FUTURE ONCOLOGY
TECHNOLOGY, PRODUCTS, MARKETS AND SERVICE OPPORTUNITIES
A NEW MEDICINE PUBLICATION NOVEMBER 15, 2003 VOLUME 7, NUMBER 8/9

STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

PANCREATIC CANCER — PART I
EPIDEMIOLOGY, ETIOLOGY, MOLECULAR MARKERS, DIAGNOSIS, AND STAGING

STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

PANCREATIC CANCER — PART I
EPIDEMIOLOGY, ETIOLOGY, MOLECULAR MARKERS, DIAGNOSIS, AND STAGING

In 2003, pancreatic cancer was the 4th leading cause of cancer deaths in the USA and, in the rest of the developed world. Pancreatic cancer is one of the most lethal malignancies, with a 5-year survival rate below 5%. The fact that it is usually detected after it has spread, either locally or distally, contributes to its dismal outcome.

TYPES OF PANCREATIC CANCER

Most pancreatic tumors are pancreatic ductal adenocarcinomas (PDAC) arising in the ductal epithelium of the exocrine pancreas. However, there are also several other types of rare pancreatic malignancies. Because prognosis and treatment options differ between the major pancreatic tumor types, accurate diagnosis is extremely important.

Pancreatic Intraductal/Intraepithelial Neoplasia (PanIN)

PDAC, like most other epithelial tumors, evolves through a multistep model of progression that begins in the normal pancreatic ducts, proceeds through noninvasive intraductal lesions, and culminates in invasive adenocarcinoma. A growing body of morphologic, clinical, and genetic observations suggests PDAC progression develops through a series of noninvasive percusor duct lesions, designated as pancreatic intraductal/intraepithelial neoplasia (PanIN)-1a (mucinous duct lesions) and -1b, -2 and -3, according to their increasing dysplastic grade. It is currently theorized that pancreatic cancer proceeds in a stepwise manner, with each advancing stage involving various genetic alter-ations. For instance, PanIN-1 may remain unchanged for a long period of time, while PanIN-3 potentially progresses rapidly to invasive PDCA.

PanIN are histologically well defined precursor lesions in the small ducts and ductules of the pancreas. They are true "clonal" neoplasms, rather than either hyperplasia, or extension of invasive pancreatic cancer into ducts. Molecular studies have established that PanIN harbor many of the same well characterized genetic alterations seen in PDAC; these genetic alterations can even be detected in pancreatic secretions more than a year before a neoplasm is clinically apparent.

A mouse model, the EL-Kras transgenic mouse, has been created that develops ductal carcinoma *in situ* apparently identical to human PanIN. EL-Kras mice have demonstrated expression of mutant K-ras (glycine to aspartate at codon 12) in the pancreas, with the protein being localized in some normal-appearing acinar tissue and carcinoma cells.

To compare the frequency and clinical correlates of PanIN with benign lesions in PDAC, investigators at Harper Hospital (Detroit, MI) analyzed pancreatectomy specimens from 82 patients with PDAC and 152 patients who underwent pancreatectomy for reasons other than primary malignancy (trauma, pancreatitis, and tumors metastasized to the pancreas), for the presence, grade, and number of foci of PanIN. Cases were graded by the highest grade of PanIN focus identified. Overall, the frequency of PanIN in patients with PDAC, including PanIN-1a, was 82%, which was significantly higher than the 54% seen in benign pancreata. There was a progressive increase from normal pancreas, to pancreatitis, to PDAC, in the frequency of overall PanIN (16%, 60%, and 82%, respectively) and PanIN-3 (0%, 4%, and 40%, respectively). In most instances, in any given case of higher grade PanIN, there were also several foci of lower grade lesions. The frequency of PanIN-2 and -3 in resected PDAC was 59%, significantly higher than the 17% seen in those without primary carcinoma. This progressive increase in frequency of PanIN from incidental pancreatectomies (presumed to have a nonpathologic pancreas) to pancreatitis (considered a risk factor for carcinoma), to PDAC, constitutes an indirect support for the precancerous role attributed to PanIN. The relatively high 43% absolute occurrence of PanIN-1a in benign conditions suggests that this group represents a combination of neoplastic and non-neoplastic lesions (Andea A, etal, Modern Pathology 2003;16:996-1006).

Another study, conducted at the University of Kiel, and the University of Bochum, in Germany, tested whether molecular genetic alterations correlated with the PanIN classification, to further categorize the various PanIN grades. Frequencies of allelic loss were determined for chromosomal arms 9p, 17p, and 18q in 81 microdissected duct lesions of various PanIN grades, using a combination of whole genome amplification and microsatellite analysis. In addition, protein expression patterns of p53 and Dpc4 were profiled by immunohistochemical analysis. There were no allelic losses in PanIN-1, while such losses were found in increasing frequency in PanIN-2, and were particularly high in those lesions with moderate grade dysplasia; allelic losses were 20%, 33% and 17%, at 9p, 17p, and 18q, respectively, in low grade PanIN-2, and 46%, 77%, and 58%, respectively, in moderate grade PanIN. PanIN-3 and invasive carcinomas exhibited abundant losses. Abnormal p53 and Dpc4 protein expression was only rarely identified in PanIN-2, but occurred frequently in PanIN-3 and invasive carcinoma. Based on these findings, combined genetic and protein expression data supports a model in which allelic loss is the first step in the biallelic inactivation of p53 and Dpc4 tumor-suppressor genes. In addition, allelic loss analysis may be useful in separating PanIN-2 with low grade dysplasia from PanIN-2 with moderate grade dysplasia, each potentially representing a distinct progression step toward invasive carcinoma (Lüttges J, etal, Am J Path 2001;158:1677-1683).

In order to establish early events in the development of PanIN, investigators at Garvan Institute of Medical Research, St. Vincent's Hospital (Darlinghurst, NSW, Australia) examined protein expression of four functionally related genes, p21(waf1/cip1/CDKN1A), p53, cyclin D1 (CCND1), and Dpc4/Smad4 (MADH4), in 451 PanIN from the pancreata of 60 patients. Overexpression of p21 was present in the normal ducts of 9% of patients and increased progressively to 16% of patients with PanIN-1a, to 32% with PanIN-1b, 56% with PanIN-2, 80% with PanIN-3, and 85% with invasive carcinoma, while p53 and cyclin D1 overexpression was detected predominantly in PanIN-3, and loss of Dpc4 expression occurred predominantly in PanIN-3 and invasive carcinoma. In addition, p21 overexpression occurred independently of p53 and Dpc4/Smad4 expression in invasive carcinoma, and PanIN-3. Cyclin D1 overexpression or loss of Dpc4 expression was observed in 85% of invasive carcinomas, but in only 14% of PanIN-2. Therefore, overexpression of p21 occurs early in the development of PanIN, before alterations in p53, cyclin D1, and Dpc4 expression. Independently of p53 and/or Dpc4 expression, p21 overexpression may reflect increased ras activity, either directly through activating K-ras mutations, or as a consequence of HER-2/neu overexpression, both of which are common in pancreatic cancer, and are early events in the development of PanIN. These observations further support the progression model for pancreatic cancer, and demonstrate that aberrant expression of key cell-cycle regulatory genes may be important in the early development and progression of PanIN (Biankin AV, etal, Cancer Res, 15 Dec 2001;61(24):8830-7).

