

**National Academy of Clinical Biochemistry (NACB) Guidelines for the Use of Tumor Markers in Pancreatic Ductal Adenocarcinoma**

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**Abbreviations:** AGA, American Gastroenterological Association; EGTM, European Group on Tumor Markers; ERCP, endoscopic retrograde cholangiopancreatograph; EUS, endoscopic ultrasound; FNA, fine needle aspirate; IPMN, intraductal papillary mucinous neoplasm; NCCN, National Comprehensive Cancer Network; PanIN, pancreatic intraepithelial neoplasia.

## **INTRODUCTION**

Pancreatic ductal adenocarcinoma is the fourth leading cause of cancer death in men and women in the USA and has the lowest survival rate for any solid cancer (1, 2). Over 31,000 individuals are diagnosed with pancreatic cancer in the USA each year, of whom ~31,000 will die of the disease. Similar mortality figures are reported in the UK, with only 2-3% of patients surviving 5 years after a diagnosis of pancreatic cancer. One important reason for this poor survival is that only 10-15% of patients are diagnosed with small, resectable cancers (1). The lifetime risk of developing pancreatic cancer is ~1/150, and the median age at diagnosis is the mid 60s. However, the age-specific incidence of pancreatic cancer is low prior to the age of 50 where the annual incidence in the general population is less than 10 cases per 100,000 (3).

The diagnosis of pancreatic cancer is usually suspected from complaints of progressive obstructive jaundice, profound weight loss and pain in the abdomen or mid-back. Chronic pancreatitis can be difficult to distinguish from pancreatic cancer with the use of clinical, imaging, and biochemical parameters. Less frequently, patients with pancreatic cancer can present with diabetes mellitus, thrombophlebitis migrans, depression, or evidence of metastatic disease. Generally, the diagnosis is established using computerized tomography (CT), endoscopic ultrasound (EUS) or ERCP (endoscopic retrograde cholangiopancreatography) with histological (or cytological) confirmation. If curative resection is considered, staging investigations using EUS or angiography are performed searching for evidence of metastases to lymph nodes, peritoneum, liver and vascular invasion of the splenic or portal vein. Helical CT with contrast usually provides good visualization of the peripancreatic vasculature without the need for formal angiography. A diagnosis of pancreatic cancer may be delayed in some patients for a variety of reasons including having non-specific symptoms, having a cancer that is small or that diffusely infiltrates the pancreas without forming a mass, delay in access to diagnostic services such as endoscopic ultrasound and fine needle aspiration, or due to the sub-optimal sensitivity of fine needle aspiration cytology (1, 4). Imaging and endoscopy can readily be used to diagnose unresectable lesions but are less effective at diagnosing small, surgically resectable cancers.

The management of pancreatic ductal adenocarcinoma depends on several factors including the patient's symptoms, the performance status of the patient, the histological classification of the cancer, the stage of the disease, and the presence of complications. Surgery (Whipple resection for tumors of the pancreatic head) remains the only realistic curative modality for pancreatic cancer. The Whipple procedure involves the resection of the head of the pancreas, the duodenum, distal common bile duct, local lymph nodes and peripancreatic tissue.

Operative mortality rates vary considerably with the experience of the surgeons and the number of patients served by the treating institution (5), but rates in expert centers are as low as 1 to 3%. Unfortunately, approximately 75% of individuals who undergo Whipple operations will die of their disease within 5 years, with a median survival of ~18 months. Many patients who undergo curative resection for pancreatic adenocarcinoma are offered adjuvant or neo-chemoradiotherapy (6, 7). Recent controversial studies suggest that radiotherapy may not be effective for most patients (8, 9). Precancerous lesions can also present with symptoms and can appear as pancreatic solid and cystic masses including intraductal papillary mucinous neoplasms (IPMNs). It is important to diagnose these neoplasms which can present in the same way as invasive pancreatic cancers but are usually curable with surgery (10).

There are few chemotherapeutic agents that are active against pancreatic cancer (11-15). Agents such as gemcitabine and 5-fluorouracil (5FU) are effective in only 10-20% of patients with the disease. Other chemotherapeutics under investigation include 5FU prodrugs (capecitabine and S-1), rubitecan (9-nitrocamptothecin, RFS 2000) and taxotere. A number of experimental approaches are being tried, including tumor vaccines (16), but their efficacy has yet to be established.

Since most patients will die of their disease, it is important to improve patient quality of life as much as possible. Measures to alleviate pain are usually necessary, generally opiate analgesia frequently given in the form of infusion pump. Destruction of the nerves around the pancreas ('celiac axis nerve block') achieved by the percutaneous, EUS-guided or intra-operative injection of 100% alcohol, provides pain relief for many patients (17-20). Stenting the main pancreatic duct has also been tried to relieve pain from elevated intraductal pressure in patients with pancreatic cancer (21). Weight loss is a common problem which is usually multifactorial and is often refractory to treatment (22). Anorexia, maldigestion and cachexia are important factors. Pancreatic enzymes and appetite stimulants may help. Of the many agents tried to combat cachexia, fish oils appear promising in early clinical trials (23). In the laboratory, fish oils appear to block the effects of cancer on muscle and fat wasting.

Many patients with pancreatic cancer will die of the complications of common bile duct obstruction. Biliary drainage can relieve symptoms and is achieved with biliary stents introduced during ERCP or percutaneously (percutaneous transhepatic cholangiography, PTC). Biliary stents can be placed as an outpatient procedure with minimal patient discomfort. Unfortunately, these stents invariably block due to progressive tumor growth, necessitating removal and replacement of the existing stent.

Since 15-40% of patients with a resectable pancreatic cancer survive 5 years after surgical resection (24), the earlier diagnosis of pancreatic cancer could save lives. Indeed, Canto et al. have shown that EUS based screening of individuals whose family history indicates that they have an increased risk of developing pancreatic cancer can detect early (non-invasive) pancreatic neoplasms (25-28). The role of screening using imaging tests is still under investigation. However, screening using EUS and spiral CT scanning (in the context of counseling about cancer risk and inherited susceptibility) appears to be effective at detecting, treating and probably curing early pancreatic cancers and precancerous lesions (intraductal papillary mucinous neoplasms), although this information is based on relatively small numbers of individuals (25, 28, 29). Potential candidates for screening include individuals who have multiple first-degree relatives with familial pancreatic cancer (27), those with germ line mutations in the *BRCA2* gene, the *STK11* gene (who have the Peutz-Jeghers syndrome), or the *p16* gene (with familial atypical mole melanoma syndrome), and those with hereditary pancreatitis due to germ line mutations in the cationic trypsinogen gene (30). Currently, screening should only be considered in individuals with a significantly increased risk of developing pancreatic neoplasia and only in the context of clinical research protocols.

Better serum markers of pancreatic cancer are urgently needed to improve pancreatic cancer diagnosis. Existing tumor markers are not sufficiently sensitive nor are they specific enough to differentiate benign from malignant disease.

## **TUMOR MARKERS IN PANCREATIC CANCER: NACB RECOMMENDATIONS**

Table 1 summarizes NACB recommendations for the use of CA19-9 in pancreatic cancer, together with those of the European Group on Tumor Markers (EGTM) and the American Gastroenterological Association (AGA). The NACB guidelines are derived from published studies of pancreatic cancer serum and tissue markers that are described below. The levels of evidence grading system used is based on that described by Hayes *et al* [see Section 1].

A tumor marker has been defined, as “a naturally occurring molecule that is measured in serum or plasma, or other body fluids or in tissue extracts or paraffin-embedded tissue to identify the presence of cancer, to assess patient prognosis, or to monitor a patient’s response to therapy with the overall goal of improving the clinical management of the patient”(31). The greatest limitation of most studies of serum markers is that they fail to limit their analyses to patients with small potentially curable pancreatic cancers.

### **Tumor markers investigated for use in pancreatic cancer**

In the discussion presented here, tumor markers have been classified according to the evidence available to support their clinical use. For markers in Category A, “accepted clinical use”, there is clear evidence that the marker is clinically useful and contributes to patient care, and that its use is widely accepted. For those in Category B, “evaluation”, there is substantial information on the marker, typically from larger cohorts, which validates its diagnostic performance, but there is no clear evidence yet that the marker is clinically useful. For markers in category C, “research or discovery”, typically only initial, discovery-phase data have been published; with further evaluation using larger cohorts required to determine the diagnostic potential.

Previously evaluated markers which have been demonstrated to be inferior to the current standard marker, CA19-9, include Amylin (IAPP) (32, 33), CA 50 (34-40), CA195 (41-44), tumor associated trypsin inhibitor (TATI) (44-46), pancreatic oncofetal antigen (POA) (47-49), YKL-40 (50), TUM2-PK (tumor M2 pyruvate kinase) (51-55) and HIP/PAP (56-60). Markers for which conflicting evidence exists as to their diagnostic potential include carcinoembryonic antigen (CEA) (61-64), CA72-4 (65-67), DUPAN-2 (35, 68), and SPan-1 (69, 70)]. None of these markers will be discussed further.

### **Tumor markers with accepted clinical use in pancreatic cancer (Category A)**

#### **CA 19-9**

CA19-9, the sialylated Lewis<sup>a</sup> blood group antigen, is still the current standard serum tumor marker for pancreatic adenocarcinoma, and the one against which others are compared. Recommendations regarding its use in the management of patients with pancreatic cancer have been issued from the European Group on Tumor Markers (EGTM) and the American Gastroenterological Association (AGA) (3, 71). The clinical usefulness of CA19-9 in the screening, diagnosis, prognosis and monitoring of therapy for pancreatic adenocarcinoma is reviewed below (see also Table 1). The US Food and Drug Administration (FDA) has approved several CA19-9 assays for monitoring patients with pancreatic cancer.

*Diagnosis.* The utility of serum CA19-9 in the diagnosis of pancreatic adenocarcinoma has been extensively investigated in numerous retrospective and prospective studies that have enrolled multiple control groups (chronic pancreatitis, obstructive jaundice, abdominal pain, healthy subjects) [for review see (72-76)]. The optimal diagnostic cutoff value of CA19-9 has been evaluated extensively. In one study CA 19-9 levels were measured in 160 patients with

pancreatic diseases (90 with pancreatic cancer, 70 with benign disease), 322 patients with biliary tract diseases (152 with biliary cancer, 170 with benign disease), and 20,035 asymptomatic controls. The mean serum concentration of CA19-9 in asymptomatic individuals was 9.42 +/- 9.95 U/ml and levels above 37 U/mL were determined to be most accurate for discriminating pancreatic cancer from benign pancreatic disease (sensitivity and specificity of 77 and 87 percent, respectively)(77). Similar sensitivity and specificity values for CA19-9 have been described in other studies, but have varied depending on the study populations under investigation.

Among patients presenting with symptoms suspected to be due to pancreatic cancer, CA19-9 performs somewhat better, but still has only modest sensitivity. In a study of 261 such patients, serum CA19-9 had a sensitivity of 70%, and a specificity of 87%. In this population, the positive predictive value of an elevated CA19-9 was 59%, and the negative predictive value was 92% (78). A meta-analysis of similar studies, performed in 1994, reported serum CA19-9 had a mean sensitivity of 81% (range 69-93%) and a specificity of 91% (range 76-99%) using a cutoff level of 37 U/L (72). Higher cutoff values increase the diagnostic specificity: for a cutoff level of 100 U/L, diagnostic specificity of 97% has been reported (79), and for CA19-9 levels >1000 U/L, specificity approaches 100% (72, 80). Importantly, CA19-9's performance characteristics improve as a patient's pancreatic cancer becomes more advanced (62, 72). Consequently, for small tumors (<3 cm), the sensitivity of CA19-9 decreases significantly (~55%) (72). CA19-9 antigen secretion depends on Lewis<sup>a</sup> antigen status. If the Lewis<sup>a</sup> antigen status is taken into account, CA19-9's overall diagnostic sensitivity can reach 92% among patients presenting with symptoms of pancreatic cancer (81). The combination of serum CA19-9 and imaging tests (sonography, CT) increase the positive predictive value (79). Unfortunately, at the current time, cure from pancreatic ductal adenocarcinoma is related to tumor stage and size (82) and thus the challenge for serum tumor markers such as CA19-9 is not simply to perform adequately among patients with advanced disease who are rarely cured of their disease, but to ensure that patients who present while they have small curative cancers can be readily identified with the help of accurate serum tumor markers. Serum CA19-9 does not have the necessary performance characteristics to meet this challenge.

Other limitations of the diagnostic usefulness of CA19-9 include its elevation in non-pancreatic gastrointestinal malignancies as well as a variety of benign disease conditions. Biliary obstruction, cholecystitis, cholangitis, hepatic cirrhosis, acute and chronic pancreatitis are common causes of elevated CA19-9 values (72, 73). For example, in one study serum CA 19-9 values ranged from 190 to 32,000 in seven patients with acute cholangitis secondary to

gallstone biliary obstruction but were normal in patients with asymptomatic cholelithiasis, common duct obstruction without cholangitis, or acute cholecystitis (83).

In view of these limitations the NACB Panel does not recommend measuring serum CA19-9 for the diagnosis of pancreatic cancer. EGTM guidelines recommend a limited role for CA19-9 in the diagnosis of pancreatic cancer as a complement to accurate pancreatic imaging procedures (3, 71). If measured, CA19-9 should be used in conjunction with an imaging test (CT, EUS). Appropriately interpreted CA19-9 values can guide further invasive testing (ERCP, EUS FNA, laparoscopy, laparotomy) in the appropriate clinical context.

