendorff C, Jensen BV, Bjerregaard JK, Nielsen KR, Bojesen SE, Calatayud D, Nielsen SE, Yilmaz M, Holländer NH, Andersen KK, Johansen JS. MicroRNA biomarkers in whole blood for detection of pancreatic cancer. *JAMA* 2014; **311**: 392-404 [PMID: 24449318 DOI: 10.1001/jama.2013.284664]

122 **Wang J**, Chen J, Chang P, LeBlanc A, Li D, Abbruzzesse JL, Frazier ML, Killary AM, Sen S. MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. *Cancer Prev Res* (Phila) 2009; **2**: 807-813 [PMID: 19723895 DOI: 10.1158/1940-6207.CAPR-09-0094]

123 **Leelawat K**, Narong S, Wannaprasert J, Ratanashu-ek T. Prospective study of MMP7 serum levels in the diagnosis of cholangiocarcinoma. *World J Gastroenterol* 2010; **16**: 4697-4703 [PMID: 20872971]

124 **Prakobwong S**, Charoensuk L, Hiraku Y, Pinlaor P, Pairojkul C, Mairiang E, Sithithaworn P, Yongvanit P, Khuntikeo N, Pinlaor S. Plasma hydroxyproline, MMP-7 and collagen I as novel predictive risk markers of hepatobiliary disease-associated cholangiocarcinoma. *Int J Cancer* 2012; **131**: E416-E424 [PMID: 21935919 DOI: 10.1002/ijc.26443]

125 **Cheon YK**, Cho YD, Moon JH, Jang JY, Kim YS, Kim YS, Lee MS, Lee JS, Shim CS. Diagnostic utility of interleukin-6 (IL-6) for primary bile duct cancer and changes in serum IL-6 levels following photodynamic therapy. *Am J Gastroenterol* 2007; **102**: 2164-2170 [PMID: 17617204 DOI: 10.1111/j.1572-0241.2007.01403.x]

126 **Sripa B**, Thinkhamrop B, Mairiang E, Laha T, Kaewkes S, Sithithaworn P, Periago MV, Bhudhisawasdi V, Yonglitthipagon P, Mulvenna J, Brindley PJ, Loukas A, Bethony JM. Elevated plasma IL-6 associates with increased risk of advanced fibrosis and cholangiocarcinoma in individuals infected by Opisthorchis viverrini. *PLoS Negl Trop Dis* 2012; **6**: e1654 [PMID: 22629477 DOI: 10.1371/journal.pntd.0001654]

127 **Tangkijvanich P**, Thong-ngam D, Theamboonlers A, Hanvivatvong O, Kullavanijaya P, Poovorawan Y. Diagnostic role of serum interleukin 6 and CA 19-9 in patients with cholangiocarcinoma. *Hepatogastroenterology* 2004; **51**: 15-19 [PMID: 15011822]

128 **Selaru FM**, Olaru AV, Kan T, David S, Cheng Y, Mori Y, Yang J, Paun B, Jin Z, Agarwal R, Hamilton JP, Abraham J, Georgiades C, Alvarez H, Vivekanandan P, Yu W, Maitra A, Torbenson M, Thuluvath PJ, Gores GJ, LaRusso NF, Hruban R, Meltzer SJ. MicroRNA-21 is overexpressed in human cholangiocarcinoma and regulates programmed cell death 4 and tissue inhibitor of metalloproteinase 3. *Hepatology* 2009; **49**: 1595-1601 [PMID: 19296468 DOI: 10.1002/hep.22838]

129 **Wang S**, Yin J, Li T, Yuan L, Wang D, He J, Du X, Lu J. Upregulated circulating miR-150 is associated with the risk of intrahepatic cholangiocarcinoma. *Oncol Rep* 2015; **33**: 819-825 [PMID: 25482320 DOI: 10.3892/or.2014.3641]

130 **Silakit R**, Loilome W, Yongvanit P, Chusorn P, Techasen A, Boonmars T, Khuntikeo N, Chamadol N, Pairojkul C, Namwat N. Circulating miR-192 in liver fluke-associated cholangiocarcinoma patients: a prospective prognostic indicator. *J Hepatobiliary Pancreat Sci* 2014; **21**: 864-872 [PMID: 25131257 DOI: 10.1002/jhbp.145]