Expressed in dividing cells, Ki-67 has been extensively used as a proliferation marker. To investigate its expression in different grades of PanIN, 76 PanIN from 41 patients were histologically graded according to recently established criteria, and were immunolabeled with a monoclonal antibody (MAb) against Ki-67 (Mib-1). Normal ducts and invasive ductal adenocarcinomas were also labeled with the MAb. In 15 normal ducts, only 0.41% of the epithelial cells expressed Ki-67, but Ki-67–labeling indices for the increasing grades of PanIN were 0.69% in

PanIN-1a, 2.33% in PanIN-1b, 14.08% in PanIN-2, and 22.01% in PanIN-3. The average labeling index of 15 invasive PDAC was 36.99%. The difference in Ki-67 labeling among these groups was statistically significant. This pattern of proliferation supports the proposed pancreatic progression model. It also correlates well with known molecular changes, such as activating point mutations in the K-ras oncogene, and loss of Dpc4 and p16 gene expression. Ki-67 staining may be useful as an adjunct in the diagnosis of precancerous lesions in the pancreas and may provide a reliable way to identify lesions at high risk for the subsequent development of infiltrating carcinoma (Klein WM, etal, Mod Pathol 2002;15:441-447).

Telomere shortening is also nearly universal in PanIN. Telomeres may be an essential gatekeeper for maintaining chromosomal integrity and, thus, normal cellular physiology of the pancreatic ductal epithelium. Evidence for telomeric dysfunction has been demonstrated in invasive pancreatic cancer. In order to investigate this phenomenon in the context of noninvasive precursor lesions, investigators at Brady Urological Institute at Johns Hopkins Medical Institutions (Baltimore, MD), using an *in situ* hybridization technique in archival samples, assessed telomere length in tissue microarrays containing a variety of noninvasive pancreatic ductal lesions, including 82 PanIN of all histologic grades (PanIN-1a=24, PanIN-1b=23, PanIN-2=24, and PanIN-3=11) that were selected from pancreatectomy specimens from either adenocarcinoma or chronic pancreatitis. Telomere fluorescence intensities in PanIN were compared with adjacent normal pancreatic ductal epithelium and acini in 62/82 (76%) lesions, or with stromal fibroblasts and islets of Langerhans in 20/82 (24%) lesions. Telomere signals were strikingly reduced in 79/82 (96%) PanIN compared to adjacent normal structures. Interestingly, a dramatic reduction of telomere fluorescence intensity was seen in 21/23 (91%) foci in PanIN-1a, the earliest precursor lesion. Reduction of telomere signal was observed in all PanIN associated with chronic pancreatitis, but telomere length in atrophic and inflammatory ductal lesions was normal. Telomere fluorescence intensity in PanIN did not correlate with proliferation measured by quantitative Ki-67-labeling index or topoisomerase II expression. This data indicate that telomere shortening is an early genetic abnormality in the progression model of PDAC. A critical shortening of telomere length in PanIN may predispose these noninvasive ductal lesions to accumulate progressive chromosomal abnormalities, and evolve into invasive carcinoma (van Heek NT, Amer J Path 2002;161:1541-1547).

Another marker present in PanIN is cyclooxygenase-2 (COX-2). In one analysis, COX-2 expression was detected in 36.3% of PanIN, compared to 19.2% in normal ducts. Significant differences in COX-2 expression were demonstrable in PanIN versus normal ducts, and PanIN-2, or -3 versus PanIN-1a or 1b. In general, the pattern of COX-2 expression increased from normal to PanIN to adenocarcinoma. Upregulation of COX-2 in a subset of noninvasive precursor lesions makes it a potential target for chemoprevention with selective COX-2 inhibitors (Maitra A, etal, Am J Clin Pathol 2002;118(2):194-201).

Also, although intraductal papillary-mucinous tumors (IPMT) are entirely distinct from PanIN, IPMT of small size resemble PanIN morphologically. Loss of Dpc4 expression has been reported in the invasive component of IPMT, as well as in PanIN-3 and invasive ductal carcinoma. Also, MUC1-positive MUC2-negative IPMT may not be distinguishable from PanIN. There may be overlapping lesions between PanIN and IPMT. Should the paradigm of the ductal origin of invasive ductal carcinoma be accepted, PanIN and a fraction of IPMT would represent precursors of PDAC (Takaori K, etal, J Hepatobiliary Pancreat Surg, 2003;10(2):125-36).

Exocrine Pancreatic Cancer

Exocrine pancreatic cancer, primarily PDAC, accounts for over 90% of all pancreatic cancer. Usually, reference to pancreatic cancer implies PDAC.

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a paucity of neoplastic cells embedded in a densely desmoplastic stroma. The majority of cancers of the pancreas are PDAC, comprising between 85% to 90% of all pancreatic tumors. PDAC occur more frequently in men than in women. They mostly affect elderly individuals between the ages of 60 to 80, and are rare in those <40 years-of-age. Between 60% and 70% of pancreatic tumors are located at the head of the pancreas, resulting in jaundice, pruritus, epigastric pain and weight loss. Carcinomas located in the body or tail of the pancreas are associated with more weight loss and pain. However, more than 50% of jaundiced patients report no pain at the time of diagnosis.

PDAC is a catastrophic malignancy. Once it occurs, the tumor rapidly invades local tissues and metastasizes to local and distant sites. The precise reasons for the biological and clinical behavior of PDAC are not known, and no single molecular event has been linked to its origin and course (Real FX, Gastroenterology, Jun 2003;124(7):1958-64). To date, numerous genetic mutations and alterations of protein expression have been associated with PDAC (Exhibit 6). Acquired mutations have been identified in the oncogenes K-ras and HER2/neu, and in the tumor-suppressor genes p16, p53, SMAD4, and BRCA2.

Mucinous cystic neoplasms of the exocrine pancreas comprise a small fraction of pancreatic tumors. Cystic neoplasms of the pancreas are rare, accounting for 10%-13% of all pancreatic cysts and 1% of all pancreatic carcinomas. Cystic abnormalities of the pancreas consist of a variety of lesions from nonmalignant pseudocyst to malignant tumors. Cystic neoplasms of the pancreas include serous cystadenomas; papillary cystic tumors; cystic islet cell tumors; mucinous cystic neoplasms (MCN), compris-

ing cystadenomas and cystadenocarcinomas; and intraductal mucin-hypersecreting pancreatic neoplasms, also known as mucinous ductal ectasia (MDE).