*Screening.* The low prevalence of pancreatic cancer in the general population means that screening asymptomatic persons for pancreatic cancer using even highly accurate serum tumor markers would yield too many false positives and cannot be recommended. This is illustrated by a recent study in which 70,940 asymptomatic subjects were screened using CA19-9. Four cases of pancreatic cancer were detected along with 1059 false-positives, yielding a positive predictive value of 0.9% (84).

When screening high-risk populations, many lesions detected by imaging are precancerous masses and in this setting, serum CA19-9 is often normal (25, 28, 29). For example, in one study CA19-9 levels were all within normal range in 14 high-risk individuals who underwent total pancreatectomy. These 14 patients had pancreatic imaging abnormalities and were found to have histologic evidence of dysplasia in their pancreatectomy specimens (85). Similarly, since many pancreatic adenocarcinomas arise from microscopic dysplastic lesions now called "PanIN" (pancreatic intraepithelial neoplasia), there is concern that neither imaging nor serum tumor markers will detect many pancreatic neoplasms until they have become invasive cancers (86-88). Serum tumor markers could still be very valuable for detecting early asymptomatic invasive pancreatic cancer, but this information must await prospective studies as there is currently only limited information about the role of tumor markers in this setting. The American Gastroenterology Association recommendations published in 1999 did not recommend a screening strategy as at that time no strategy had been shown to detect early pancreatic cancers in patients with an increased risk for developing pancreatic cancer.

*Prognosis.* Serum CA19-9 levels carry independent predictive value for the determination of resectability of pancreatic cancer (62, 80) and of overall patient survival (81, 89-91). For example, a retrospective review of 104 pancreatic cancer patients treated with radiotherapy found the CA19-9 level at diagnosis to be a significant prognostic indicator, with median survival

rates of 8 and 20 months for patients with a CA19-9 level above or below the median (680 U/L) (91). Similarly, in a cohort study of patients undergoing resection for pancreatic cancer (n=347), the median survival time for patients with the same tumor stage was significantly longer in patients whose CA19-9 levels returned to normal after resection, than in those whose CA19-9 did not normalize (81). In another study of 28 patients with advanced pancreatic cancer and elevated baseline CA19-9 treated with gemcitabine, pretreatment CA19-9 level was an independent, and significant predictor of survival, and was more informative than CA19-9 response ( $p = .0497$ ) (92). Most recently, undetectable preoperative CA 19-9 levels have been shown to correlate with improved survival for patients with resectable pancreatic adenocarcinoma. The authors suggest that patients who present with undetectable preoperative CA19-9 levels and potentially resectable pancreatic cancer, regardless of advanced stage, should be considered candidates for aggressive therapy (93).

While the AGA guidelines do not comment on this topic specifically, the EGTM guidelines state that CA19-9 has the potential to assess prognosis for pancreatic cancer, but its use for that purpose is unproven (71).

The NACB Panel recommends that serum CA19-9 levels should be taken into account for risk stratification of pancreatic cancer patients. Although high levels of CA19-9 are an adverse prognostic indicator, it is only one of many parameters that influence prognosis and decisions about therapy.

*Monitoring of therapy.* Substantial evidence is available regarding the role of serial CA19-9 measurements in the monitoring of palliative chemotherapy for pancreatic cancer. In two recent studies of palliative gemcitabine chemotherapy (n=89 for the 2 studies combined), a decline in CA19-9 of >20% compared to baseline after 8 weeks therapy (2 cycles) has been shown to be a better indicator of response and survival than CT imaging (94, 95). No patient had decreasing CA19-9 levels in the presence of progressive disease and all patients with an objective response (partial or complete) had CA19-9 reductions of >20%. The independent prognostic value of serial CA19-9 kinetics has also been reported using different palliative combination chemotherapy regimens (95-97). In a study of 21 patients with advanced pancreatic cancer treated with gemcitabine and cisplatin, all 4 patients who achieved complete remission demonstrated a return of CA19-9 levels into the normal range (98), while the 4 patients who achieved partial remission showed a decrease in their CA19-9 values (98).

Serial CA19-9 measurements have greater sensitivity and specificity for determining response than individual measurements. In one study, a serial drop in CA19-9 levels had a

sensitivity of 67% for predicting partial response and a serial elevations a sensitivity of 86% for predicting progressive disease (97). One study concluded that decreasing CA19-9 values suggest tumor progression is unlikely and treatment should be continued, while patients with an increase of CA19-9 during chemotherapy with gemcitabine had a poor prognosis and in the absence of a significant improvement in clinical response, further chemotherapy is of questionable value (94).

Similar studies have been performed investigating the role of CA19-9 in predicting the response to radiotherapy for pancreatic cancer (91, 99, 100). Serial measurements to monitor response radiotherapy, however, are less practicable, since radiotherapy is limited by a maximum tolerated dose and is of short duration in contrast to palliative chemotherapy. When used to follow the prognosis of patients after chemoradiotherapy, serial CA19-9 predicted disease relapse with 100% sensitivity and 88% specificity (99).

Current guidelines from the AGA, NCCN and EGTM state that CA19-9 measurements cannot yet be recommended for monitoring the efficacy of treatment of pancreatic cancer.

The NACB Panel recommends that serial CA19-9 measurements be used in conjunction with imaging to monitor patient's response to therapy, particularly palliative chemotherapy. Serial CA19-9 monitoring is also recommended in the follow-up of patients after potentially curative surgery, although the utility of detecting early rises in CA19-9 and instituting therapy prior to other evidence of relapse has yet to be demonstrated.

#### *Gene testing for inherited susceptibility*

Individuals with inherited mutations of BRCA2, p16 (Familial atypical multiple mole melanoma), STK11 (Peutz-Jeghers syndrome), cationic trypsinogen (hereditary pancreatitis), FANCC, FANCG and occasionally mismatch repair gene defects (hereditary non-polyposis colorectal cancer) are at increased risk of developing inherited pancreatic cancer (101-109). Most of the known inherited causes of pancreatic cancer are due to germ line *BRCA2* mutations, which are found in ~10% of all familial pancreatic cancers (103, 110-112) and ~5% of patients with apparently sporadic pancreatic cancer (102, 103). Kindred with germ line *BRCA2* mutations may have multiple pancreatic cancers in the absence of breast or ovarian cancer. Therefore, at many centers in the USA patients with a strong family history of pancreatic cancer are recommended to undergo genetic testing for germ line *BRCA2* mutations after appropriate genetic counseling. This is based on expert opinion and applies to populations where *BRCA2* mutations have been demonstrated in patients with familial pancreatic cancer (103, 110, 111). In addition to considerations regarding prophylactic mastectomy and oophorectomy, patients

with germ line *BRCA2* mutations may benefit from screening to look for evidence of pancreatic neoplasia (26), but this is still under investigation. The other known genes cause only a very small percentage of familial pancreatic cancers. Pancreatic cancer families with a malignant melanoma will occasionally harbor germ line *p16* mutations, but there is not enough evidence to recommend these patients for *p16* gene testing (113-115). Individuals with pancreatic cancer who are from families having multiple members with melanomas may have familial atypical melanoma mole syndrome (FAMM) and in this setting *p16* gene testing is appropriate (Table 2).

#### *Tissue markers of pancreatic cancer*

Infiltrating adenocarcinomas of the pancreas differentially express a large number of proteins and many of these proteins can be detected using commercially available antibodies (see table 3). Some of these tissue markers may aid in establishing the diagnosis of cancer, but the diagnosis of pancreatic cancer should never be based solely on immunohistochemical labeling of tissues.

All adenocarcinomas of the pancreas express cytokeratin (CK) and almost all will label with the anti-cytokeratin antibodies AE1/AE3 and CAM 5.2 (116-118). Antibodies are now available to a number of cytokeratins of different molecular weights and CK 7, CK 8, CK 18 and CK 19 are expressed in 70-100% of pancreatic cancers (115). CK 17 is expressed in 50-70%, and CK 20 in <20% (116). The pattern of immunolabeling for cytokeratins can be diagnostically useful, as most acinar neoplasms and most well differentiated endocrine neoplasms of the pancreas do not express CK7.

Ductal adenocarcinomas of the pancreas also express epithelial membrane antigen (EMA), and a variety of tumor antigens including carcinoembryonic antigen (CEA), carcinoma antigen 19-9 (CA19-9), CA 125, and DuPan 2 (116, 119, 120). The expression of CEA may be particularly useful in distinguishing infiltrating adenocarcinoma from reactive glands.

Adenocarcinomas of the pancreas express several mucins including MUC1 (a pan-epithelial mucin equivalent to EMA), MUC3, MUC4, and MUC5AC (a gastric foveolar mucin) (121). A quarter of ductal adenocarcinomas will express MUC6 (a pyloric-gland mucin), but <10% will express MUC2. This pattern of mucin expression can help distinguish ductal adenocarcinomas from other tumor types in the pancreas. For example, the majority of intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) of the pancreas express MUC2 but not MUC1 (121-124). MUC4 expression is under investigation for its utility for pancreatic cancer diagnosis and may help distinguish pancreatic cancer tissue

samples from those with chronic pancreatitis (125). MUC4 expression also becomes increasingly likely in PanINs of advanced grade (126).

The SMAD4 (DPC4/MADH4) tumor suppressor gene is genetically inactivated in 55% of pancreatic cancers and mutations in the *DPC4* gene are highly correlated with loss of protein expression. Cancers showing an abnormal loss of Smad4 protein expression have a poorer prognosis (127, 128). Since loss of Smad4 expression is only occasionally found in other cancers and is not a feature of non-neoplastic pancreatic conditions, Smad4 expression has been demonstrated to facilitate the interpretation of diagnostically difficult cytological specimens (129).

### **Tumor markers under evaluation for use in pancreatic cancer [Category B (Table 4)]**

#### *CA242.*

CA242, the non-fucosylated precursor sialyl-Lewis<sup>c</sup> antigen, is a mucin-based serum marker. The serum levels of this marker are not dependent on Lewis<sup>a</sup> secretor status (61). Substantial published data are available on the diagnostic utility of CA242 as a pancreatic cancer marker. Depending on the population studied, sensitivities of 41%-75% and specificities of 85-95% for the diagnosis of pancreatic cancer have been described (130-132). Overall, the reported diagnostic performance is similar to that of CA19.9 (130, 133-135). In a study of 42 consecutive patients with pancreatic carcinoma, CA19.9 (at a cut-off value of 37 U/ml) and CA242 (cut-off value 20 U/ml) had essentially the same performance characteristics. CA242 was better than CA19.9 with respect to specificity (>90%), whereas CA19.9 had better sensitivity (>70%) (136). CA242 levels also carry prognostic significance. In one study, patients with resected pancreatic cancer and a preoperative CA242 <25 U/ml had a significantly better prognosis than those with higher CA242 levels, independent of resectability and of CA19-9 levels (137). The advantages of CA242 over CA19-9 include its independence of Lewis antigen status and that its levels are less influenced by cholestasis (133). In conclusion, CA242 has been shown to have similar, but not superior diagnostic performance compared to CA19-9. In selected situations, such as Lewis<sup>a</sup> non-secretor status, its determination may be clinically useful.

#### *CAM 17.1*

CAM 17.1, a sialylated blood group type-1 antigen, probably sialyl-I (61, 138), is also a mucin-based marker. Initial reports using sera from patients with pancreatic cancer and various controls (chronic pancreatitis, healthy controls, those with other GI cancers) describe

sensitivities of 67-78% and specificities of 76-91% (139, 140). The diagnostic sensitivity of CAM 17.1 is similar to that of CA19-9, but CAM 17.1 may have higher specificity (139). One large prospective cohort study of serum CAM 17.1 in 250 patients suspected to have pancreatic cancer demonstrated a sensitivity for predicting pancreatic cancer of 86% and specificity of 91%; with 89% sensitivity and 94% specificity for patients who did not have jaundice (141). The combination of CAM 17.1 and abdominal sonography further increased the sensitivity to 94%. In the same study, high CAM17.1 levels (>200U/L) also predicted non-resectability. Expression of CAM 17.1 is influenced by Lewis<sup>a</sup> antigen status (138); consequently the same limitation (7-10% non-secretor in the general population) seen with CA19-9 applies. The potential superiority to CA19-9 in terms of specificity has not been confirmed in other studies.

#### *Tissue polypeptide specific antigen (TPS)*

Two retrospective cohort studies have investigated the role of tissue polypeptide specific antigen (TPS), an epitope from soluble cytokeratin 18 fragments, in the monitoring of palliative chemotherapy for advanced pancreatic cancer. One of these studies showed a superior performance in comparison with CA 19-9 (41, 142). However, both studies only included a limited number of patients (n=9 and 20). TPS has also been investigated for its ability to differentiate patients with chronic pancreatitis from those with pancreatic cancer but results are inconclusive (41, 143, 144). In one study of 122 patients with suspected pancreatic carcinoma or chronic pancreatitis an elevated TPS level (>100 U/L) was found in 100% (46/46) of the patients with pancreatic cancer, whereas an elevated CA 19-9 (>37U/ mL) was seen in 32/46 (70%) patients. Elevations of TPS and CA 19-9 levels were found in 22% and 19% of 74 patients with chronic pancreatitis, respectively. If a cut-off value of 200 U/L was used, TPS had a sensitivity of 97% and specificity of 98% in discriminating pancreatic cancer from chronic pancreatitis (145). These data suggest that TPS may have a role in the detection of early pancreatic cancer and in the monitoring of responses to therapy, but further study is warranted before definitive recommendations can be made.