131 **Wongkham S**, Sheehan JK, Boonla C, Patrakitkomjorn S, Howard M, Kirkham S, Sripa B, Wongkham C, Bhudhisawasdi V. Serum MUC5AC mucin as a potential marker for cholangiocarcinoma. *Cancer Lett* 2003; **195**: 93-99 [PMID: 12767517]

132 **Bamrungphon W**, Prempracha N, Bunchu N, Rangdaeng S, Sandhu T, Srisukho S, Boonla C, Wongkham S. A new mucin antibody/enzyme-linked lectin-sandwich assay of serum MUC5AC mucin for the diagnosis of cholangiocarcinoma. *Cancer Lett* 2007; **247**: 301-308 [PMID: 16793202 DOI: 10.1016/j.canlet.2006.05.007]

133 **Li Y**, Li DJ, Chen J, Liu W, Li JW, Jiang P, Zhao X, Guo F, Li XW, Wang SG. Application of Joint Detection of AFP, CA19-9, CA125 and CEA in Identification and Diagnosis of Cholangiocarcinoma. *Asian Pac J Cancer Prev* 2015; **16**: 3451-3455 [PMID: 25921161]

134 **Furmanczyk PS**, Grieco VS, Agoff SN. Biliary brush cytology and the detection of cholangiocarcinoma in primary sclerosing cholangitis: evaluation of specific cytomorphologic features and CA19-9 levels. *Am J Clin Pathol* 2005; **124**: 355-360 [PMID: 16191503 DOI: 10.1309/J030-JYPW-KQTH-CLNJ]

135 **Siqueira E**, Schoen RE, Silverman W, Martin J, Rabinovitz M, Weissfeld JL, Abu-Elmaagd K, Madariaga JR, Slivka A. Detecting cholangiocarcinoma in patients with primary sclerosing cholangitis. *Gastrointest Endosc* 2002; **56**: 40-47 [PMID: 12085033]

136 **Moreno Luna LE**, Kipp B, Halling KC, Sebo TJ, Kremers WK, Roberts LR, Barr Fritcher EG, Levy MJ, Gores GJ. Advanced cytologic techniques for the detection of malignant pancreatobiliary strictures. *Gastroenterology* 2006; **131**: 1064-1072 [PMID: 17030177 DOI: 10.1053/j.gastro.2006.08.021]

137 **Kipp BR**, Stadheim LM, Halling SA, Pochron NL, Harmsen S, Nagorney DM, Sebo TJ, Therneau TM, Gores GJ, de Groen PC, Baron TH, Levy MJ, Halling KC, Roberts LR. A comparison of routine cytology and fluorescence in situ hybridization for the detection of malignant bile duct strictures. *Am J Gastroenterol* 2004; **99**: 1675-1681 [PMID: 15330900 DOI: 10.1111/j.1572-0241.2004.30281.x]

138 **Lindberg B**, Arnelo U, Bergquist A, Thörne A, Hjerpe A, Granqvist S, Hansson LO, Tribukait B, Persson B, Broomé U. Diagnosis of biliary strictures in conjunction with endoscopic retrograde cholangiopancreaticography, with special reference to patients with primary sclerosing cholangitis. *Endoscopy* 2002; **34**: 909-916 [PMID: 12430077 DOI: 10.1055/s-2002-35298]

139 **Boberg KM**, Jebsen P, Clausen OP, Foss A, Aabakken L, Schrumpf E. Diagnostic benefit of biliary brush cytology in cholangiocarcinoma in primary sclerosing cholangitis. *J Hepatol* 2006; **45**: 568-574 [PMID: 16879890 DOI: 10.1016/j.jhep.2006.05.010]

140 **Levy MJ**, Baron TH, Clayton AC, Enders FB, Gostout CJ, Halling KC, Kipp BR, Petersen BT, Roberts LR, Rumalla A, Sebo TJ, Topazian MD, Wiersema MJ, Gores GJ. Prospective evaluation of advanced molecular markers and imaging techniques in patients with indeterminate bile duct strictures. *Am J Gastroenterol* 2008; **103**: 1263-1273 [PMID: 18477350 DOI: 10.1111/j.1572-0241.2007.01776.x]