MCN and MDE evolve from the pancreatic duct epithelium, produce an abundance of mucin, and may be premalignant or malignant. While MCN primarily affect middle-aged women with lesions occurring predominantly in the body and tail of the pancreas, MDE primarily affects men in the sixth or seventh decade with lesions more often located in the head of the pancreas. Survival rates associated with both of these types of tumors are better than those reported for PDAC. Because intraductal papillary neoplasms with or without MDE represent a spectrum of main-duct papillary tumors ranging from adenoma to carcinoma, it is suggested that the term intraductal papillary mucinous tumors (IPMT) of the pancreas should encompass both tumors.

PDAC and IPMT differ in their expression of mucin markers MUC1 and MUC2, while both tumors express MUC5AC. Noninvasive mucinous cystic neoplasms lack MUC1 expression (except for an eosinophilic variant), but express it when they become invasive. Therefore, MCU1 expression might be used as a marker indicating the step of progression from noninvasiveness to invasiveness (Luttges J, etal, Am J Surg Pathol 2002 Apr;26(4):466-71).

Most completely resected mucinous cystic neoplasms follow a benign course. Investigators at Johns Hopkins University School of Medicine reviewed the gross and microscopic findings and outcomes of 61 mucinous cystic neoplasms diagnosed between March 20, 1984 and July 8, 1998. Based on histology, each neoplasm was placed into one of four categories, i. e., invasive mucinous cystadenocarcinoma (n=20), mucinous cystic neoplasm with *in situ* carcinoma (n=9), borderline mucinous cystic neoplasm (n=5), and mucinous cystadenoma (n=27). Patient outcomes were obtained from hospital records and patient and physician follow-up. Disease-specific survival rates of patients with invasive mucinous cystadenocarcinomas were 67% at 2 years and 33% at 5 years, at a mean follow-up of survivors at 4.2 years. None of mucinous cystadenoma, borderline mucinous cystic neoplasm, or mucinous cystic neoplasm with *in situ* carcinoma recurred or metastasized at mean follow-up of survivors of 4.1 years, 5.6 years and 5.1, respectively, and no patient died of the disease. The difference in disease-specific survival rates between patients with invasive mucinous cystadenocarcinoma, and those with noninvasive tumors was significant (Wilentz RE, etal, Am J Surg Pathol, Nov 1999;23 (11):1320-7).

Intraductal papillary mucinous tumors (IPMT), or neoplasms (IPMN), are distinguished from mucinous cystic neoplasms, serous cystic neoplasms, and pseudopapillary cystic tumors. The World Health Organization (WHO) classified IPMT as a subgroup of pancreatic cancer in 1996. Because of their favorable prognosis, an extensive diagnostic workup for IPMT should be performed in patients presenting with cystic lesions of the pancreas. Surgical resection is the therapy of choice for IPMT (Schmitz-Winnenthal FH, etal, Curr Gastroenterol Rep, Apr 2003; 5(2):133-40).

Like PDAC, IPMT is considered to develop by a multistage process. It is generally thought that mutational inactivation of tumor-suppressor genes, including p16, Dpc4, and p53, that is a major mechanism underlying PDAC carcinogenesis, is relatively uncommon in IPMT. Incidence of K-ras mutations in IPMT is almost similar to those in PDAC. However, there is wide discrepancy between findings regarding the incidence and type of mutations indicative of IPMT.

Molecular mechanisms underlying tumorigenesis of IPMT include allelic losses detected at 9p, 17p, and 18q, suggesting that inactivation of tumor-suppressor genes at these loci play a role in the tumorigenesis of IPMT. In 38 IPMT (hyperplasia=9, adenoma=16, carcinoma tissues=13), analyzed for expression of p53, Ki-67, p16, and SMAD4, nuclear p53 expression was observed in 5/13 (38%) carcinoma tissues, but not in hyperplasia or adenoma tissues. Partial loss of p16 expression was observed in 9/16 (56%) adenoma, and 12/13 (92%) carcinoma tissues. Partial loss of p16 expression was observed even in adenomas with mild atypia. Partial loss of SMAD4 expression was observed in 6/6 (38%) adenoma and 12/13 (92%) carcinoma tissues. The SMAD4 negative index was significantly higher in invasive carcinomas than noninvasive ones. These results suggest that loss of p16 is an early event, and p53 alteration is a late event during the progression of IPMT. SMAD4 inactivation appears to be an early event but is also involved in invasive tumor growth. These alterations accumulate during the progression of IPMT, showing a stepwise accumulation of genetic changes during progression (Sasaki S, etal, Oncol Rep, Jan-Feb 2003;10(1):21-5).

Different results were reported by investigators at the University of Ulm, in Germany, who examined the prevalence of p53, p16/MTS1 and K-ras mutations in benign and malignant IPMT in order to assess their role in tumor progression. Among 13 different archival tumor specimens, 3 cases involved invasive tumor. K-ras mutations were seen in 4/13 benign and malignant IPMT, whereas an alteration of the coding p53 gene sequence could only be detected in the intraductal and invasive component of one malignant tumor. None of the tissue specimens revealed mutations in exon 2 of p16/MTS1. In contrast to K-ras mutations, alterations in the p53 gene may characterize IPMT, and may, therefore, play a role in the early diagnosis of the disease. Lack of mutations in the p16/MTS1 gene suggests that other genes may be involved in the formation of IPMT (Mueller J, etal, Hepatogastroenterology, Mar-Apr 2003;50(50):541-4).

One of the critical pathways that contribute to the malignant progression of IPMT is *de novo* methylation of multiple CpG islands (Sato N, etal, AACR02, Abs. 5589).

This aberrant methylation of CpG islands in IPMT increases with histologic grade.

Neuroendocrine Pancreatic Tumors

Only a very small percentage of all tumors (<5%) arise in the endocrine compartment of the pancreas, involved in producing hormones such as insulin. Neuroendocrine tumors of the pancreas include insulinoma, gastrinoma, WDHA (watery diarrhea hypokalemia hypochlorhydria syndrome or VIP oma), glucagonoma and somatostatinoma. About one third of neuroendocrine pancreatic tumors do not present any hormone-related symptoms, and are referred to as nonfunctioning. Most neuroendocrine tumors, with the exception of insulinomas, which are usually benign, may metastasize if left untreated. Generally, neuroendocrine digestive tumors are very rare slow growing neoplasms that metastasize later than ductal and acinar carcinomas. Diagnosis is late because of symptom diversity.

Neuroendocrine tumors occur with the same frequency in men and women. Unlike most other pancreatic malignancies, these tumors are diagnosed in young patients with the median age at diagnosis being 53 years (range=18-80). The annual incidence of neuroendocrine pancreatic tumors is about 0.35 to 0.40 per 100,000. Some of the tumors can be hereditary as a part of a syndrome referred to as multiple endocrine neoplasia type 1 (MEN 1), in which other hormone-producing organs such as the parathyroid and pituitary glands, may also be involved.