### **Potential tumor markers for pancreatic cancer still in the research or discovery stage [Category C (Table 4)]**

#### *Serum macrophage inhibitory cytokine 1 (MIC-1)*

Serum macrophage inhibitory cytokine 1 (MIC-1) was initially identified as being over expressed in pancreatic and colorectal cancer by gene expression studies (146, 147) and is also over expressed in prostate and other cancers (148). Serum MIC-1 level and genotype has

been associated with progression and prognosis of colorectal carcinoma (149). Recently, the diagnostic performance of MIC-1 was investigated using a custom-designed ELISA to assay 326 sera samples from patients with resectable pancreatic cancer (n=80), resectable ampullary and cholangiocarcinoma (n=30), other pancreatic neoplasms (n=42), chronic pancreatitis (n=77) and healthy controls (n=97). Serum MIC-1 had a sensitivity of 71% and specificity of 78% (cutoff 1070 pg/ml). CA19-9 had similar diagnostic utility in this cohort (ROC AUC 0.81 for MIC-1 and 0.77 for CA 19-9). The combination of MIC-1 and CA19-9 significantly improved the diagnostic accuracy compared to each single marker (ROC 0.87). More recently, MIC-1 was compared to CA19-9 and other investigational markers after some minor modifications in assay performance. Serum MIC-1 was elevated in 96% of individuals with resectable pancreatic cancer, as well as in 42% of those with chronic pancreatitis. The diagnostic accuracy of MIC-1 by ROC curve analysis was significantly better than CA19-9 (Koopmann et al, unpublished).

#### *Osteopontin*

Since first identified as a transformation-associated protein, osteopontin (OPN) has been recognized as important in the processes of tumorigenicity and metastasis (150). OPN is over expressed in lung, breast, prostate, gastric, esophageal and ovarian cancers (151). Gene expression profiling technology has also been used to demonstrate increased osteopontin (OPN) mRNA expression in pancreatic cancers (152). As a secreted molecule, serum OPN can be measured using an ELISA assay designed to limit osteopontin factor H interactions in the serum. Serum OPN was measured in patients with resectable pancreatic cancer (n=50) and in normal controls (n=22) and found to outperform CA19-9, with 97% specificity and 80% sensitivity for OPN (cutoff 334 ng/ mL).

#### *Tissue inhibitor of metalloproteinase type 1 (TIMP-1)*

Tissue inhibitor of metalloproteinase type 1 (TIMP-1) was first identified as a potential marker of pancreatic cancer following the discovery that it was over expressed in pancreatic cancer tissues (153). Plasma TIMP-1 levels have been reported to be increased in patients with colorectal cancers, with levels having prognostic significance in patients with colorectal carcinoma (154) and primary breast cancer (155). Evaluation of TIMP-1 using a commercial ELISA that detects free and complexed forms showed significantly higher levels in the sera of patients with pancreatic cancer (n=85) compared to normal controls (n=98). While serum TIMP-1 did not outperform CA19-9, in one study the combined measurement of TIMP-1, CEA and CA19-9 levels achieved 100% specificity at 60% sensitivity (specificity optimized cutoff values)

and 95% specificity at 81% sensitivity (sensitivity optimized cutoff values), illustrating the potential utility of combining markers (153). Further study of the diagnostic utility of TIMP-1 measurement for pancreatic cancer diagnosis is required including further evaluation of assays and the best body fluid for analysis (plasma vs. serum).

#### *Serum Proteomic markers*

Since the serum proteome contains many potential biomarkers for disease detection, several proteomic approaches are being used to identify novel protein markers of disease. One of the most commonly used analytical platform for high-throughput proteomic studies uses a ProteinChip® Biomarker System-II, also known as surface-enhanced laser desorption/ionization (SELDI), and a low-resolution time-of-flight (TOF) mass spectrometer. Pilot studies utilizing SELDI have identified candidate biomarkers in ovarian, breast, prostate and pancreatic cancers (156-161). In addition, recently a large multicenter study of ovarian cancer sera identified three peptides that were diagnostically useful in discriminating ovarian cancer from control sera. These markers, having been discovered in patients from one center were validated using patients from other centers (162). In a case-control study, Koopmann *et al.* compared serum samples from patients with resectable pancreatic adenocarcinoma using SELDI to those of a variety of disease and healthy controls (158). They found two discriminating peptide peaks could differentiate patients with pancreatic cancer from healthy controls with a sensitivity of 78% and specificity of 97%, outperforming CA19-9 ( $p < 0.05$ ). The diagnostic accuracy of these two peptides was improved by using them in combination with CA19-9. SELDI markers were also better than CA19-9 in distinguishing patients with pancreatic cancer from those with pancreatitis. Although these results indicate the potential diagnostic utility of assaying small serum peptide markers of pancreatic cancer, large multicenter studies are needed to confirm these findings and to precisely identify peptide markers so that more specific assays can be designed for their detection.

#### **Markers of pancreatic neoplasia detectable in pancreatic juice (Table 5)**

Serum markers play an important role in the diagnosis of many cancers, but they are less useful for identifying non-invasive neoplasms. The need to identify very small non-invasive pancreatic neoplasms has led to interest in identifying novel markers of pancreatic neoplasia that can be applied to pancreatic juice specimens. Accurate molecular markers of pancreatic neoplasia that could be applied to test inconclusive cytological or biopsy specimens would be valuable for evaluating patients with suspected pancreatic cancer. Samples of pancreatic

secretions (pancreatic juice) are also attractive diagnostic specimens because they have high concentrations of neoplastic DNA and proteins and could be a useful clinical specimen to use when diagnosing symptomatic patients as well as when screening high-risk individuals for evidence of early pancreatic cancer or of precancerous neoplasms of the pancreas, an approach analogous to using nipple aspirates for breast cancer diagnosis.

Multiple studies have determined the utility of using pancreatic juice samples to detect DNA methylation changes, DNA mutations, and protein over expression. For example, mutant *K-ras* genes are readily detected in pancreatic juice, but in plasma these mutations are usually detectable only after patients have inoperable cancer (163-165). Pure pancreatic juice collection requires ERCP, but pancreatic juice can also be collected in the duodenum and assayed for the presence of cancer DNA during routine upper-gastrointestinal endoscopy after secretin stimulation without the need for ERCP. Pancreatic juice markers and the assays designed for their detection are currently still undergoing evaluation and have not yet been demonstrated to be useful in clinical practice.

### **DNA alterations in pancreatic juice as markers of pancreatic cancer**

#### *Mutant DNA*

The diagnostic potential of DNA-based markers has improved in recent years, with technological developments including chip-based technology, and quantitative polymerase chain reaction (PCR) (166). DNA mutations can be detected at very low concentrations, even when a mutant allele is admixed with many hundreds or even thousands of wild-type alleles. Despite the accuracy of many DNA-based detection methods, and the multiple genes targeted for mutation in pancreatic cancers (167), few somatic mutations have been identified that have ideal diagnostic characteristics. An ideal genetic marker would be present in virtually all pancreatic cancers and would be readily detectable. The easiest mutations to detect are those that are limited to a single codon or to a very specific portion of the targeted gene such as *K-ras* or *BRAF* (163-165, 168-170). Unfortunately *K-ras* mutations are not specific for invasive pancreatic cancer; studies of *K-ras* gene mutations in pancreatic tissues, pancreatic juice and stool find they occur in patients with chronic pancreatitis, in individuals who smoke, and in PanINs from patients without pancreatic cancer (171-175).

*TP53* gene mutations generally occur relatively late in the neoplastic to invasive pancreatic cancer, and the detection of *TP53* gene mutations has been widely investigated as a potentially specific diagnostic marker in various cancers. In pancreatic ductal adenocarcinoma, *TP53* gene mutations are found in ~70% of invasive cancers (176). Although a few nucleotide hot spots of

*TP53* gene mutations are known to exist, mutations occur throughout the gene (177). In one study, assaying for eight common *TP53* gene mutations in stool with the use of a mismatch ligation, investigators identified 59% of stool samples from patients with colon cancer (178), but other studies have found a much lower percentage of recurrent *TP53* gene mutations (179). Hot spots of *TP53* gene mutation arise because of specific environmental factors, such as smoking or aflatoxin exposure, thus the prevalence of *TP53* mutational hotspots vary with the population under study. Most studies of *TP53* mutations as a cancer marker have used assays such as chip technologies, single-strand conformational polymorphism, and temperature gradient capillary electrophoresis that have the potential to identify the complete spectrum of *TP53* gene mutations. The sensitivity and limit of detection of these technologies are not as good as strategies that detect single nucleotide mutations. Assays such as single-strand conformational polymorphism (SSCP) and temperature gradient capillary electrophoresis can detect mutations that are present in at least 1% and more often 5-10% of the total DNA (180, 181). Using SSCP, investigators have reported the presence of *TP53* gene mutations, within exons 5-8, in pancreatic juice samples and in brush cytology specimens of 40%--50% of patients with pancreatic cancers (182). This figure is close to the number of mutations that one would expect to find in the primary pancreatic cancers from these patients (182). One study using temperature gradient gel electrophoresis demonstrated that pancreatic juice from patients with chronic pancreatitis rarely harbors *TP53* gene mutations (183). Gene chip technology has also been used for *TP53* gene mutation detection and it has the advantage that it can identify, in a single assay, a large percentage of possible *TP53* gene mutations in a given DNA sample as long as the mutation is present in the sample at sufficient concentration relative to normal DNA (>1% of DNA) (184). Although these gene chips are very effective in identifying missense mutations, they may miss the small deletions and insertions that represent ~10%--20% of all *TP53* gene mutations. In studies using Affymetrix™ *p53* gene chips, investigators were able to identify ~80% of all *TP53* gene mutations in non--small cell lung cancer tissues (179, 185). The concentration of mutant DNA in the pancreatic juice of patients with pancreatic cancer can be less than 1% of total DNA (186) so the utility of using *TP53* gene chip assays for pancreatic cancer diagnosis remains to be determined.

The emergence of chip technologies has also facilitated investigations into the diagnostic utility of mitochondrial mutations. Mitochondrial mutations are commonly found in multiple cancers types (187-191). One advantage of mitochondrial DNA as a marker for cancer is that each cell has many more copies of the mitochondrial genome than of nuclear DNA, and the amount of mitochondrial DNA in cancer cells is several times more abundant than it is in normal

tissues (187, 188, 190, 192). To efficiently interrogate the mitochondrial genome, a “MitoChip” has been developed (189), and initial studies suggest that this chip can be used to detect mitochondrial mutations in pancreatic juice samples obtained from patients with pancreatic cancer as well as from the urine of patients with urinary tract cancers (189). These results are preliminary and require confirmation in larger prospective studies.

### *Methylated DNA*

Since aberrant hypermethylation of tumor suppressor genes is common during carcinogenesis, DNA methylation abnormalities may be particularly suitable for use in early-detection strategies (193, 194). Several dozen genes, such as *SPARC*, *ppENK*, *p16*, *TSLC1* and *TFPI-2*, have been identified as aberrantly methylated in pancreatic carcinomas: some of these markers are aberrantly methylated in ~90% of pancreatic cancers (194-198). The detection of such aberrant methylation changes in clinical samples is feasible with methylation-specific PCR (MSP) (199). MSP requires bisulfite modification of the DNA to convert all unmethylated cytosines to uracils. MSP has been used successfully to identify methylated DNA in most biologic fluids, including blood, fine needle aspirates, saliva, and sputum from cancer patients (200). In addition, quantification of methylated DNA using real-time MSP is being employed in an attempt to distinguish more accurately cancers from non-neoplastic diseases. Initial studies in patients with pancreatic disease have demonstrated that aberrant DNA methylation patterns can be detected in pancreatic juice samples from patients with pancreatic cancer (195, 196, 201), and are only rarely found in pancreatic juice samples from patients without pancreatic cancer. Although these data are promising, several biologic features of DNA methylation require consideration when DNA methylation markers are considered for use in clinical practice as cancer markers. These include tissue-specific differences in normal methylation patterns, increases in DNA methylation with patient age, limitations of bisulfite modification, and the potential for methylated DNA markers to detect early neoplasia with little propensity for cancer (202-206). For example, some genes normally unmethylated in the pancreas and commonly methylated in pancreatic cancers undergo low-level methylation in non-neoplastic duodenal tissues. Some methylation in normal tissues such as duodenum is more likely to be present in older patients (201). This suggests that assaying for DNA methylation in pure pancreatic juice collected directly from the pancreatic duct will be preferable as it avoids possible duodenal contamination (201). Because MSP requires a bisulfite modification step that causes DNA degradation, MSP is not as sensitive as simple PCR, and has a lower limit of detection of ~10-20 copies (201). Even at this level of detection, MSP may

detect low-level methylation that arises either from normal aging or in lesions of low malignant potential. For example, low-grade PanINs develop with increasing frequency in the pancreas with increasing age, and some of these PanINs harbor methylation changes that are not found in normal pancreatic epithelium. For this reason, genes that only undergo methylation in high grade PanINs and invasive cancers are likely to have more diagnostic accuracy for detecting pancreatic cancer as opposed to those that are methylated in early PanIN (207).