141 **Kipp BR**, Fritcher EG, Clayton AC, Gores GJ, Roberts LR, Zhang J, Levy MJ, Halling KC. Comparison of KRAS mutation analysis and FISH for detecting pancreatobiliary tract cancer in cytology specimens collected during endoscopic retrograde cholangiopancreatography. *J Mol Diagn* 2010; **12**: 780-786 [PMID: 20864634 DOI: 10.2353/jmoldx.2010.100016]

142 **Boberg KM**, Schrumpf E, Bergquist A, Broomé U, Pares A, Remotti H, Schjölberg A, Spurkland A, Clausen OP. Cholangiocarcinoma in primary sclerosing cholangitis: K-ras mutations and Tp53 dysfunction are implicated in the neoplastic development. *J Hepatol* 2000; **32**: 374-380 [PMID: 10735605]

143 **Glasbrenner B**, Ardan M, Boeck W, Preclik G, Möller P, Adler G. Prospective evaluation of brush cytology of biliary strictures during endoscopic retrograde cholangiopancreatography. *Endoscopy* 1999; **31**: 712-717 [PMID: 10604612 DOI: 10.1055/s-1999-73]

144 **Ponchon T**, Gagnon P, Berger F, Labadie M, Liaras A, Chavaillon A, Bory R. Value of endobiliary brush cytology and biopsies for the diagnosis of malignant bile duct stenosis: results of a prospective study. *Gastrointest Endosc* 1995; **42**: 565-572 [PMID: 8674929]

145 **Jailwala J**, Fogel EL, Sherman S, Gottlieb K, Flueckiger J, Bucksot LG, Lehman GA. Triple-tissue sampling at ERCP in malignant biliary obstruction. *Gastrointest Endosc* 2000; **51**: 383-390 [PMID: 10744806]

146 **Baron TH**, Harewood GC, Rumalla A, Pochron NL, Stadheim LM, Gores GJ, Therneau TM, De Groen PC, Sebo TJ, Salomao DR, Kipp BR. A prospective comparison of digital image analysis and routine cytology for the identification of malignancy in biliary tract strictures. *Clin Gastroenterol Hepatol* 2004; **2**: 214-219 [PMID: 15017605]

147 **Draganov PV**, Chauhan S, Wagh MS, Gupte AR, Lin T, Hou W, Forsmark CE. Diagnostic accuracy of conventional and cholangioscopy-guided sampling of indeterminate biliary lesions at the time of ERCP: a prospective, long-term follow-up study. *Gastrointest Endosc* 2012; **75**: 347-353 [PMID: 22248602 DOI: 10.1016/j.gie.2011.09.020]

148 **Ryan ME**, Baldauf MC. Comparison of flow cytometry for DNA content and brush cytology for detection of malignancy in pancreaticobiliary strictures. *Gastrointest Endosc* 1994; **40**: 133-139 [PMID: 8013809]

149 **Macken E**, Drijkoningen M, Van Aken E, Van Steenbergen W. Brush cytology of ductal strictures during ERCP. *Acta Gastroenterol Belg* 2000; **63**: 254-259 [PMID: 11189981]

150 **Schoefl R**, Haefner M, Wrba F, Pfeffel F, Stain C, Poetzi R, Gangl A. Forceps biopsy and brush cytology during endoscopic retrograde cholangiopancreatography for the diagnosis of biliary stenoses. *Scand J Gastroenterol* 1997; **32**: 363-368 [PMID: 9140159]

151 **Sugiyama M**, Atomi Y, Wada N, Kuroda A, Muto T. Endoscopic transpapillary bile duct biopsy without sphincterotomy for diagnosing biliary strictures: a prospective comparative study with bile and brush cytology. *Am J Gastroenterol* 1996; **91**: 465-467 [PMID: 8633492]