EPIDEMIOLOGY

Worldwide incidence of pancreatic cancer is estimated at 222,434 (Exhibit 1), and mortality at 219,599 (Exhibits 2). The high mortality rate results from a high incidence of metastatic disease at diagnosis, and lack of effective systemic therapy. The only chance of cure remains surgical resection, but only 15% to 20% of patients have resectable disease. Most patients have either locally advanced or metastatic pancreatic cancer at presentation. Pancreatic cancer is most often diagnosed in the aged, making it a disease of the developed world (Exhibits 3 and 4).

In 2002, approximately 30,300 were diagnosed with pancreatic cancer in the USA, and 29,700 died from the disease making it the 4th leading cause of death from cancer, after lung, prostate, breast, and colorectal cancer. Incidence of pancreatic cancer is estimated at 30,700 in the USA in 2003, and deaths are estimated at 30,000.

In the USA, pancreatic cancer is a disease of the aged (Exhibit 5). It hardly ever occurs in people under 50 years-of-age, and it is rare in the <65 years age group. It emerges as a risk in people over 55 and its incidence jumps dramatically among those over 75. For unknown reasons, in the USA, compared to other racial and ethnic groups, African Americans are the most vulnerable to pancreatic cancer, followed by whites. The incidence of pancreatic cancer is significantly higher among African Americans within all age groups. In the 55-69 years age group, incidence rates in this population exceed those for whites by about 60%, and this difference diminishes somewhat among those >70 years old. Incidence rates for Japanese men and women exceed those for the white population in the oldest age group. Racial/ethnic patterns in mortality rates by age group closely follow those seen in the incidence rates.

ETIOLOGY OF PANCREATIC CANCER

Both genetic and environmental factors may play a role in the development of pancreatic cancer. Germline mutations in a few genes including p16 and BRCA2 have been implicated in a small fraction of cases. Mutations or deletions in such genes as K-ras, p16, p53, Dpc4, and BRCA2 increase the risk of developing pancreatic cancer. Smoking, alcohol and coffee consumption, and exposure to organochlorine or hydrocarbon solvents, have all been associated with the frequency and spectrum of K-ras mutation in pancreatic tumors. Cigarette smoking, an established risk factor for pancreatic cancer, may play a role in the development of these mutations. Active smokers are at two to three times the risk of developing pancreatic cancer when compared to nonsmokers.

The association between diet and pancreatic cancer is rather tenuous, but higher risk is attributed to consumption of smoked or processed meats, and animal foods, in general, and a lower risk to consumption of fruits and vegetables. Evidence that alcohol drinking and coffee consumption increase the risk is not sufficient, although an association with higher level of consumption remains a possibility. Colonization by Helicobacter pylori appears to increase risk. Diabetes mellitus, long standing diabetes in particular, may also be a risk factor for pancreatic cancer. Individuals with hereditary pancreatitis, or non-hereditary chronic pancreatitis are possibly at increased risk of pancreatic cancer (Lin Y, etal, Asian Pac J Cancer Prev 2001;2(4):271-280).

DNA adducts derived from exposure to polycyclic aromatic hydrocarbon, aromatic amines, and heterocyclic amines have been detected in human pancreatic tissues. DNA damages derived from oxidative stress and lipid peroxidation are also present in the pancreas. However, to date, no study has demonstrated a main effect of carcinogen-metabolizing genes and DNA repair genes on pancreatic cancer risk. Dietary folate intake and serum levels of folate have been associated with the risk of pancreatic cancer among male smokers (Li D and Jiao L, Int J Gastrointest Cancer 2003;33(1):3-14).

Epigenetic Events

Epigenetic events, including aberrant methylation of tumor suppressor gene promoter regions, may play a central role in tumorigenesis involving both the exocrine and endocrine pancreas. Understanding the contribution of gene promoter methylation to the mechanisms of pancreatic cancer evolution may lead to sensitive molecular markers for early detection. Methylation changes of spe-

Exhibit 1
Worldwide Incidence of Pancreatic Cancer in 2002

Location	Male New Cases (#)	Rate (Crude)* per 100,000	Female New Cases (#)	Rate (Crude)* per 100,000	Total Cases (#)
World	**118,845**	**3.79**	**103,589**	**3.35**	**222,434**
Eastern Africa	1,031	0.82	975	0.77	2,006
Middle Africa	162	0.75	495	2.28	657
Northern Africa	944	0.99	675	0.72	1,619
Southern Africa	226	0.93	184	0.74	410
Western Africa	507	0.41	493	0.40	1,000
Total Africa	**2,870**		**2,822**		**5,692**
Caribbean	735	4.19	521	2.96	1,256
Central America	2,605	3.71	2,400	3.35	5,005
South America	6,804	3.82	6,613	3.65	13,417
North America	16,962	10.80	17,568	10.81	34, 530
Total Americas	**27,106**		**27,102**		**20,242**
Eastern Asia	33,759	4.68	25,233	3.67	58,992
South-Eastern Asia	2,874	1.38	2,537	1.21	5,411
South Central Asia	6,642	0.89	4,442	0.63	11,084
Western Asia	1,960	2.19	1,286	1.52	3,246
Total Asia	**45,235**		**33,498**		**78,733**
Eastern Europe	8,212	10.94	6,998	8.49	15,210
Northern Europe	5,058	10.79	5,261	10.84	10,319
Southern Europe	7,836	11.49	7,239	10.12	15,075
Western Europe	8,838	10.73	8,490	9.86	17,328
Europe Total	**29,944**		**27,988**		**57,932**
Australia/ New Zealand	**1,048**	**8.98**	**1,090**	**9.25**	**2,175**

**Source: Ferlay J, etal, Globocan 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0. IARC CancerBase No. 5. Lyon, IARCPress, 2001.*

cific gene promoter sites were examined in 29 fresh frozen tissue samples, including acinar cell carcinoma (n=3), adenocarcinoma (n=18), islet cell tumors (n=7), and small cell carcinoma (n=1), obtained from resected neoplasia involving the exocrine or endocrine pancreas. There were methylation changes in 14 promoter regions of tumor suppressor genes. The frequency of methylation was APC=52%, p73=35%, BRCA1=35%, p16=24%, p15=24%, MLH1=21%, MGMT=14%, RARβ2=14%, TIMP3=10%, E-cadherin=7%, GSTπ=4%, DAP-K=4%, p14=0%. There was at least one methylation change in 86% (25/29) of these tumors while 52% (15/29) contained two or more methylated genes. Histologic differences among the pancreatic neoplasms were reflected in subtle differences in methylation patterns, with 100% (3/3) of acinar cell carcinoma samples having a multigene methylation profile, whereas 83 % (15/18) of adenocarcinoma samples having at least one gene methylated, and 39% >2 genes methylated. At least one methylated promoter region was detected in 100% (7/7) of the islet cell tumors; 71% (5/7) of these endocrine tumors possessed more than two methylated sites. The methylation of promoter regions associated with tumor suppressor genes, especially APC, p73, BRCA1, p16, p15, and MLH1, may play a central role in the evolution of neoplastic disease of the pancreas. Also, there are differences between patterns of methylation in endocrine and exocrine pancreatic tumors (Guo M, etal, AACR02, Abs. 29).