### **Tissue Markers under investigation**

Many proteins that are differentially expressed in pancreatic cancer have been identified through global analyses of gene expression (208-217). For example, mesothelin (expressed in nearly 100% of pancreatic cancers) may prove to be a useful tumor antigen (218). Other over expressed proteins include prostate stem cell antigen (expressed in 60%), sea urchin fascin homolog (95%), claudin-4, 14-3-3-sigma, transglutaminase 2, CDC25B, ADAM9, cdc2/p34, heat shock protein 47, trefoil factor-2 (219) and topoisomerase II alpha (95%) (220-225). Two kallikreins, KLK6 and KLK10 are significantly up-regulated in pancreatic cancer (226), as are a number of members of the S100 protein family (227, 228), and aurora kinase (229). The clinical usefulness of detecting these markers has not yet been demonstrated. Gene expression microarray analysis is being studied as a prognostic tool for several cancers, but because of the dismal prognosis of pancreatic cancer, prognostic studies of pancreatic cancer markers are not being extensively investigated. Some markers may be useful for predicting if IPMNs have an associated infiltrating pancreatic carcinoma (such as claudin 4, S100A4, and mesothelin) (230). Such markers may prove useful to assay on suspected IPMNs before surgery to help predict whether an IPMN has an associated invasive cancer to help decide on surgery. The potential therapeutic/chemoprevention target cyclooxygenase-2 is expressed in most pancreatic cancers as are mediators of the sonic hedgehog pathway including PTCH (231, 232).

Increasingly, it is becoming important to identify molecular targets of novel cancer therapies. There is considerable hope that the future of pancreatic cancer diagnosis will include molecular profiling to identify therapeutic targets for novel therapies. One example of this paradigm is the genetic inactivation of the Fanconi anemia pathway in a small percentage of pancreatic adenocarcinomas (108). Cells with inactivation of the Fanconi pathway are hypersensitive to certain chemotherapeutics, such as mitomycin, which may explain previous clinical evidence of occasional treatment responses to mitomycin (233). These encouraging findings highlight the need to identify which pancreatic cancers harbor inactivation of the Fanconi anemia pathway. It is expected that as additional therapeutics are identified that target

specific pathways, accurate tumor markers will be needed to evaluate the status of these pathways to help determine whether a particular therapeutic is an appropriate target for an individual's pancreatic cancer.

## **CONCLUSIONS**

In conclusion, while CA19-9 remains the most commonly used pancreatic cancer tumor marker, there are currently many additional candidate markers under investigation. Additional markers are needed not only to facilitate the early diagnosis of pancreatic cancer, but also to help diagnose pancreatic cancer precursor lesions. Our knowledge of pancreatic neoplasia has increased years, and with it the evidence from clinical studies screening individuals at high risk of developing pancreatic cancer. These studies lend support to the idea that the best way to reduce the mortality of pancreatic cancer is to use molecular markers and pancreatic imaging to identify patients with precancerous lesions of the pancreas that are likely to progress to pancreatic cancer and treat these patients while they still have best chance of being cured.

**Table 1. Recommendations by different Expert Groups for use of CA 19-9 as a tumor marker for pancreatic cancer**

<b>Application</b>	<b>EGTM 1999 (74)</b>	<b>AGA 1999 (3)</b>	<b>NACB 2005</b>	<b>LOE [NACB]</b>
Screening	No specific recommendations	No specific screening method for high-risk subjects recommended; best strategy perhaps CT/EUS and CA 19-9. Clinical benefit unproven	Not for screening of the general population. CA19-9 is often normal in high-risk subjects with a strong family history of pancreatic cancer undergoing EUS/CT screening of the pancreas (ref. 69-70).	III
Diagnosis	Is a diagnostic aid for pancreatic malignancy, but with limited value, especially in early stages and the presence of cholestasis	No specific recommendations	If it is used, CA19-9 should be used in conjunction with an imaging test (CT, EUS). Appropriately interpreted CA19-9 values can guide further invasive testing (ERCP, EUS FNA, laparoscopy, laparotomy) in the appropriate clinical context.	I
Prognosis	Routine use for prognostic purposes of unproven value	No specific recommendations	Has independent prognostic value with regard to resectability and survival; Clinical decisions should be based on all available information	I
Monitoring	Routine use for monitoring of unproven value	Not an accepted test for anti-tumor efficacy	Serial measurements during palliative chemotherapy can be used in conjunction with imaging tests to determine response. Serial measurements recommended for follow-up after potentially curative surgery	I

CT, computed tomography; ERCP, endoscopic retrograde cholangiopancreatograph; EUS, endoscopic ultrasound; FNA, fine needle aspirate; LOE=level of evidence. I: Evidence from well-powered prospective controlled studies, or pooled or meta-analysis or level II or III studies. II: Marker evidence determined during a prospective therapeutic trial. III: Evidence from large prospective studies. IV: Evidence from small retrospective studies. V: Evidence from small pilot studies.

**Table 2: Gene testing for hereditary pancreatic cancer**

<b>Gene test</b>	<b>Characteristics and clinical implications</b>	<b>LOE</b>	<b>References</b>
<i>BRCA2</i>	Mutated in ~5% of patients with apparently sporadic pancreatic cancer and in 5-17% of patients with familial pancreatic cancer	I	101,102, 109-111
<i>STK11</i>	Germ line mutations only found in those with the Peutz Jeghers syndrome phenotype. Affected patients have ~30% risk of developing pancreatic cancer	I	103
<i>Cationic trypsinogen</i>	Gene testing in the context of hereditary pancreatitis	I	29,104, 105
<i>p16</i>	Considered for patients with pancreatic cancer occurring in families suspected of having familial atypical melanoma mole syndrome (FAMM). Prevalence of germ line p16 mutations not determined for families with pancreatic cancer and melanoma who do not meet criteria for FAMM	II	112-114

LOE=level of evidence.

**Table 3: Tissue markers for pancreatic ductal adenocarcinoma.**

<b>Tissue markers</b>	<b>Characteristics and clinical implications</b>	<b>LOE</b>	<b>Reference</b>
CK 7, CK 8, CK 18 and CK 19	Expressed in 70-100%	I	115-117
CK 17	Expressed in 50-70%	I	115-117
CK 20	Expressed in < 20%	I	115-117
CEA	Distinguish infiltrating adenocarcinoma from reactive glands	II	119
MUC 2	Expressed in < 10% pancreatic adenocarcinoma; but in majority of IPMN	II	124-126
MUC 6	Expressed in 25%;	II	124-126
Trefoil factor-2	Expressed in minority of pancreatic adenocarcinoma, but in >90% of IPMN and MCN	IV	216
Mesothelin	Expressed in close to 100%; pilot studies suggest protein expression by immunohistochemistry can aid in cytology interpretation	IV	215
MADH4/SMAD4/DPC4 tumor suppressor gene	Down-regulated in 55%; associated with poor prognosis	I	124-126

CK, cytokeratin; LOE=level of evidence.

**Table 4: Candidate serum protein markers of pancreatic ductal adenocarcinoma**

Marker	Phase of Development	Uses and Potential Uses	LOE*	References
CA19-9	Accepted clinical use	Monitoring of disease burden, adjunct to diagnosis	I	70-98
CA242	Evaluation	Diagnosis; not superior to CA19-9	III	126-133
CAM17.1	Evaluation	Diagnosis; not superior to CA19-9	III	134-137
TPS	Evaluation	Diagnosis; not superior to CA19-9	III	43, 138-141
MIC-1	Research/Discovery	Diagnosis; maybe more sensitive than CA19-9, but also often elevated in patients with chronic pancreatitis	III	142-145
Osteopontin	Research/Discovery	Diagnosis; studies using plasma and accurate assays required	IV	146-148
TIMP-1	Research/Discovery	Diagnosis; studies using plasma and accurate assays required	IV	149-152
SELDI proteomic profiling	Research/Discovery	Diagnosis; pilot studies have identified peptide markers, but require confirmatory studies	IV	155-158

LOE=level of evidence.

**Table 5: Candidate DNA markers applicable to pancreatic juice analysis**

Marker	Phase of Development	Potential Uses	LOE	References
Mutant p53	Evaluation	Diagnosis: Mutations thought to be highly specific for neoplasia but better assays are needed to detect the spectrum of p53 mutations, to detect them at low concentrations and to use assays that can be routinely applied in clinical labs.	I	173-183
Mutant KRAS	Evaluation	Diagnosis: KRAS mutations are not specific for pancreatic cancer. Further studies ongoing to evaluate the diagnostic accuracy of assays that quantify as opposed to merely detecting mutant KRAS levels	I	164-172
Methylated DNA	Evaluation	Diagnosis: Numerous candidate markers including SPARC, TFPI-2, ppENK, and others. Each are moderately sensitive and highly specific for pancreatic cancer using MSP in pure pancreatic juice. Ongoing studies are evaluating their diagnostic accuracy	IV	191-196-198
Mitochondrial DNA mutations	Evaluation	Diagnosis: Pilot studies using chip technology	IV	186

LOE=level of evidence.

## REFERENCES

1. Association AG. American Gastroenterological Association medical position statement: Epidemiology, diagnosis, and treatment of pancreatic ductal adenocarcinoma. *Gastroenterology* 1999;117(6):1463-1484.
2. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics 2003. *CA Cancer J. Clin.* 2003;53(1):5-26.
3. DiMagno EP, Reber HA, Tempero MA. AGA technical review on the epidemiology, diagnosis, and treatment of pancreatic ductal adenocarcinoma. American Gastroenterological Association. *Gastroenterology* 1999;117(6):1464-84.
4. Rosty C, Goggins M. Early detection of pancreatic carcinoma. *Hematol. Oncol. Clin. North. Am.* 2002;16(1):37-52.
5. Sosa JA, Bowman HM, Gordon TA, Bass EB, Yeo CJ, Lillemoe KD, et al. Importance of hospital volume in the overall management of pancreatic cancer. *Ann Surg* 1998;228(3):429-38.
6. Yeo CJ, Abrams RA, Grochow LB, Sohn TA, Ord SE, Hruban RH, et al. Pancreaticoduodenectomy for pancreatic adenocarcinoma: postoperative adjuvant chemoradiation improves survival. A prospective, single- institution experience. *Ann Surg* 1997;225(5):621-33; discussion 633-6.
7. Abrams RA, Grochow LB, Chakravarthy A, Sohn TA, Zahurak ML, Haulk TL, et al. Intensified adjuvant therapy for pancreatic and periampullary adenocarcinoma: survival results and observations regarding patterns of failure, radiotherapy dose and CA19-9 levels. *Int J Radiat Oncol Biol Phys* 1999;44(5):1039-46.
8. Choti MA. Adjuvant therapy for pancreatic cancer--the debate continues. *N Engl J Med* 2004;350(12):1249-51.
9. Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med* 2004;350(12):1200-10.
10. Sohn TA, Yeo CJ, Cameron JL, Iacobuzio-Donahue CA, Hruban RH, Lillemoe KD. Intraductal papillary mucinous neoplasms of the pancreas: an increasingly recognized clinicopathologic entity. *Ann Surg* 2001;234(3):313-21; discussion 321-2.
11. Kroep JR, Pinedo HM, van Groeningen CJ, Peters GJ. Experimental drugs and drug combinations in pancreatic cancer. *Ann Oncol* 1999;10(Suppl 4):234-8.
12. van Groeningen CJ. Intravenous and intra-arterial chemotherapeutic possibilities in biliopancreatic cancer. *Ann Oncol* 1999;10(Suppl 4):305-7.
13. van Riel JM, van Groeningen CJ, Pinedo HM, Giaccone G. Current chemotherapeutic possibilities in pancreaticobiliary cancer. *Ann Oncol* 1999;10(Suppl 4):157-61.
14. Rich TA. Chemoradiation for pancreatic and biliary cancer: current status of RTOG studies. *Ann Oncol* 1999;10(Suppl 4):231-3.
15. Veenhof CH. Pancreatic endocrine tumours, immunotherapy and gene therapy: chemotherapy and interferon therapy of endocrine tumours. *Ann Oncol* 1999;10(Suppl 4):185-7.
16. Jaffee EM, Abrams R, Cameron J, Donehower R, Duerr M, Gossett J, et al. A phase I clinical trial of lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene for the treatment of pancreatic adenocarcinoma. *Hum Gene Ther* 1998;9(13):1951-71.
17. Sohn TA, Lillemoe KD, Cameron JL, Huang JJ, Pitt HA, Yeo CJ. Surgical palliation of unresectable periampullary adenocarcinoma in the 1990s. *J Am Coll Surg* 1999;188(6):658-66; discussion 666-9.