152 **Kubota Y**, Takaoka M, Tani K, Ogura M, Kin H, Fujimura K, Mizuno T, Inoue K. Endoscopic transpapillary biopsy for diagnosis of patients with pancreaticobiliary ductal strictures. *Am J Gastroenterol* 1993; **88**: 1700-1704 [PMID: 8213710]

153 **Kim HJ**, Kim MH, Lee SK, Yoo KS, Seo DW, Min YI. Tumor vessel: a valuable cholangioscopic clue of malignant biliary stricture. *Gastrointest Endosc* 2000; **52**: 635-638 [PMID: 11060188 DOI: 10.1067/mge.2000.108969]

154 **Howell DA**, Beveridge RP, Bosco J, Jones M. Endoscopic needle aspiration biopsy at ERCP in the diagnosis of biliary strictures. *Gastrointest Endosc* 1992; **38**: 531-535 [PMID: 1327937]

155 **Van Laethem JL**, Bourgeois V, Parma J, Delhaye M, Cochaux P, Velu T, Devière J, Cremer M. Relative contribution of Ki-ras gene analysis and brush cytology during ERCP for the diagnosis of biliary and pancreatic diseases. *Gastrointest Endosc* 1998; **47**: 479-485 [PMID: 9647372]

156 **McNally ME**, Collins A, Wojcik SE, Liu J, Henry JC, Jiang J, Schmittgen T, Bloomston M. Concomitant dysregulation of microRNAs miR-151-3p and miR-126 correlates with improved survival in resected cholangiocarcinoma. *HPB* (Oxford) 2013; **15**: 260-264 [PMID: 23458262 DOI: 10.1111/j.1477-2574.2012.00523.x]

157 **Li B**, Han Q, Zhu Y, Yu Y, Wang J, Jiang X. Down-regulation of miR-214 contributes to intrahepatic cholangiocarcinoma metastasis by targeting Twist. *FEBS J* 2012; **279**: 2393-2398 [PMID: 22540680 DOI: 10.1111/j.1742-4658.2012.08618.x]

158 **Giovannetti E**, Del Tacca M, Mey V, Funel N, Nannizzi S, Ricci S, Orlandini C, Boggi U, Campani D, Del Chiaro M, Iannopollo M, Bevilacqua G, Mosca F, Danesi R. Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. *Cancer Res* 2006; **66**: 3928-3935 [PMID: 16585222 DOI: 10.1158/0008-5472.CAN-05-4203]

159 **Fujita H**, Ohuchida K, Mizumoto K, Itaba S, Ito T, Nakata K, Yu J, Kayashima T, Souzaki R, Tajiri T, Manabe T, Ohtsuka T, Tanaka M. Gene expression levels as predictive markers of outcome in pancreatic cancer after gemcitabine-based adjuvant chemotherapy. *Neoplasia* 2010; **12**: 807-817 [PMID: 20927319]

160 **Murata Y**, Hamada T, Kishiwada M, Ohsawa I, Mizuno S, Usui M, Sakurai H, Tabata M, Ii N, Inoue H, Shiraishi T, Isaji S. Human equilibrative nucleoside transporter 1 expression is a strong independent prognostic factor in UICC T3-T4 pancreatic cancer patients treated with preoperative gemcitabine-based chemoradiotherapy. *J Hepatobiliary Pancreat Sci* 2012; **19**: 413-425 [PMID: 21898089 DOI: 10.1007/s00534-011-0440-3]

161 **Greenhalf W**, Ghaneh P, Neoptolemos JP, Palmer DH, Cox TF, Lamb RF, Garner E, Campbell F, Mackey JR, Costello E, Moore MJ, Valle JW, McDonald AC, Carter R, Tebbutt NC, Goldstein D, Shannon J, Dervenis C, Glimelius B, Deakin M, Charnley RM, Lacaine F, Scarfe AG, Middleton MR, Anthoney A, Halloran CM, Mayerle J, Oláh A, Jackson R, Rawcliffe CL, Scarpa A, Bassi C, Büchler MW. Pancreatic cancer hENT1 expression and survival from gemcitabine in patients from the ESPAC-3 trial. *J Natl Cancer Inst* 2014; **106**: djt347 [PMID: 24301456 DOI: 10.1093/jnci/djt347]