Exhibit 2
Worldwide Mortality from Pancreatic Cancer in 2002

Location	Male Deaths (#)	Rate (Crude)* per 100,000	Female Deaths	Rate (Crude)* per 100,000	Total Deaths (#)
World	**115,082**	**3.67**	**104,517**	**3.38**	**219,599**
Eastern Africa	1,006	0.80	963	0.76	1,969
Middle Africa	160	0.74	484	2.23	644
Northern Africa	925	0.97	656	0.70	1,581
Southern Africa	216	0.89	177	0.71	393
Western Africa	470	0.38	456	0.37	926
Total Africa	**2,777**		**2,736**		**5,513**
Caribbean	732	4.17	558	3.17	1,290
Central America	3,054	4.35	2,902	4.05	5,956
South America	6,982	3.92	6,812	3.76	13,794
North America	16,130	10.27	17,065	10.50	33,195
Total Americas	**26,898**		**27,337**		**54,235**
Eastern Asia	29,792	4.13	23,789	3.46	53,581
Southeastern Asia	2,624	1.26	2,432	1.16	5,056
South Central Asia	6,493	0.87	4,371	0.62	10,864
Western Asia	1,826	2.04	1,244	1.47	3,070
Total Asia	**40,735**		**31,836**		**72,571**
Eastern Europe	7,731	10.30	6,668	8.09	14,399
Northern Europe	5,578	11.90	5,839	12.03	11,417
Southern Europe	7,536	11.05	6,960	9.73	14,496
Western Europe	10,757	13.06	11,107	12.90	21,864
Europe Total	**31,602**		**30,574**		**62,176**
Australia/ New Zealand	**989**	**8.47**	**1,031**	**8.75**	**2,054**

**Source: Ferlay J, etal, Globocan 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0. IARC CancerBase No. 5. Lyon, IARCPress, 2001.*

To investigate the relationship between DNA hypomethylation and gene overexpression in pancreatic cancer, researchers at Johns Hopkins analyzed the methylation status of a subset of 18 genes previously identified by global gene expression studies as being overexpressed in pancreatic cancer tissues compared with normal pancreata. For comparison, the methylation status of 14 genes not known to be overexpressed in pancreatic cancer was also determined. Methylation-specific PCR analysis revealed that 19 of these 32 genes were methylated at their 5' CpG in normal pancreas. When these 19 genes were analyzed for their methylation pattern in pancreatic cancer, all 7 of the genes (claudin4, lipocalin2, 14-3-3sigma, trefoil factor2, S100A4, mesothelin, and prostate stem cell antigen) that were overexpressed in the neoplastic cells of pancreatic cancer and not expressed in normal pancreatic duct, displayed a high prevalence of hypomethylation in pancreatic cancer cell lines and primary pancreatic carcinomas. By contrast, only 1 of 12 genes not overexpressed in pancreatic cancer demonstrated hypomethylation. In pancreatic cancer cell lines that retained methylation of 1 or more of the 7 aforementioned overexpressed and hypomethylated genes, treatment with 5-aza-2'-deoxycytidine or with trichostatin A, either alone or in combination, almost invariably reactivated the transcription of each of these 7 genes. These results indicate that gene hypomethylation is a frequent epigenetic event in pancreatic cancer and is commonly associated with the overexpression of affected genes (Sato N, Cancer Res, 15 Jul 2003;63(14):4158-66).

Hereditary Factors

Hereditary syndromes that have a known predisposition for development of pancreatic cancer include familial multiple endocrine neoplasia type 1 (men-1), including Werner Syndrome; hereditary pancreatitis; familial atypical multiple mole melanoma (FAMM) syndrome; Peutz-Jeghers syndrome; familial breast cancer (BRCA-2); hereditary nonpolyposis colorectal cancer syndrome (HNPCC); and Li-Fraumeni syndrome. Germline mutations of the BRCA2 gene are present in 5-10% of patients with pancreatic cancer who, typically, do not have a family history of pancreatic cancer and are mistaken as having sporadic disease. Germline mutations of the men-1 gene are responsible for the men-1 syndrome, known to be associated with pancreatic endocrine tumors.

High frequency genetic changes associated with PDAC include mutations of the k-ras oncogene, and inactivating alterations of the p53, p16, and Dpc4 tumor-suppressor genes. Currently it is believed that 3% to 5% of all cases of pancreatic cancer may be caused by a genetic predisposition, and only 10% of patients with pancreatic cancer have a familial predisposition to the disease (Cowgill SM and Muscarella P, Am J Surg, Sep 2003;186(3):279-86, Chappuis PO, etal, Cancer Invest 2001;19:65-75, and Goggins M, etal, Ann Oncol 1999;10 (Suppl 4):4-8).

The quest to identify a hereditary genetic predisposition to pancreatic cancer has led to the formation of pancreatic cancer registries around the world, with voluntary screening of patients and siblings for hereditary genetic defects. Establishment of such registries allows the study of patterns of inheritance of pancreatic cancer on a scale not possible before. In addition, advances in molecular genetics have enabled testing members of these families for germline mutations in known cancer-causing genes. As a result, some of the genetic alterations responsible for the familial aggregation of pancreatic cancer have been identified in some families.

The National Familial Pancreas Tumor Registry (NFPTR) at Johns Hopkins University, identified a number of families in which multiple members have been diagnosed with pancreatic cancer. As of 1999, the NFPTR had enrolled 362 families in which at least one family member had been diagnosed with pancreatic cancer, including 151 families in which at least two first-degree relatives had been diagnosed with pancreatic cancer. Analysis of these families revealed that even second-degree relatives of patients from these families are at increased risk of developing pancreatic cancer. Based on tests for germline mutations in known cancer causing genes in a number of kindreds, germline mutations in BRCA2 have been shown to predispose to both breast and pancreatic cancer, germline mutations in p16 to melanoma and pancreatic cancer (FAMMM syndrome), and genetic mutations in STK11/LKB1 to pancreatic cancer in patients with the Peutz-Jeghers Syndrome (PJS). These findings indicate that pancreatic cancer aggregates in some families, and relatives of patients with pancreatic cancer have an increased risk of developing pancreatic cancer themselves (Hruban RH, etal, Ann Oncol 1999;10 Suppl 4:69-73).

When 29 samples from patients with pancreatic cancer, enrolled in the NFPTR, were analyzed for mutations in tumor suppressor genes, BRCA2 gene sequencing identified 5 mutations (17.2%) believed to be deleterious and one new point mutation. Two of the five BRCA2 mutation carriers reported a family history of breast cancer, and none reported a family history of ovarian cancer. These findings confirm the increased risk of pancreatic cancer in individuals with BRCA2 mutations, and identifies germ-line BRCA2 mutations as the most common inherited genetic alteration yet identified in familial pancreatic cancer (Murphy KM, etal, Cancer Res, 1 Jul 2002; 62(13):3789-93).