18. Brown DL, Caswell RE, Wong GY, Nauss LA, Offord KP. Referral of patients with pain from pancreatic cancer for neurolytic celiac plexus block. *Mayo Clin Proc* 1997;72(9):831-4.
19. Wiersema MJ, Wiersema LM. Endosonography-guided celiac plexus neurolysis. *Gastrointest Endosc* 1996;44(6):656-62.
20. Russell RC. Palliation of pain and jaundice: an overview. *Ann Oncol* 1999;10(Suppl 4):165-9.
21. Tham TC, Lichtenstein DR, Vandervoort J, Wong RC, Slivka A, Banks PA, et al. Pancreatic duct stents for "obstructive type" pain in pancreatic malignancy. *Am J Gastroenterol* 2000;95(4):956-60.
22. Body JJ. The syndrome of anorexia-cachexia. *Curr Opin Oncol* 1999;11(4):255-60.
23. Barber MD, Ross JA, Voss AC, Tisdale MJ, Fearon KC. The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer [In Process Citation]. *Br J Cancer* 1999;81(1):80-6.
24. Yeo CJ, Cameron JL, Lillemoe KD, Sitzmann JV. Pancreaticoduodenectomy for cancer of the head of the pancreas:201 patients. *Ann. Surg.* 1995;221(6):721-31.
25. Canto MI GM, Yeo CJ, Griffin C, Axilbund JE, Brune K, Ali SZ, Jagannath S, Petersen GM, Fishman EK, Piantadosi S, Giardiello FM, and Hruban RH. Screening for pancreatic neoplasia in high risk individuals. *Clinical Gastroenterology and Hepatology* 2003;2:606-21.
26. Canto MI, Goggins M, Yeo CJ, Griffin C, Axilbund JE, Brune K, Ali SZ, Jagannath S, Petersen GM, Fishman EK, Piantadosi S, Giardiello FM, and Hruban RH. Screening for pancreatic neoplasia in high risk individuals. *Clin Gastro Hepatol* 2004;2:606-21.
27. Klein AP, Brune KA, Petersen GM, Goggins M, Tersmette AC, Offerhaus GJ, et al. Prospective risk of pancreatic cancer in familial pancreatic cancer kindreds. *Cancer Res* 2004;64(7):2634-8.
28. Brentnall TA, Bronner MP, Byrd DR, Haggitt RC, Kimmey MB. Early diagnosis and treatment of pancreatic dysplasia in patients with a family history of pancreatic cancer. *Ann Intern Med* 1999;131(4):247-55.
29. M. Canto MG, C. Yeo, EK Fishman, S. Jagannath, S. Kantsevov, C. Griffin, J. Axilbund, K. Brune, S. Ali, J. Richman, G. Petersen, F. Giardiello, R Hruban, AN Kalloo. Screening for pancreatic neoplasia in asymptomatic high risk individuals: A prospective, controlled study. AACR/Lustgarten Foundation Pancreatic Cancer Research Meeting, San Francisco, June 25, 2004 2004.
30. Ulrich CD. Pancreatic cancer in hereditary pancreatitis: consensus guidelines for prevention, screening and treatment. *Pancreatology* 2001;1(5):416-22.
31. Fleisher M, A.M. D, Sturgeon CM, Lamerz R, Witliff JL. Tumor markers: Physiology, Pathobiology, Technology and Clinical Applications. Chicago: AACCC press; 2002.
32. Chari ST, Klee GG, Miller LJ, Raimondo M, DiMagno EP. Islet amyloid polypeptide is not a satisfactory marker for detecting pancreatic cancer. *Gastroenterology* 2001;121(3):640-5.
33. Brand RE, Ding XZ, Young CM, Adrian TE. The specificity of amylin for the diagnosis of pancreatic adenocarcinoma. *Int J Gastrointest Cancer* 2002;31(1-3):123-8.
34. Lucarotti ME, Habib NA, Kelly SB, Rothnie ND, Nelson O, Lindholm L, et al. Clinical evaluation of combined use of CEA, CA19-9 and CA50 in the serum of patients with pancreatic carcinoma. *Eur J Surg Oncol* 1991;17(1):51-3.
35. Satake K, Takeuchi T. Comparison of CA19-9 with other tumor markers in the diagnosis of cancer of the pancreas. *Pancreas* 1994;9(6):720-4.
36. Kawa S, Kato M, Oguchi H, Hsue GL, Kobayashi T, Koiwai T, et al. Clinical evaluation of pancreatic cancer-associated mucin expressing CA19-9, CA50, Span-1, sialyl SSEA-1, and Dupan-2. *Scand J Gastroenterol* 1992;27(8):635-43.

37. Brockhaus M, Magnani JL, Herlyn M, Blaszczyk M, Stepiewski Z, Koprowski H, et al. Monoclonal antibodies directed against the sugar sequence of lacto-N-fucopentaose III are obtained from mice immunized with human tumors. *Arch Biochem Biophys* 1982;217(2):647-51.
38. Haglund C, Kuusela P, Jalanko H, Roberts PJ. Serum CA 50 as a tumor marker in pancreatic cancer: a comparison with CA 19-9. *Int J Cancer* 1987;39(4):477-81.
39. Kuusela P, Haglund C, Roberts PJ, Jalanko H. Comparison of CA-50, a new tumour marker, with carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) in patients with gastrointestinal diseases. *Br J Cancer* 1987;55(6):673-6.
40. Palsson B, Masson P, Andren-Sandberg A. Tumour marker CA 50 levels compared to signs and symptoms in the diagnosis of pancreatic cancer. *Eur J Surg Oncol* 1997;23(2):151-6.
41. Kornek G, Schenk T, Raderer M, Djavarnmad M, Scheithauer W. Tissue polypeptide-specific antigen (TPS) in monitoring palliative treatment response of patients with gastrointestinal tumours. *Br J Cancer* 1995;71(1):182-5.
42. Taylor OM, Cooper EH, Benson EA, McMahon MJ. The prognostic value of the tumour markers CA 195 and CEA in patients with adenocarcinoma of the pancreas. *Eur J Surg Oncol* 1992;18(5):508-13.
43. Hyoty M, Hyoty H, Aaran RK, Airo I, Nordback I. Tumour antigens CA 195 and CA 19-9 in pancreatic juice and serum for the diagnosis of pancreatic carcinoma. *Eur J Surg* 1992;158(3):173-9.
44. Masson P, Palsson B, Andren-Sandberg A. Evaluation of CEA, CA 19-9, CA-50, CA-195, and TATI with special reference to pancreatic disorders. *Int J Pancreatol* 1991;8(4):333-44.
45. Pasanen PA, Eskelinen M, Partanen K, Pikkarainen P, Penttila I, Alhava E. Tumour-associated trypsin inhibitor in the diagnosis of pancreatic carcinoma. *J Cancer Res Clin Oncol* 1994;120(8):494-7.
46. Aroasio E, Piantino P. Tumor-associated trypsin inhibitor in pancreatic diseases. *Scand J Clin Lab Invest Suppl* 1991;207:71-3.
47. Shahangian S, Fritsche HA, Jr., Hughes JI, Gelder FB. Pancreatic oncofetal antigen and carbohydrate antigen 19-9 in sera of patients with cancer of the pancreas. *Clin Chem* 1989;35(3):405-8.
48. Zhao XY, Yu SY, Da SP, Bai L, Guo XZ, Dai XJ, et al. A clinical evaluation of serological diagnosis for pancreatic cancer. *World J Gastroenterol* 1998;4(2):147-149.
49. Schmiegel WH, Kreiker C, Eberl W, Arndt R, Classen M, Greten H, et al. Monoclonal antibody defines CA 19-9 in pancreatic juices and sera. *Gut* 1985;26(5):456-60.
50. Fukushima N, Koopmann J, Sato N, Prasad N, Leach SD, Hruban RH, Goggins M. Gene expression alterations in the non-neoplastic parenchyma adjacent to infiltrating pancreatic ductal adenocarcinoma. *Mod Pathol* 2005;in press.
51. Schneider J, Schulze G. Comparison of tumor M2-pyruvate kinase (tumor M2-PK), carcinoembryonic antigen (CEA), carbohydrate antigens CA 19-9 and CA 72-4 in the diagnosis of gastrointestinal cancer. *Anticancer Res* 2003;23(6D):5089-93.
52. Oremek GM, Eigenbrodt E, Radle J, Zeuzem S, Seiffert UB. Value of the serum levels of the tumor marker TUM2-PK in pancreatic cancer. *Anticancer Res* 1997;17(4B):3031-3.
53. Cerwenka H, Aigner R, Bacher H, Werkgartner G, el-Shabrawi A, Quehenberger F, et al. TUM2-PK (pyruvate kinase type tumor M2), CA19-9 and CEA in patients with benign, malignant and metastasizing pancreatic lesions. *Anticancer Res* 1999;19(1B):849-51.
54. Schulze G. The tumor marker tumor M2-PK: an application in the diagnosis of gastrointestinal cancer. *Anticancer Res* 2000;20(6D):4961-4.

55. Hardt PD, Ngoumou BK, Rupp J, Schnell-Kretschmer H, Kloer HU. Tumor M2-pyruvate kinase: a promising tumor marker in the diagnosis of gastro-intestinal cancer. *Anticancer Res* 2000;20(6D):4965-8.
56. Cervello M, Giannitrapani L, La Rosa M, Notarbartolo M, D'Alessandro N, Virruso L, et al. Expression of HIP/PAP mRNA in human hepatoma cell lines. *Ann N Y Acad Sci* 2002;963:53-8.
57. Christa L, Simon MT, Brezault-Bonnet C, Bonte E, Carnot F, Zylberberg H, et al. Hepatocarcinoma-intestine-pancreas/pancreatic associated protein (HIP/PAP) is expressed and secreted by proliferating ductules as well as by hepatocarcinoma and cholangiocarcinoma cells. *Am J Pathol* 1999;155(5):1525-33.
58. Iovanna JL, Keim V, Nordback I, Montalto G, Camarena J, Letoublon C, et al. Serum levels of pancreatitis-associated protein as indicators of the course of acute pancreatitis. Multicentric Study Group on Acute Pancreatitis. *Gastroenterology* 1994;106(3):728-34.
59. Rosty C, Christa L, Kuzdzal S, Baldwin WM, Zahurak ML, Carnot F, et al. Identification of hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein I as a biomarker for pancreatic ductal adenocarcinoma by protein biochip technology. *Cancer Res* 2002;62(6):1868-75.
60. Cerwenka H, Aigner R, Bacher H, Werkgartner G, El-Shabrawi A, Quehenberger F, et al. Pancreatitis-associated protein (PAP) in patients with pancreatic cancer. *Anticancer Res* 2001;21(2B):1471-74.
61. Lamerz R. Role of tumour markers, cytogenetics. *Ann Oncol* 1999;10 Suppl 4:145-9.
62. Schlieman MG, Ho HS, Bold RJ. Utility of tumor markers in determining resectability of pancreatic cancer. *Arch Surg* 2003;138(9):951-5; discussion 955-6.
63. Audisio RA, Veronesi P, Maisonneuve P, Chiappa A, Andreoni B, Bombardieri E, et al. Clinical relevance of serological markers in the detection and follow-up of pancreatic adenocarcinoma. *Surg Oncol* 1996;5(2):49-63.
64. Gattani AM, Mandeli J, Bruckner HW. Tumor markers in patients with pancreatic carcinoma. *Cancer* 1996;78(1):57-62.
65. Carpelan-Holmstrom M, Louhimo J, Stenman UH, Alfthan H, Haglund C. CEA, CA 19-9 and CA 72-4 improve the diagnostic accuracy in gastrointestinal cancers. *Anticancer Res* 2002;22(4):2311-6.
66. Heptner G, Domschke S, Domschke W. Comparison of CA 72-4 with CA 19-9 and carcinoembryonic antigen in the serodiagnostics of gastrointestinal malignancies. *Scand J Gastroenterol* 1989;24(6):745-50.
67. Wu JT, Carlisle P. Low frequency and low level of elevation of serum CA 72-4 in human carcinomas in comparison with established tumor markers. *J Clin Lab Anal* 1992;6(1):59-64.
68. Kawa S, Oguchi H, Kobayashi T, Tokoo M, Furuta S, Kanai M, et al. Elevated serum levels of Dupan-2 in pancreatic cancer patients negative for Lewis blood group phenotype. *Br J Cancer* 1991;64(5):899-902.
69. Satake K, Chung YS, Umeyama K, Takeuchi T, Kim YS. The possibility of diagnosing small pancreatic cancer (less than 4.0 cm) by measuring various serum tumor markers. A retrospective study. *Cancer* 1991;68(1):149-52.
70. Frena A. SPan-1 and exocrine pancreatic carcinoma. The clinical role of a new tumor marker. *Int J Biol Markers* 2001;16(3):189-97.
71. Tumour markers in gastrointestinal cancers--EGTM recommendations. European Group on Tumour Markers. *Anticancer Res* 1999;19(4A):2811-5.
72. Steinberg W. The clinical utility of the CA 19-9 tumor-associated antigen. *Am J Gastroenterol* 1990;85(4):350-5.
73. Duffy MJ. CA 19-9 as a marker for gastrointestinal cancers: a review. *Ann Clin Biochem* 1998;35 ( Pt 3):364-70.