162 **Park BK**, Paik YH, Park JY, Park KH, Bang S, Park SW, Chung JB, Park YN, Song SY. The clinicopathologic significance of the expression of vascular endothelial growth factor-C in intrahepatic cholangiocarcinoma. *Am J Clin Oncol* 2006; **29**: 138-142 [PMID: 16601431 DOI: 10.1097/01.coc.0000204402.29830.08]

163 **Tannapfel A**, Benicke M, Katalinic A, Uhlmann D, Köckerling F, Hauss J, Wittekind C. Frequency of p16(INK4A) alterations and K-ras mutations in intrahepatic cholangiocarcinoma of the liver. *Gut* 2000; **47**: 721-727 [PMID: 11034592]

164 **Xu RF**, Sun JP, Zhang SR, Zhu GS, Li LB, Liao YL, Xie JM, Liao WJ. KRAS and PIK3CA but not BRAF genes are frequently mutated in Chinese cholangiocarcinoma patients. *Biomed Pharmacother* 2011; **65**: 22-26 [PMID: 21051183 DOI: 10.1016/j.biopha.2010.06.009]

165 **Zou S**, Li J, Zhou H, Frech C, Jiang X, Chu JS, Zhao X, Li Y, Li Q, Wang H, Hu J, Kong G, Wu M, Ding C, Chen N, Hu H. Mutational landscape of intrahepatic cholangiocarcinoma. *Nat Commun* 2014; **5**: 5696 [PMID: 25526346 DOI: 10.1038/ncomms6696]

166 **Isa T**, Tomita S, Nakachi A, Miyazato H, Shimoji H, Kusano T, Muto Y, Furukawa M. Analysis of microsatellite instability, K-ras gene mutation and p53 protein overexpression in intrahepatic cholangiocarcinoma. *Hepatogastroenterology* 2002; **49**: 604-608 [PMID: 12063950]

167 **Goldenberg D**, Rosenbaum E, Argani P, Wistuba II, Sidransky D, Thuluvath PJ, Hidalgo M, Califano J, Maitra A. The V599E BRAF mutation is uncommon in biliary tract cancers. *Mod Pathol* 2004; **17**: 1386-1391 [PMID: 15181454 DOI: 10.1038/modpathol.3800204]

168 **Wang P**, Dong Q, Zhang C, Kuan PF, Liu Y, Jeck WR, Andersen JB, Jiang W, Savich GL, Tan TX, Auman JT, Hoskins JM, Misher AD, Moser CD, Yourstone SM, Kim JW, Cibulskis K, Getz G, Hunt HV, Thorgeirsson SS, Roberts LR, Ye D, Guan KL, Xiong Y, Qin LX, Chiang DY. Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. *Oncogene* 2013; **32**: 3091-3100 [PMID: 22824796 DOI: 10.1038/onc.2012.315]

169 **Andresen K**, Boberg KM, Vedeld HM, Honne H, Hektoen M, Wadsworth CA, Clausen OP, Karlsen TH, Foss A, Mathisen O, Schrumpf E, Lothe RA, Lind GE. Novel target genes and a valid biomarker panel identified for cholangiocarcinoma. *Epigenetics* 2012; **7**: 1249-1257 [PMID: 22983262 DOI: 10.4161/epi.22191]

170 **Andresen K**, Boberg KM, Vedeld HM, Honne H, Jebsen P, Hektoen M, Wadsworth CA, Clausen OP, Lundin KE, Paulsen V, Foss A, Mathisen Ø, Aabakken L, Schrumpf E, Lothe RA, Lind GE. Four DNA methylation biomarkers in biliary brush samples accurately identify the presence of cholangiocarcinoma. *Hepatology* 2015; **61**: 1651-1659 [PMID: 25644509 DOI: 10.1002/hep.27707]

171 **Sriraksa R**, Zeller C, El-Bahrawy MA, Dai W, Daduang J, Jearanaikoon P, Chau-In S, Brown R, Limpaiboon T. CpG-island methylation study of liver fluke-related cholangiocarcinoma. *Br J Cancer* 2011; **104**: 1313-1318 [PMID: 21448164 DOI: 10.1038/bjc.2011.102]