Investigators at Philipps University (Marburg, Germany), established, in July 1999, a German case collection, to obtain and evaluate data on familial pancreatic cancer to examine its prevalence and characteristics. The prevalence of pancreatic cancer as well as other tumors and diseases was studied in families with at least 2 first-degree relatives with histologically confirmed pancreatic cancer, and in families of patients with pancreatic cancer and a first-degree relative with malignant melanoma. In an 18-month period, 73 independent kindreds with potential familial pancreatic cancer contacted the national case collection, of whom 20 fulfilled the criteria for familial pancreatic cancer and underwent complete workups. Most families exhibited an autosomal dominant pattern of inheritance. Among these 20 families, 12 cases involved isolated accumulation of pancreatic cancer. However, in 8/20 (35%) families, additional tumor types such as melanoma, breast and prostate cancer also occurred. The observed phenotypic heterogeneity indicates an association with predisposing tumor-suppressor genes p16 and BRCA2 in up to 30% of cases of familial pancreatic cancer. Whereas none of the 18 families with pancreatic cancer alone carried p16INK4a mutations, 2/5 (40%) with pancreatic cancer and melanoma carried such mutations. Mutations to the CDKN2A gene were uncommon in familial pancreatic cancer without a concurrent family history of malignant melanoma. These results support the existence of a pancreatic cancer-melanoma syndrome (Bartsch DK, etal, Pancreatology 2001;1(5):510-6).

In 14 patients with both pathologically verified PDAC and melanoma, 2 variant germline CDKN2A mutations were identified in 2/14 patients; both variants lead to compromised CDKN2A function. Occurrence of both pancreatic cancer and melanoma in the same patient signals an inherited susceptibility to cancer, and this predisposition is, in some cases, attributable to germline CDKN2A mutations (Lal G, etal, Genes Chromosomes Cancer, Apr 2000;27(4):358-61). Therefore, patients with one of these diseases should be questioned for a family history of the other disease, and families with both should be counseled about possible genetic screening of family members.

In another study, in the course of research on hereditary pancreatic cancer, investigators observed the joint occurrence of pancreatic cancer and basal-cell carcinoma in 3 German families. The median age of onset of pancreatic cancer was 67 years (range=43-81) and of basal-cell carcinoma 62 years (range=56-73). No other malignancies, diseases, or malformations were identified in living members of these families, or at necropsy at death. Because basal-cell carcinoma is the most common skin cancer in Germany, with an incidence of 1 per 1,000 people, while incidence of pancreatic cancer is rare, about 1 per 10,000, a coincidental concurrence of both tumors in these families cannot be ruled out. However, the occurrence of pancreatic cancer and basal-cell carcinoma in one patient, and the presence of multiple basal-cell carcinomas in 3 members of one family, suggest an inherited predisposition for the tumors. There is one type of basal cell carcinoma, a rare autosomal dominant inherited disease, referred to as the Gorlin or basal-cell naevus syndrome, characterized by multiple lesions in association with multiple cysts within the jaws. Germline mutations of the human homolog of the Drosophila patched (PTCH) gene were identified in about 40% of patients with basal-cell naevus syndrome (Lench NJ, etal, Hum Genet 1997;100:497-502). Also, one third of cases of sporadic basal-cell carcinoma show somatic PTCH mutations (Gailani MR, etal, JNCI 1997;89:1103-09).

Because PTCH is part of the sonic hedgehog signaling pathway and involved in the development of the pancreas, it may be that errors in this pathway could promote human pancreatic disorders (Hebrok M, etal, Development 2000;127:4905-13). Thus, the concurrence of pancreatic cancer and basal-cell carcinoma may point to a new syndrome in which inherited alterations of the hedgehog signaling pathway could cause the development of tumors (Sina-Frey M, The Lancet 11 Jan 2003;361:9352).

Peutz-Jeghers syndrome (PJS) is an autosomal dominant disorder characterized by hamartomatous polyps in the gastrointestinal (GI) tract, and by pigmented macules of the lips, buccal mucosa, and digits. PJS also predisposes patients to an increased risk for GI and pancreatic cancer. Germline and somatic genetic alterations of the STK11/LKB1 gene, encoding a serine/threonine kinase, may play a causal role in carcinogenesis and contribute to the development of both sporadic and familial forms of cancer (Su GH, etal, Am J Pathol, Jun 1999;154(6):1835-40). Investigators at M. D. Anderson Cancer Center (Houston, TX) have generated conditional knock out mice for LKB1 gene, using an lkb1 targeting vector introduced into mice. This tissue specific lkb1-/- mouse model should be a useful tool for the study of PJS and may aid in the new therapies for this disease (Chongjuan Wei C, etal, AACR03, Abs. 6120).

Smoking

Cigarette smoking has been established as a risk factor for pancreatic cancer, based on findings from almost all epidemiologic studies, but it has been only modestly associated with such risk, with smokers being at a 2- to 3-fold risk compared to nonsmokers. Smoking is estimated to account for between 25% and 29% of pancreatic cancer incidence. Long-term smoking cessation may reduce this risk.

Experimental and epidemiologic evidence suggests that both carcinogen-induced DNA damage, and cellular damage induced by inflammation, play a role in pancreatic carcinogenesis. For instance, long-term cigarette smoke inhalation leads to chronic pancreatic inflammation in the rat pancreas, a factor known to increase the risk for pancreatic cancer (Wittel UA, etal, AACR02, Abs. LB82). Aromatic amines and nitrosamines, present in cigarette smoke, are thought to play a role in pancreatic carcinogenesis via activation by cytochrome P-450. Cigarette smoke, which has been shown to induce DNA damage and mutations in target cells, also contains and generates free radicals and oxidants. Oxidative stress and free radical generation occur in pancreatitis, an inflammatory disease of the pancreas that is itself a risk factor for pancreatic cancer, and for which smoking is a risk factor.

Investigators conducted a population-based case-control study in six San Francisco Bay area counties from 1994 to 2001 to identify any association between polymorphisms in genes for two carcinogen metabolizing enzymes, cytochrome P450 1A1 (CYP1A1) and glutathione S-transferase (GST), smoking, and adenocarcinoma of the exocrine pancreas (Duell EJ, etal, JNCI 2002;94:297-306). Blood samples obtained from 309 case subjects and 964 control subjects were analyzed to determine their genotypes for three CYP1A1 polymorphisms (m1, m2, and m4) and for homozygous deletions of two GST genes, GSTM1 and GSTT1. Control subjects were frequency matched to case subjects by age and sex. All statistical tests were two-sided.