74. Ritts RE, Pitt HA. CA 19-9 in pancreatic cancer. *Surg Oncol Clin N Am* 1998;7(1):93-101.
75. Posner MR, Mayer RJ. The use of serologic tumor markers in gastrointestinal malignancies. *Hematol Oncol Clin North Am* 1994;8(3):533-53.
76. Sawabu N, Watanabe H, Yamaguchi Y, Ohtsubo K, Motoo Y. Serum tumor markers and molecular biological diagnosis in pancreatic cancer. *Pancreas* 2004;28(3):263-7.
77. Kim HJ, Kim MH, Myung SJ, Lim BC, Park ET, Yoo KS, et al. A new strategy for the application of CA19-9 in the differentiation of pancreaticobiliary cancer: analysis using a receiver operating characteristic curve. *Am J Gastroenterol* 1999;94(7):1941-6.
78. Pleskow DK, Berger HJ, Gyves J, Allen E, McLean A, Podolsky DK. Evaluation of a serologic marker, CA19-9, in the diagnosis of pancreatic cancer. *Ann Intern Med* 1989;110(9):704-9.
79. Ritts RE, Jr., Nagorney DM, Jacobsen DJ, Talbot RW, Zurawski VR, Jr. Comparison of preoperative serum CA19-9 levels with results of diagnostic imaging modalities in patients undergoing laparotomy for suspected pancreatic or gallbladder disease. *Pancreas* 1994;9(6):707-16.
80. Forsmark CE, Lambiase L, Vogel SB. Diagnosis of pancreatic cancer and prediction of unresectability using the tumor-associated antigen CA19-9. *Pancreas* 1994;9(6):731-4.
81. Safi F, Schlosser W, Falkenreck S, Beger HG. CA 19-9 serum course and prognosis of pancreatic cancer. *Int J Pancreatol* 1996;20(3):155-61.
82. Yeo CJ, Cameron JL. Prognostic factors in ductal pancreatic cancer. *Langenbecks Arch Surg* 1998;383(2):129-33.
83. Albert MB, Steinberg WM, Henry JP. Elevated serum levels of tumor marker CA19-9 in acute cholangitis. *Dig Dis Sci* 1988;33(10):1223-5.
84. Kim JE, Lee KT, Lee JK, Paik SW, Rhee JC, Choi KW. Clinical usefulness of carbohydrate antigen 19-9 as a screening test for pancreatic cancer in an asymptomatic population. *J Gastroenterol Hepatol* 2004;19(2):182-6.
85. Brentnall TA, Bronner MP, Byrd DR, Haggitt RC, Kimmey MB. Early diagnosis and treatment of pancreatic dysplasia in patients with a family history of pancreatic cancer. *Ann. Intern. Med.* 1999;131(4):247-55.
86. Hruban RH, Goggins M, Parsons J, Kern SE. Progression model for pancreatic cancer. *Clin Cancer Res* 2000;6(8):2969-72.
87. Hruban RH, Adsay NV, Albores-Saavedra J, Compton C, Garrett ES, Goodman SN, et al. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. *Am J Surg Pathol* 2001;25(5):579-86.
88. Hruban RH, Takaori K, Klimstra DS, Adsay NV, Albores-Saavedra J, Biankin AV, et al. An Illustrated Consensus on the Classification of Pancreatic Intraepithelial Neoplasia and Intraductal Papillary Mucinous Neoplasms. *Am J Surg Pathol* 2004;28(8):977-987.
89. Grem J. The prognostic importance of tumor markers in adenocarcinomas of the gastrointestinal tract. *Curr Opin Oncol* 1997;9(4):380-7.
90. Montgomery RC, Hoffman JP, Riley LB, Rogatko A, Ridge JA, Eisenberg BL. Prediction of recurrence and survival by post-resection CA 19-9 values in patients with adenocarcinoma of the pancreas. *Ann Surg Oncol* 1997;4(7):551-6.
91. Katz A, Hanlon A, Lanciano R, Hoffman J, Coia L. Prognostic value of CA 19-9 levels in patients with carcinoma of the pancreas treated with radiotherapy. *Int J Radiat Oncol Biol Phys* 1998;41(2):393-6.
92. Saad ED, Machado MC, Wajsbrodt D, Abramoff R, Hoff PM, Tabacof J, et al. Pretreatment CA 19-9 level as a prognostic factor in patients with advanced pancreatic cancer treated with gemcitabine. *Int J Gastrointest Cancer* 2002;32(1):35-41.
93. Berger AC, Meszoely IM, Ross EA, Watson JC, Hoffman JP. Undetectable Preoperative Levels of Serum CA 19-9 Correlate with Improved Survival for Patients with Resectable Pancreatic Adenocarcinoma. *Ann Surg Oncol* 2004;11(7):644-9.

94. Ziske C, Schlie C, Gorschluter M, Glasmacher A, Mey U, Strehl J, et al. Prognostic value of CA 19-9 levels in patients with inoperable adenocarcinoma of the pancreas treated with gemcitabine. *Br J Cancer* 2003;89(8):1413-7.
95. Halm U, Schumann T, Schiefke I, Witzigmann H, Mossner J, Keim V. Decrease of CA 19-9 during chemotherapy with gemcitabine predicts survival time in patients with advanced pancreatic cancer. *Br J Cancer* 2000;82(5):1013-6.
96. Stemmler J, Stieber P, Szymala AM, Schalhorn A, Schermuly MM, Wilkowski R, et al. Are serial CA 19-9 kinetics helpful in predicting survival in patients with advanced or metastatic pancreatic cancer treated with gemcitabine and cisplatin? *Onkologie* 2003;26(5):462-7.
97. Gogas H, Lofts FJ, Evans TR, Daryanani S, Mansi JL. Are serial measurements of CA19-9 useful in predicting response to chemotherapy in patients with inoperable adenocarcinoma of the pancreas? *Br J Cancer* 1998;77(2):325-8.
98. Heinemann V, Schermuly MM, Stieber P, Schulz L, Jungst D, Wilkowski R, et al. CA19-9: a predictor of response in pancreatic cancer treated with gemcitabine and cisplatin. *Anticancer Res* 1999;19(4A):2433-5.
99. Micke O, Bruns F, Kurowski R, Horst E, deVries AF, Hausler JW, et al. Predictive value of carbohydrate antigen 19-9 in pancreatic cancer treated with radiochemotherapy. *Int J Radiat Oncol Biol Phys* 2003;57(1):90-7.
100. Ohara K, Tatsuzaki H, Molotkova NG, Oda T, Yuzawa K, Saida Y, et al. Utility of serum CA 19-9 monitoring in preoperative radiotherapy for pancreatic cancer. *Hepatogastroenterology* 2001;48(39):859-63.
101. Klein AP, Beaty TH, Bailey-Wilson JE, Brune KA, Hruban RH, Petersen GM. Evidence for a major gene influencing risk of pancreatic cancer. *Genet Epidemiol* 2002;23(2):133-49.
102. Ozcelik H, Schmocker B, Di Nicola N, Shi XH, Langer B, Moore M, et al. Germline BRCA2 6174delT mutations in Ashkenazi Jewish pancreatic cancer patients. *Nat Genet* 1997;16(1):17-8.
103. Goggins M, Schutte M, Lu J, Moskaluk CA, Weinstein CL, Petersen GM, et al. Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. *Cancer Res* 1996;56:5360-5364.
104. Giardiello FM, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. *Gastroenterology* 2000;119(6):1447-53.
105. Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, et al. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group [see comments]. *N Engl J Med* 1993;328(20):1433-7.
106. Lowenfels AB, Maisonneuve P, DiMagno EP, Elitsur Y, Gates LK, Jr., Perrault J, et al. Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. *J Natl Cancer Inst* 1997;89(6):442-6.
107. Goldstein AM, Fraser MC, Struewing JP, Hussussian CJ, Ranade K, Zametkin DP, et al. Increased risk of pancreatic cancer in melanoma-prone kindreds with p16INK4 mutations. *N Engl J Med* 1995;333: 970-974.
108. van der Heijden MS, Yeo CJ, Hruban RH, Kern SE. Fanconi anemia gene mutations in young-onset pancreatic cancer. *Cancer Res* 2003;63(10):2585-8.
109. Petersen GM, Hruban RH. Familial pancreatic cancer: where are we in 2003? *J Natl Cancer Inst* 2003;95(3):180-1.
110. Murphy KM, Brune KA, Griffin C, Sollenberger JE, Petersen GM, Bansal R, et al. Evaluation of Candidate Genes MAP2K4, MADH4, ACVR1B, and BRCA2 in Familial Pancreatic Cancer: Deleterious BRCA2 Mutations in 17%. *Cancer Res* 2002;62(13):3789-93.
111. Hahn SA, Greenhalf B, Ellis I, Sina-Frey M, Rieder H, Korte B, et al. BRCA2 germline mutations in familial pancreatic carcinoma. *J Natl Cancer Inst* 2003;95(3):214-21.

112. Real FX, Malats N, Lesca G, Porta M, Chopin S, Lenoir GM, et al. Family history of cancer and germline BRCA2 mutations in sporadic exocrine pancreatic cancer. *Gut* 2002;50(5):653-7.
113. H.T.Lynch RMF, J.L. Lynch, C.R. Kapler, L. Fusaro, R. Brand, M. Goggins, S.E. Kern. Genetic counseling and testing for germline p16 mutations in two pancreatic cancer-prone families. *Gastroenterology* 2000;in press.
114. Lynch HT, Brand RE, Hogg D, Deters CA, Fusaro RM, Lynch JF, et al. Phenotypic variation in eight extended CDKN2A germline mutation familial atypical multiple mole melanoma-pancreatic carcinoma-prone families. *Cancer* 2002;94(1):84-96.
115. Rutter JL, Bromley CM, Goldstein AM, Elder DE, Holly EA, Guerry Dt, et al. Heterogeneity of risk for melanoma and pancreatic and digestive malignancies: a melanoma case-control study. *Cancer* 2004;101(12):2809-16.
116. Solcia E, Capella, C., and Klöppel, G. Atlas of tumor pathology: Tumors of the pancreas. 3rd series ed. Washington, DC: Armed Forces Institute of Pathology; 1997.
117. Iacobuzio-Donahue C, Hruban, R. H. Gene expression in neoplasms of the pancreas: applications to diagnostic pathology. *Adv Anat Pathol* 2003;10:125-134.
118. Iacobuzio-Donahue CA, Hruban, R. H. Expression Profiling of Pancreatic Ductal Adenocarcinoma. In: Gerald MLaWL, editor. *Expression Profiling of Human Tumors: Diagnostic and Research Applications*: Totowa: Humana Press Inc.; 2003. p. 257-275.
119. Balague C, Audie, J. P., Porchet, N., and Real, F. X. In situ hybridization shows distinct patterns of mucin gene expression in normal, benign, and malignant pancreas tissues. *Gastroenterology* 1995;109:953-964.
120. Shimizu M, Saitoh, Y., Ohyanagi, H., and Itoh, H. Immunohistochemical staining of pancreatic cancer with CA19-9, KM01, unabsorbed CEA, and absorbed CEA. *Arch Pathol Lab Med* 1990;114:195-200.
121. Terada T, Ohta, T., Sasaki, M., Nakanuma, Y., and Kim, Y. S. Expression of MUC apomucins in normal pancreas and pancreatic tumours. *J Pathol* 1996;180:160-5.
122. Lüttges J, Zamboni, G., Longnecker, D. S., and Klöppel, G. The immunohistochemical mucin expression pattern distinguishes different types of intraductal papillary mucinous neoplasms of the pancreas and determines their relationship to mucinous noncystic carcinoma and ductal adenocarcinoma. *Am.J.Surg.Pathol.* 2001;25:942-8.
123. Lüttges J, Feyerabend, B., Buchelt, T., Pacena, M., and Klöppel, G. The mucin profile of noninvasive and invasive mucinous cystic neoplasms of the pancreas. *Am J Surg.Pathol* 2002;26:466-71.
124. Adsay NV, Merati, K., Andea, A., Sarkar, F., Hruban, R. H., Wilentz, R. E., Goggins, M., Iacobuzio-Donahue, C., Longnecker, D. S., and Klimstra, D. S. The dichotomy in the preinvasive neoplasia to invasive carcinoma sequence in the pancreas: differential expression of MUC1 and MUC2 supports the existence of two separate pathways of carcinogenesis. *Mod Pathol* 2002;15:1087-95.
125. Andrianifahanana M, Moniaux N, Schmied BM, Ringel J, Friess H, Hollingsworth MA, et al. Mucin (MUC) gene expression in human pancreatic adenocarcinoma and chronic pancreatitis: a potential role of MUC4 as a tumor marker of diagnostic significance. *Clin Cancer Res* 2001;7(12):4033-40.
126. Swartz MJ, Batra SK, Varshney GC, Hollingsworth MA, Yeo CJ, Cameron JL, et al. MUC4 expression increases progressively in pancreatic intraepithelial neoplasia. *Am J Clin Pathol* 2002;117(5):791-6.
127. Tascilar M, Skinner HG, Rosty C, Sohn T, Wilentz RE, Offerhaus GJ, et al. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2001;7(12):4115-21.