172 **Uhm KO**, Lee ES, Lee YM, Kim HS, Park YN, Park SH. Aberrant promoter CpG islands methylation of tumor suppressor genes in cholangiocarcinoma. *Oncol Res* 2008; **17**: 151-157 [PMID: 18773859]

173 **Amornpisutt R**, Proungvitaya S, Jearanaikoon P, Limpaiboon T. DNA methylation level of OPCML and SFRP1: a potential diagnostic biomarker of cholangiocarcinoma. *Tumour Biol* 2015; **36**: 4973-4978 [PMID: 25652468 DOI: 10.1007/s13277-015-3147-2]

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| **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)index 2: (miR-26b, miR-34a, miR-122, miR-126, miR-145, miR-150, miR-223, miR-505, miR-636, miR-885.5p)[121] | Denmark | 409 | Index 1: 77Index 2: 80 | Index 1: 66Index 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 |
| miR-1921[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 1Western 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.

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| **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 |
| Cytology + p53 + KRAS[76] | The Netherlands | 23 | 10 | 100 | 79 |
| **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 (cholangioacarcinoma, 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; ERCP: Endoscopic retrograde cholangio-pancreatography; 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.

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| **Table 3 Prognostic markers in pancreatic ductal adenocarcinoma and cholangiocarcinoma** |
| **Pancreatic cancer** | **Marker** | **Country** | **Total patients, *n*** | **Marker positive PDAC** | **Type of dysregulation** | **Prognostic value, OS months** | **HR or *P*-value for OS** |
| 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 |
| **CCA** | **Marker** | **Country** | **Total Patients, n** | **CCA patients, n** | **Type of dysregulation** | **Prognostic value** | **HR (95%CI) or *P*-value for OS** |
| miR-1921[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) |
| miR-211[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-223Group 2: miR-122, miR-145, miR-146a, miR-200c, miR-221, and miR-222[44] | Greece | 179 | 21 | Group 1: Up-regulatedGroup 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] |

1Liver 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.

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| **Table 4 Theranostic markers in pancreatic ductal adenocarcinoma and cholangiocarcinoma** |
| **Pancreatic cancer** | **Marker(drug)** | **Countries** | **Patients with + marker** | **Staining** | **Median survival (mo)** | **HR** |
| *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: 12High Hent1: 9 | Low: 4High: 13 | 1 |
| Italy[158] | 83 | Low Hent1: 27Inter: 28High Hent1: 26 | Low: 8.5Inter:15.7High: 25.7 | 4.21 |
| United States[97] | 91 | Low Hent1: 39High Hent1: 34 | 2 | 0.51 |
| Belgium[98] | 45 | Low Hent1: 26High Hent1: 19 | Low: 13.3High: 18.7 | 4.31 (HR for death)*P* = 0.0001 (OS) |
| Japan[159] | 40 | Low Hent1: 26High Hent1: 14 | Low: 8High:25 | *P* = 0.011 (OS) |
| Japan[160] | 55 | Low Hent1: 16High Hent1: 39 | Low: 11.8High: 24.9 | 3.15 (OS) |
| BelgiumFrance[86] | 243 | Low Hent1: 142High Hent1: 92 | 2 | 0.34 |
| Worldwide Multicenter[99] | 177 | Low Hent1: 118High Hent1: 59 | Low: 6.1High: 5.2 | 1.147 |
| England[161] | 176 | Low Hent1: 77High Hent1:99 | Low: 17.1High: 26.2 | 0.6 |
| CCA | **Marker** | **Countries** | **CCA patients** | **% mutated** | **Type of mutation** | **Potential theranostic value** |
| 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] | USA, 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 | 10-22.31 | 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]Panel: CDO1, CNRIP1,SEPT9, and VIM[170] | Norway | 3930 | 8785 | Promoter methylation | Anti-methylation therapy |
| SFRP1[169,171-173] | Norway, United Kingdom, South Korea, Thailand | 255 | 59-83.6 | Promoter methylation | Tumor suppression with gene therapy (RNAi) |

1Not reported; 2Results 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.