None of the genetic polymorphisms themselves affected the risk of pancreatic cancer among Caucasian study participants. However, an interaction was observed between GSTT1-null genotype and cigarette smoking among Caucasians that was more prominent among women than men. Relative to never smokers with the GSTT1-present genotype, the age-adjusted odds ratio (OR) of pancreatic cancer for heavy smokers with the GSTT1-null genotype were 5.0 for women and 3.2 for men; for heavy smokers with the GSTT1-present genotype, OR was 2.0 for women and 2.1 for men. OR for pancreatic cancer among heavy smokers with both GSTT1-null and GSTM1-null genotypes were similar in magnitude to those among heavy smokers with the GSTT1-null genotype alone. There was no evidence of an interaction between CYP1A1 polymorphisms and smoking. The combination of heavy smoking, and a deletion polymorphism in GSTT1 is associated with an increased risk of pancreatic cancer among Caucasians, with the associations possibly stronger in women than in men.

Exhibit 3
Incidence of Pancreatic Cancer in the Developed World in 2002

Location	Male Cases (#)	Crude Rate* (per 100,000)	Female Cases (#)	Crude Rate* (per 100,000)	Total Cases (#)
Europe	**60,576**		**56,330**		**116,906**
Eastern Europe	**8,212**	**10.94**	**6,998**	**8.49**	**15,210**
Belarus	518	10.69	397	7.22	915
Bulgaria	440	11.94	289	7.34	729
Czech Republic	828	16.60	800	15.17	1,628
Hungary	860	17.93	820	15.55	1,680
Moldova	180	8.54	127	5.46	307
Poland	2,014	10.74	1,814	9.13	3,828
Romania	1,169	10.74	786	6.88	1,955
Slovakia	323	12.27	244	8.75	567
Ukraine	2,295	10.26	2,001	7.69	4,296
Northern Europe	**5,058**	**10.79**	**5,261**	**10.84**	**10,319**
Denmark	297	11.20	329	12.13	626
Estonia	103	15.65	90	11.84	193
Finland	294	11.63	334	12.59	628
Iceland	10	7.37	12	8.61	22
Ireland	187	9.74	180	9.18	367
Latvia	175	16.06	147	11.47	322
Lithuania	215	12.76	189	9.87	404
Norway	267	11.95	295	12.90	562
Sweden	459	10.45	501	11.16	960
United Kingdom	3,048	10.31	3,186	10.50	6,234
Southern Europe	**7,836**	**11.49**	**7,239**	**10.12**	**15,075**
Albania	107	6.19	92	5.08	199
Croatia	296	13.90	261	11.53	557
Greece	633	12.07	475	8.79	1,108
Italy	3,980	14.07	3,836	12.94	7,816
Malta	19	9.55	16	7.76	35
Portugal	484	10.00	410	7.83	894
Slovenia	100	10.60	115	11.66	215
Spain	1,811	9.22	1,596	7.78	3,407
Yugoslavia	501	9.67	507	9.27	1,008
Western Europe	**8,838**	**10.73**	**8,490**	**9.86**	**17,328**
Austria	454	11.39	498	11.90	952
Belgium	547	10.89	519	9.88	1,066
France	2,490	8.52	1,870	6.09	4,360
Germany	5,047	12.52	5,200	12.37	10,247
Luxembourg	21	9.33	22	9.66	43
Switzerland	460	12.77	384	10.37	844
Japan	**11,081**	**17.84**	**8,665**	**13.34**	**19,746**
North America	**16,962**	**10.80**	**17,568**	**10.81**	**34, 530**
Canada	1,592	10.09	1,625	10.89	3,217
USA	15,385	10.89	15,943	10.89	31,328
Total	**88,619**		**82,563**		**171,182**

**Source: Ferlay J, etal, Globocan 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0. IARC CancerBase No. 5. Lyon, IARCPress, 2001.*

Exhibit 4
Mortality from Pancreatic Cancer in the Developed World in 2002

Location	Male Deaths (#)	Crude Rate* (per 100,000)	Female Deaths (#)	Crude Rate* (per 100,000)	Total Deaths (#)
Europe	**63,915**		**61,539**		**125,454**
Eastern Europe	**7,731**	**10.3**	**6,668**	**8.09**	**14,399**
Belarus	461	9.53	366	6.66	827
Bulgaria	416	11.29	278	7.06	694
Czech Republic	803	16.10	771	14.62	1,574
Hungary	819	17.06	792	15.01	1,611
Moldova	167	7.92	117	5.05	284
Poland	1,904	10.15	1,745	8.78	3,649
Romania	1,103	10.13	744	6.51	1,847
Slovakia	301	11.42	240	8.61	541
Ukraine	2,150	9.61	1,882	7.23	4,032
Northern Europe	**5,578**	**11.90**	**5,839**	**12.03**	**11,417**
Denmark	335	12.60	363	13.38	698
Estonia	88	13.42	63	8.25	151
Finland	339	13.40	396	14.92	735
Iceland	14	9.95	15	10.86	29
Ireland	199	10.33	194	9.91	393
Latvia	85	7.81	73	5.70	158
Lithuania	230	13.64	175	9.14	405
Norway	295	13.17	293	12.81	588
Sweden	670	15.25	745	16.61	1,415
United Kingdom	3,314	11.21	3,523	11.61	6,837
Southern Europe	**7,536**	**11.05**	**6,960**	**9.73**	**14,496**
Albania	85	4.88	72	3.97	157
Croatia	249	11.70	215	9.51	464
Greece	618	11.78	468	8.67	1,086
Italy	3,816	13.49	3,682	12.42	7,498
Malta	24	12.17	21	10.36	45
Portugal	466	9.62	400	7.63	866
Slovenia	113	12.01	140	14.17	253
Spain	1,950	9.93	1,727	8.42	3,677
Yugoslavia	347	6.69	336	6.14	683
Western Europe	**10,757**	**13.06**	**11,107**	**12.9**	**21,864**
Austria	572	14.35	651	15.55	1,223
Belgium	624	12.42	619	11.79	1,243
France	3,653	12.50	3,273	10.66	6,926
Germany	5,627	13.96	6,146	14.62	11,773
Luxembourg	24	10.74	26	11.21	50
Switzerland	452	12.54	414	11.19	866
Japan	**10,118**	**16.29**	**8,398**	**12.93**	**18,516**
North America	**16,130**	**10.27**	**17,065**	**10.50**	**33,195**
Canada	1,691	10.72	1,717	10.65	3,408
USA	14,439	10.22	15,342	10.48	29,787
Total	**90,163**		**87,002**		**177,165**

**Source: Ferlay J, etal, Globocan 2000: Cancer Incidence, Mortality and Prevalence Worldwide, Version 1.0. IARC CancerBase No. 5. Lyon, IARCPress, 2001.*

Preliminary studies indicated that patients with pancreatic cancer who smoked (n=153) developed this disease 5 years earlier than nonsmokers (n=272), i.e., at a mean age of 59 years versus 64 years. Significantly higher levels of the carcinogenic tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), an established pancreatic carcinogen in animal studies, was detected in the pancreatic juice of smokers as opposed to nonsmokers. Human pancreas metabolizes NNK and, therefore, tobacco-specific nitrosamines play a role in the development of pancreatic cancer in cigarette smokers (Prokopczyk B, etal, AACR02, Abs. 3415).