128. Wilentz RE, Iacobuzio-Donahue CA, Argani P, McCarthy DM, Parsons JL, Yeo CJ, et al. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. *Cancer Res* 2000;60(7):2002-6.
129. Tascilar M, Offerhaus GJ, Altink R, Argani P, Sohn TA, Yeo CJ, et al. Immunohistochemical labeling for the Dpc4 gene product is a specific marker for adenocarcinoma in biopsy specimens of the pancreas and bile duct. *Am J Clin Pathol* 2001;116(6):831-7.
130. Haglund C, Lundin J, Kuusela P, Roberts PJ. CA 242, a new tumour marker for pancreatic cancer: a comparison with CA 19-9, CA 50 and CEA. *Br J Cancer* 1994;70(3):487-92.
131. Ventrucchi M, Ubalducci GM, Cipolla A, Panella MA, Ligabue A. Serum CA 242: the search for a valid marker of pancreatic cancer. *Clin Chem Lab Med* 1998;36(3):179-84.
132. Ozkan H, Kaya M, Cengiz A. Comparison of tumor marker CA 242 with CA 19-9 and carcinoembryonic antigen (CEA) in pancreatic cancer. *Hepatogastroenterology* 2003;50(53):1669-74.
133. Kawa S, Tokoo M, Hasebe O, Hayashi K, Imai H, Oguchi H, et al. Comparative study of CA242 and CA19-9 for the diagnosis of pancreatic cancer. *Br J Cancer* 1994;70(3):481-6.
134. Nilsson O, Johansson C, Glimelius B, Persson B, Norgaard-Pedersen B, Andren-Sandberg A, et al. Sensitivity and specificity of CA242 in gastro-intestinal cancer. A comparison with CEA, CA50 and CA 19-9. *Br J Cancer* 1992;65(2):215-21.
135. Plebani M, Basso D, Navaglia F, D'Angeli F, Panozzo MP, Del Giudice G, et al. Is CA242 really a new tumour marker for pancreatic adenocarcinoma? *Oncology* 1995;52(1):19-23.
136. Banfi G, Bravi S, Ardemagni A, Zerbi A. CA 19.9, CA 242 and CEA in the diagnosis and follow-up of pancreatic cancer. *Int J Biol Markers* 1996;11(2):77-81.
137. Lundin J, Roberts PJ, Kuusela P, Haglund C. Prognostic significance of serum CA 242 in pancreatic cancer. A comparison with CA 19-9. *Anticancer Res* 1995;15(5B):2181-6.
138. Eccleston DW, Milton JD, Hoffman J, Bara J, Rhodes JM. Pancreatic tumour marker anti-mucin antibody CAM 17.1 reacts with a sialyl blood group antigen, probably I, which is expressed throughout the human gastrointestinal tract. *Digestion* 1998;59(6):665-70.
139. Gansauge F, Gansauge S, Parker N, Beger M, Poch B, Link KH, et al. CAM 17.1--a new diagnostic marker in pancreatic cancer. *Br J Cancer* 1996;74(12):1997-2002.
140. Parker N, Makin CA, Ching CK, Eccleston D, Taylor OM, Milton JD, et al. A new enzyme-linked lectin/mucin antibody sandwich assay (CAM 17.1/WGA) assessed in combination with CA 19-9 and peanut lectin binding assay for the diagnosis of pancreatic cancer. *Cancer* 1992;70(5):1062-8.
141. Yiannakou JY, Newland P, Calder F, Kingsnorth AN, Rhodes JM. Prospective study of CAM 17.1/WGA mucin assay for serological diagnosis of pancreatic cancer. *Lancet* 1997;349(9049):389-92.
142. Glimelius B, Hoffman K, Einarsson R, Pahlman L, Graf W. Monitoring palliative chemotherapy in advanced gastrointestinal cancer using serial tissue polypeptide specific antigen (TPS) measurements. *Acta Oncol* 1996;35(2):141-8.
143. Plebani M, Basso D, Del Favero G, Ferrara C, Meggiato T, Fogar P, et al. Clinical utility of TPS, TPA and CA 19-9 measurement in pancreatic cancer. *Oncology* 1993;50(6):436-40.
144. Banfi G, Zerbi A, Pastori S, Parolini D, Di Carlo V, Bonini P. Behavior of tumor markers CA19.9, CA195, CAM43, CA242, and TPS in the diagnosis and follow-up of pancreatic cancer. *Clin Chem* 1993;39(3):420-3.
145. Slesak B, Harlozinska-Szmyrka A, Knast W, Sedlaczek P, van Dalen A, Einarsson R. Tissue polypeptide specific antigen (TPS), a marker for differentiation between pancreatic carcinoma and chronic pancreatitis. A comparative study with CA 19-9. *Cancer* 2000;89(1):83-8.

146. Buckhaults P, Rago C, St Croix B, Romans KE, Saha S, Zhang L, et al. Secreted and cell surface genes expressed in benign and malignant colorectal tumors. *Cancer Res* 2001;61(19):6996-7001.
147. Koopmann J, Buckhaults P, Brown DA, Zahurak ML, Sato N, Fukushima N, et al. Serum macrophage inhibitory cytokine 1 as a marker of pancreatic and other periampullary cancers. *Clin Cancer Res* 2004;10(7):2386-92.
148. Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, et al. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. *Cancer Res* 2001;61(16):5974-8.
149. Brown DA, Ward RL, Buckhaults P, Liu T, Romans KE, Hawkins NJ, et al. MIC-1 serum level and genotype: associations with progress and prognosis of colorectal carcinoma. *Clin Cancer Res* 2003;9(7):2642-50.
150. Rittling SR, Chambers AF. Role of osteopontin in tumour progression. *Br J Cancer* 2004;90(10):1877-81.
151. Furger KA, Menon RK, Tuckl AB, Bramwell VH, Chambers AF. The functional and clinical roles of osteopontin in cancer and metastasis. *Curr Mol Med* 2001;1(5):621-32.
152. Iacobuzio-Donahue CA, Maitra A, Shen-Ong GL, Van Heek T, Ashfaq R, Meyer R, et al. Discovery of novel tumor markers of pancreatic cancer using global gene expression technology. *Am. J. Pathol.* 2002;160(4):1239-1249.
153. Zhou W, Sokoll LJ, Bruzek DJ, Zhang L, Velculescu VE, Goldin SB, et al. Identifying markers for pancreatic cancer by gene expression analysis. *Cancer Epidemiol. Biomarkers Prev.* 1998;7(2):109-112.
154. Yukawa N, Yoshikawa T, Akaike M, Sugimasa Y, Takemiya S, Yanoma S, et al. Prognostic impact of tissue inhibitor of matrix metalloproteinase-1 in plasma of patients with colorectal cancer. *Anticancer Res* 2004;24(3b):2101-5.
155. Schrohl AS, Holten-Andersen MN, Peters HA, Look MP, Meijer-van Gelder ME, Klijn JG, et al. Tumor tissue levels of tissue inhibitor of metalloproteinase-1 as a prognostic marker in primary breast cancer. *Clin Cancer Res* 2004;10(7):2289-98.
156. Petricoin EF, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, et al. Use of proteomic patterns in serum to identify ovarian cancer. In: *Lancet*; 2002. p. 572-7.
157. Petricoin EF, 3rd, Ornstein DK, Pawletz CP, Ardekani A, Hackett PS, Hitt BA, et al. Serum proteomic patterns for detection of prostate cancer. In: *J Natl Cancer Inst*; 2002. p. 1576-8.
158. Koopmann J, Zhang Z, White N, Rosenzweig J, Fedarko N, Jagannath S, et al. Serum diagnosis of pancreatic adenocarcinoma using surface-enhanced laser desorption and ionization mass spectrometry. In: *Clin Cancer Res*; 2004. p. 860-8.
159. Koopmann J, Fedarko NS, Jain A, Maitra A, Iacobuzio-Donahue C, Rahman A, et al. Evaluation of osteopontin as biomarker for pancreatic adenocarcinoma. In: *Cancer Epidemiol Biomarkers Prev*; 2004. p. 487-91.
160. Koopmann J, Buckhaults P, Brown DA, Zahurak ML, Sato N, Fukushima N, et al. Serum macrophage inhibitory cytokine 1 as a marker of pancreatic and other periampullary cancers. In: *Clin Cancer Res*; 2004. p. 2386-92.
161. Rosty C, Christa L, Kuzdzal S, Baldwin WM, Zahurak ML, Carnot F, et al. Identification of hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein I as a biomarker for pancreatic ductal adenocarcinoma by protein biochip technology. In: *Cancer Res*; 2002. p. 1868-75.
162. Zhang Z, Bast RC, Jr., Yu Y, Li J, Sokoll LJ, Rai AJ, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. *Cancer Res* 2004;64(16):5882-90.

163. Yamada T, Nakamori S, Ohzato H, Oshima S, Aoki T, Higaki N, et al. Detection of K-ras gene mutations in plasma DNA of patients with pancreatic adenocarcinoma: correlation with clinicopathological features. *Clinical Cancer Research* 1998;4(6):1527-32.
164. Mulcahy HE, Lyautey J, Lederrey C, qi Chen X, Anker P, Alstead EM, et al. A prospective study of K-ras mutations in the plasma of pancreatic cancer patients. *Clinical Cancer Research* 1998;4(2):271-5.
165. Castells A, Puig P, Mora J, Boadas J, Boix L, Urgell E, et al. K-ras mutations in DNA extracted from the plasma of patients with pancreatic carcinoma: diagnostic utility and prognostic significance. *J Clin Oncol* 1999;17(2):578-84.
166. Harden SV, Sanderson H, Goodman SN, Partin AA, Walsh PC, Epstein JI, et al. Quantitative GSTP1 methylation and the detection of prostate adenocarcinoma in sextant biopsies. *J Natl Cancer Inst* 2003;95(21):1634-7.
167. Jaffee EM, Hruban RH, Canto M, Kern SE. Focus on pancreas cancer. *Cancer Cell* 2002;2(1):25-8.
168. Calhoun ES, Jones JB, Ashfaq R, Adsay V, Baker SJ, Valentine V, et al. BRAF and FBXW7 (CDC4, FBW7, AGO, SEL10) mutations in distinct subsets of pancreatic cancer: potential therapeutic targets. *Am J Pathol* 2003;163(4):1255-60.
169. Cohen Y, Xing M, Mambo E, Guo Z, Wu G, Trink B, et al. BRAF mutation in papillary thyroid carcinoma. *J Natl Cancer Inst* 2003;95(8):625-7.
170. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002;417(6892):949-54.
171. Berger DH, Chang H, Wood M, Huang L, Heath CW, Lehman T, et al. Mutational activation of K-ras in nonneoplastic exocrine pancreatic lesions in relation to cigarette smoking status [In Process Citation]. *Cancer* 1999;85(2):326-32.
172. Caldas C, Hahn SA, da Costa LT, Redston MS, Schutte M, Seymour AB, et al. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma [published erratum appears in *Nat Genet* 1994 Dec;8(4):410]. *Nat Genet* 1994;8(1):27-32.
173. Kalthoff H, Schmiegel W, Roeder C, Kasche D, Schmidt A, Lauer G, et al. p53 and K-RAS alterations in pancreatic epithelial cell lesions. *Oncogene* 1993;8(2):289-98.
174. Moskaluk CA, Hruban RH, Kern SE. p16 and K-ras mutations in the intraductal precursors of human pancreatic adenocarcinoma. *Cancer Res* 1997;57:2140-2143.
175. Tada M, Omata M, Kawai S, Saisho H, Ohto M, Saiki RK, et al. Detection of ras gene mutations in pancreatic juice and peripheral blood of patients with pancreatic adenocarcinoma. *Cancer Research* 1993;53(11):2472-4.
176. Redston MS, Caldas C, Seymour AB, Hruban RH, da Costa L, Yeo CJ, et al. p53 mutations in pancreatic carcinoma and evidence of common involvement of homocopolymer tracts in DNA microdeletions. *Cancer Res* 1994;54:3025-3033.
177. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science* 1991;253(5015):49-53.
178. Dong SM, Traverso G, Johnson C, Geng L, Favis R, Boynton K, et al. Detecting colorectal cancer in stool with the use of multiple genetic targets. *J Natl Cancer Inst* 2001;93(11):858-65.
179. Ahrendt SA, Hu Y, Buta M, McDermott MP, Benoit N, Yang SC, et al. p53 mutations and survival in stage I non-small-cell lung cancer: results of a prospective study. *J Natl Cancer Inst* 2003;95(13):961-70.
180. Yamaguchi Y, Watanabe H, Yrdiran S, Ohtsubo K, Motoo Y, Okai T, et al. Detection of mutations of p53 tumor suppressor gene in pancreatic juice and its application to diagnosis of patients with pancreatic cancer: comparison with K-ras mutation. *Clin Cancer Res* 1999;5(5):1147-53.

181. Kaino M, Kondoh S, Okita S, Hatano S, Shiraishi K, Kaino S, et al. Detection of K-ras and p53 gene mutations in pancreatic juice for the diagnosis of intraductal papillary mucinous tumors. *Pancreas* 1999;18(3):294-9.
182. Sturm PD, Hruban RH, Ramsoekh TB, Noorduyt LA, Tytgat GN, Gouma DJ, et al. The potential diagnostic use of K-ras codon 12 and p53 alterations in brush cytology from the pancreatic head region. *J Pathol* 1998;186(3):247-53.
183. Lohr M, Muller P, Mora J, Brinkmann B, Ostwald C, Farre A, et al. p53 and K-ras mutations in pancreatic juice samples from patients with chronic pancreatitis. *Gastrointest Endosc* 2001;53(7):734-43.
184. Wikman FP, Lu ML, Thykjaer T, Olesen SH, Andersen LD, Cordon-Cardo C, et al. Evaluation of the performance of a p53 sequencing microarray chip using 140 previously sequenced bladder tumor samples. *Clin Chem* 2000;46(10):1555-61.
185. Ahrendt SA, Halachmi S, Chow JT, Wu L, Halachmi N, Yang SC, et al. Rapid p53 sequence analysis in primary lung cancer using an oligonucleotide probe array. *Proc Natl Acad Sci U S A* 1999;96(13):7382-7.
186. Shi C ES, Jones D, Fukushima N, Hua L, Parker AR, Yeo CJ, Hruban RH, Goggins MG, Eshleman JR. LigAmp for sensitive detection of single-nucleotide differences. *Nature Methods* 2004;1:141-7.
187. Polyak K, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD, et al. Somatic mutations of the mitochondrial genome in human colorectal tumours. *Nat Genet* 1998;20(3):291-3.
188. Jones JB, Song JJ, Hempen PM, Parmigiani G, Hruban RH, Kern SE. Detection of mitochondrial DNA mutations in pancreatic cancer offers a "mass"-ive advantage over detection of nuclear DNA mutations. *Cancer Res* 2001;61(4):1299-304.
189. Maitra A, Cohen Y, Gillespie SE, Mambo E, Fukushima N, Hoque MO, et al. The Human MitoChip: a high-throughput sequencing microarray for mitochondrial mutation detection. *Genome Res* 2004;14(5):812-9.
190. Fliss MS, Usadel H, Caballero OL, Wu L, Buta MR, Eleff SM, et al. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. *Science* 2000;287(5460):2017-9.
191. Sanchez-Cespedes M, Parrella P, Nomoto S, Cohen D, Xiao Y, Esteller M, et al. Identification of a mononucleotide repeat as a major target for mitochondrial DNA alterations in human tumors. *Cancer Res* 2001;61(19):7015-9.
192. Nomoto S, Yamashita K, Koshikawa K, Nakao A, Sidransky D. Mitochondrial D-loop mutations as clonal markers in multicentric hepatocellular carcinoma and plasma. *Clin Cancer Res* 2002;8(2):481-7.
193. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002;3(6):415-28.
194. Sato N, Fukushima N, Maehara N, Matsubayashi H, Koopmann J, Su GH, et al. SPARC/osteonectin is a frequent target for aberrant methylation in pancreatic adenocarcinoma and a mediator of tumor-stromal interactions. *Oncogene* 2003;22(32):5021-30.
195. Sato N, Parker AR, Fukushima N, Miyagi Y, Iacobuzio-Donahue CA, Eshleman JR, et al. Epigenetic inactivation of TFPI-2 as a common mechanism associated with growth and invasion of pancreatic ductal adenocarcinoma. *Oncogene* 2005.
196. Sato N, Fukushima N, Maitra A, Matsubayashi H, Yeo CJ, Cameron JL, et al. Discovery of novel targets for aberrant methylation in pancreatic carcinoma using high-throughput microarrays. *Cancer Res* 2003;63(13):3735-42.
197. Jansen M, Fukushima N, Rosty C, Walter K, Altink R, Heek TV, et al. Aberrant Methylation of the 5' CpG Island of TSLC1 Is Common in Pancreatic Ductal Adenocarcinoma and Is First Manifest in High-Grade PanINs. *Cancer Biol Ther* 2002;1(3):293-6.
198. Sato N, Ueki T, Fukushima N, Iacobuzio-Donahue CA, Yeo CJ, Cameron JL, Hruban RH, Goggins M. Aberrant Methylation of CpG Islands in Intraductal Papillary Mucinous

- Neoplasms of the Pancreas Increases with Histological Grade. *Gastroenterology* 2002;123:1365-72.
199. Herman JG, Graff JR, Myohanen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci U S A* 1996;93(18):9821-6.
  200. Esteller M, Corn PG, Baylin SB, Herman JG. A gene hypermethylation profile of human cancer. *Cancer Res* 2001;61(8):3225-9.
  201. Fukushima N, Walter KM, Uek T, Sato N, Matsubayashi H, Cameron JL, et al. Diagnosing pancreatic cancer using methylation specific PCR analysis of pancreatic juice. *Cancer Biol Ther* 2003;2(1):78-83.
  202. Matsubayashi H, Sato N, Fukushima N, Yeo CJ, Walter KM, Brune K, et al. Methylation of cyclin D2 is observed frequently in pancreatic cancer but is also an age-related phenomenon in gastrointestinal tissues. *Clin Cancer Res* 2003;9(4):1446-52.
  203. Matsubayashi H et al, Sato N, Brune K, Blackford AL, Hruban RH, Canto M, Yeo CJ, Goggins M. Age- and Disease-Related Methylation of Multiple Genes in Non-neoplastic Duodenal tissues. *Clin Cancer Res* 2005;11:573-83.
  204. Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. *Nat Genet* 1994;7(4):536-40.
  205. Pao MM, Tsutsumi M, Liang G, Uzvolgyi E, Gonzales FA, Jones PA. The endothelin receptor B (EDNRB) promoter displays heterogeneous, site specific methylation patterns in normal and tumor cells. *Hum Mol Genet* 2001;10(9):903-10.
  206. Nguyen C, Liang G, Nguyen TT, Tsao-Wei D, Groshen S, Lubbert M, et al. Susceptibility of nonpromoter CpG islands to de novo methylation in normal and neoplastic cells. *J Natl Cancer Inst* 2001;93(19):1465-72.
  207. Sato N, Parker AR, Fukushima N, Miyagi Y, Iacobuzio-Donahue C, Eshleman JR, Goggins M. Epigenetic inactivation of TFPI-2 as a common mechanism associated with growth and invasion of pancreatic ductal adenocarcinoma. *Oncogene* 2004:in press.
  208. Iacobuzio-Donahue CA AM, Shen-Ong GL, van Heek T, Ashfaq R, Meyer R, Walter K, Berg K, Hollingsworth MA, Cameron JL, Yeo CJ, Kern SE, Goggins M, Hruban RH. Discovery of Novel Tumor Markers of Pancreatic Cancer using Global Gene Expression Technology. *Am J Pathol* 2002;160:1239-49.
  209. Iacobuzio-Donahue CA, Maitra A, Olsen M, Lowe AW, van Heek NT, Rosty C, et al. Exploration of global gene expression patterns in pancreatic adenocarcinoma using cDNA microarrays. *Am J Pathol* 2003;162(4):1151-62.
  210. Iacobuzio-Donahue CA, Ashfaq R, Maitra A, Adsay NV, Shen-Ong GL, Berg K, et al. Highly expressed genes in pancreatic ductal adenocarcinomas: a comprehensive characterization and comparison of the transcription profiles obtained from three major technologies. *Cancer Res* 2003;63(24):8614-22.
  211. Byungwoo Ryu JJ, Natalie J. Blades, Giovanni Parmigiani, Michael A. Hollingsworth, Ralph H. Hruban, and Scott E. Kern. Relationships and Differentially Expressed Genes among Pancreatic Cancers Examined by Large-scale Serial Analysis of Gene Expression. *Cancer Res* 2002;62:819-826.
  212. Ryu B, Jones J, Hollingsworth MA, Hruban RH, Kern SE. Invasion-specific Genes in Malignancy Serial Analysis of Gene Expression Comparisons of Primary and Passaged Cancers. *Cancer Res* 2001;61:1833-1838.
  213. Crnogorac-Jurcevic T, Efthimiou E, Capelli P, Blaveri E, Baron A, Terris B, et al. Gene expression profiles of pancreatic cancer and stromal desmoplasia. *Oncogene* 2001;20(50):7437-46.

214. Crnogorac-Jurcevic T, Efthimiou E, Nielsen T, Loader J, Terris B, Stamp G, et al. Expression profiling of microdissected pancreatic adenocarcinomas. *Oncogene* 2002;21(29):4587-94.
215. Han H, Bearss DJ, Browne LW, Calaluca R, Nagle RB, Von Hoff DD. Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. *Cancer Res* 2002;62(10):2890-6.
216. Cao D, Hustinx SR, Sui G, Bala P, Sato N, Martin S, et al. Identification of Novel Highly Expressed Genes in Pancreatic Ductal Adenocarcinomas through a Bioinformatics Analysis of Expressed Sequence Tags. *Cancer Biol Ther* 2004;3:1081-9.
217. Hustinx SR, Cao D, Maitra A, Sato N, Martin ST, Sudhir D, et al. Differentially Expressed Genes in Pancreatic Ductal Adenocarcinomas Identified Through Serial Analysis of Gene Expression. *Cancer Biol Ther* 2004;3:1254-61.
218. Thomas AM, Santarsiero LM, Lutz ER, Armstrong TD, Chen YC, Huang LQ, et al. Mesothelin-specific CD8(+) T cell responses provide evidence of in vivo cross-priming by antigen-presenting cells in vaccinated pancreatic cancer patients. *J Exp Med* 2004;200(3):297-306.
219. Terris B, Blaveri E, Crnogorac-Jurcevic T, Jones M, Missiaglia E, Ruzniewski P, et al. Characterization of gene expression profiles in intraductal papillary-mucinous tumors of the pancreas. *Am J Pathol* 2002;160(5):1745-54.
220. Argani P, Rosty C, Reiter RE, Wilentz RE, Murugesan SR, Leach SD, et al. Discovery of new markers of cancer through serial analysis of gene expression: prostate stem cell antigen is overexpressed in pancreatic adenocarcinoma. *Cancer Res* 2001;61(11):4320-4.
221. Argani P, Iacobuzio-Donahue C, Ryu B, Rosty C, Goggins M, Wilentz RE, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). *Clin Cancer Res* 2001;7(12):3862-8.
222. Swierczynski SL, Maitra, A., Abraham, S. C., Iacobuzio-Donahue, C. A., Ashfaq, R., Cameron, J. L., Schulick, R. D., Yeo, C. J., Rahman, A., Hinkle, D. A., Hruban, R. H., and Argani, P. Analysis of novel tumor markers in pancreatic and biliary carcinomas using tissue microarrays. *Hum.Pathol* 2004;35:357-366.
223. Maitra A, Iacobuzio-Donahue C, Rahman A, Sohn TA, Argani P, Meyer R, et al. Immunohistochemical validation of a novel epithelial and a novel stromal marker of pancreatic ductal adenocarcinoma identified by global expression microarrays: sea urchin fascin homolog and heat shock protein 47. *Am J Clin Pathol* 2002;118(1):52-9.
224. Grutzmann R, Luttges, J., Sipos, B., Ammerpohl, O., Dobrowolski, F., Alldinger, I., Kersting, S., Ockert, D., Koch, R., Kalthoff, H., Schackert, H. K., Saeger, H. D., Kloppel, G., and Pilarsky, C. ADAM9 expression in pancreatic cancer is associated with tumour type and is a prognostic factor in ductal adenocarcinoma. *Br J Cancer* 2004;90:1053-1058.
225. Guo J, Kleeff, J., Li, J., Ding, J., Hammer, J., Zhao, Y., Giese, T., Korc, M., Buchler, M. W., and Friess, H. Expression and functional significance of CDC25B in human pancreatic ductal adenocarcinoma. *Oncogene* 2004;23:71-81.
226. Yousef GM, Borgono, C. A., Popalis, C., Yacoub, G. M., Polymeris, M. E., Soosaipillai, A., and Diamandis, E. P. In-silico analysis of kallikrein gene expression in pancreatic and colon cancers. *Anticancer Res.* 2004;24:43-51.
227. Crnogorac-Jurcevic T, Missiaglia, E., Blaveri, E., Gangeswaran, R., Jones, M., Terris, B., Costello, E., Neoptolemos, J. P., and Lemoine, N. R. Molecular alterations in pancreatic carcinoma: expression profiling shows that dysregulated expression of S100 genes is highly prevalent. *J Pathol* 2003;201:63-74.
228. Rosty C, Ueki T, Argani P, Jansen M, Yeo CJ, Cameron JL, et al. Overexpression of S100A4 in Pancreatic Ductal Adenocarcinomas Is Associated with Poor Differentiation and DNA Hypomethylation. *Am J Pathol* 2002;160(1):45-50.

229. Li D, Zhu J, Firozi PF, Abbruzzese JL, Evans DB, Cleary K, et al. Overexpression of oncogenic STK15/BTAK/Aurora A kinase in human pancreatic cancer. *Clin Cancer Res* 2003;9(3):991-7.
230. Sato N, Fukushima N, Maitra A, Iacobuzio-Donahue CA, van Heek NT, Cameron JL, Yeo CJ, Hruban RH, Goggins M. Gene expression profiling identifies genes associated with invasive intraductal papillary mucinous neoplasms of the pancreas. *Am J Pathol* 2004;164:903-14.
231. Maitra A, Ashfaq, R., Gunn, C. R., Rahman, A., Yeo, C. J., Sohn, T. A., Cameron, J. L., Hruban, R. H., and Wilentz, R. E. Cyclooxygenase 2 expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasia: an immunohistochemical analysis with automated cellular imaging. *Am J Clin Pathol* 2002;118:194-201.
232. Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, et al. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature* 2003;425(6960):846-51. Epub 2003 Sep 14.
233. van der Heijden MS, Brody JR, Gallmeier E, Cunningham SC, Dezentje DA, Shen D, et al. Functional defects in the Fanconi anemia pathway in pancreatic cancer cells. *Am J Pathol* 2004;165(2):651-7.