Also, research suggests that nicotine and nicotine-like substances in cigarette smoke may interact, directly or indirectly, with nicotinic acetylcholine receptors in normal and neoplastic ductal epithelial cells in the pancreas, and perhaps influence lynx1-modulated autocrine and paracrine acetylcholine signaling in the development of pancreatic carcinoma (Sekhon HS, etal, AACR03, Abs. 416).

Other Risk Factors

Studies of dietary factors have not been entirely consistent but do suggest a higher risk for pancreatic cancer with consumption of smoked or processed meats or animal foods, in general, and lower risk with consumption of fruits and vegetables. Several studies have suggested that high dietary fat intake, particularly essential fatty acids, is associated with pancreatic cancer development and growth. For instance, lipoxygenase inhibitors attenuate growth of human pancreatic cancer xenografts and induce apoptosis through the mitochondrial pathway.

Occupational exposure to dyes, metals, polycyclic aromatic hydrocarbons (PAH) and other agents may also play a role in the development of pancreatic cancer.

Because exocrine pancreatic cancer is the human tumor with the highest prevalence of K-ras mutations at diagnosis, the relationship between mutations in codon 12 of the K-ras gene and past occupational exposure was analyzed in 107 cases of exocrine pancreatic cancer in Finland, by comparing specific occupational exposure of cases with mutated K-ras (n=83) to those with wild-type K-ras (n=24). Patients with K-ras mutations were significantly more likely (OR=4.8) than those with wild-type K-ras to have been exposed to dyes and organic pigments. There was some indication of weaker associations between K-ras mutations and occupational exposure to lead, PAH, benzo[a]pyrene, gasoline, nickel, inhalatory exposure to chromium and sedentary work. The association with chromium compounds was stronger for G to T transversions, a finding compatible with experimental studies on mutation spectra for chromium. These results somewhat support the hypothesis of indirect relationships between occupational exposure to dyes and organic pigments, and the activation of the K-ras gene in the etiopathogenesis of human exocrine pancreatic cancer (Alguacil J, etal, Int J Cancer, 20 Nov 2003;107(4):635-41).

Also, organochlorine compounds such as dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), and some polychlorinated biphenyls (PCB) could play a part in the pathogenesis of exocrine pancreatic cancer through modulation of K-ras activation (Porta M, etal, Lancet, 18-25 Dec 1999;354(9196):2125-9).

The association of nonsteroidal anti-inflammatory (NSAI) drugs, including aspirin, with pancreatic cancer took an interesting turn when data presented at the Frontiers in Cancer Prevention meeting of the American Association for Cancer Research (AACR) on October 26-30, in Phoenix, AZ, from a study conducted at Harvard Medical School and Dana-Farber Cancer Center (Boston MA), indicated a 58% higher risk of pancreatic cancer in women who took ≥2 aspirins a week for ≥20 years. These findings come from a study of 88,378 women taking part in a large and wide-ranging study of nurses and their health. Among the 161 nurses who developed pancreatic cancer over 18 years, those who took ≥14 tablets per week had an 86% greater risk of pancreatic cancer than nonusers. Those who took between 6 and 13 tablets had a 41% higher risk, while those who only took 1 to 3 aspirins a week had an 11% greater risk.

These results are a little surprising because approximately a year ago, researchers at the University of Minnesota (Minneapolis, MN) reported that nonsteroidal anti-inflammatory (NSAI) drugs, including aspirin, inhibit pancreatic cancer (Anderson KE, etal, JNCI, 7 Aug 2002;94(15):1168-71). In a prospective study, conducted between 1992 and 1999, to examine the association between the self-reported use of aspirin and other NSAID and the incidence of pancreatic cancer, among 28,283 postmenopausal women who lived in Iowa, 80 cases of pancreatic cancer were identified during 7 years of follow-up. The multivariate-adjusted relative risk of pancreatic cancer associated with any current use of aspirin versus no use was 0.57. There was a trend of decreasing risk of pancreatic cancer incidence with increasing frequency of aspirin use per week. This data indicates that aspirin might be chemopreventive for pancreatic cancer. These conflicting results may be indicative of statistical misadventures, especially when the object of the evaluation is a rare occurrence. In both of these studies, risk is being evaluated in an event affecting 0.18% to 0.28% of the total population under evaluation over several years.

MOLECULAR MARKERS

Molecular markers in pancreatic cancer are being sought to provide a means of screening/early diagnosis, a way to accurately define the specific type of tumor under evaluation and assess patient prognosis, and to monitor the evolution of the disease and the impact of treatment. In addition, molecular markers serve as targets for therapeutic interventions.

Although genetic factors, such as mutations, deletions and alterations of certain genes, and aberrant expression of

certain proteins, undoubtedly play a major role in the development and progression of pancreatic cancer, the exact link between these events and the disease has not been determined. Use of advanced processing and analytical techniques promises to provide a more accurate picture of dysregulated genes and aberrant protein expression, but current knowledge regarding these events lacks the degree necessary to be applicable in the clinic. On a regular basis, novel markers are added to a growing list of those linked to pancreatic cancer (Exhibit 6), but the utility of most of these markers remains obscure.

Currently, very few molecular markers have been shown to be relevant in the clinic. Several markers have been evaluated for diagnostic or prognostic applications, as described below, but none has proven accurate enough to be used routinely for such applications. Several pathways being evaluated as treatment targets in pancreatic cancer are the topic of upcoming part of this series on pancreatic cancer.

One challenge facing those evaluating the role of genetic and protein abnormalities in pancreatic cancer involves accurate disease models. Investigators at the University of California San Francisco (UCSF) Cancer Research Institute and UCSF Cancer Center (San Francisco, CA) are developing *in vivo* and *ex vivo* mouse models to enable systematic investigation of the role of genetics in pancreatic carcinogenesis. To this end, several transgenic mouse lines were derived harboring the avian retroviral receptor (TVA) and the tetracycline-controlled trans-activator (tTA) molecule under the transcriptional control of the cytokeratin 19 (CK19) promoter-enhancer. CK19-driven transgenes were expressed in a number of simple pancreatic, prostate and breast epithelia. Pancreatic ductal epithelial cells are being isolated from these mice to be infected with avian retroviruses carrying a number of genes commonly mutated in pancreatic cancer, to assess their role in pancreatic carcinogenesis (Dankort DL and McMahon M, AACR03, Abs. 4165).

SCREENING

Genetic alterations in pancreatic cancer may be used to potentially screen populations at risk. Although asymptomatic population screening is currently unrealistic, recognition of subpopulations at increased risk from pancreatic cancer, along with novel and sensitive detection techniques, may make early disease detection possible. For instance, when appropriate tools become available, screening may be practical for subpopulations of individuals, such as chronic smokers, those >59 years-of-age carrying some degree of risk, those with familial pancreatic cancer or related malignancies, racial groups at increased risk for pancreatic cancer, etc.

The challenge of screening asymptomatic populations for pancreatic cancer was further confirmed in a study of a mass screening between 1984 to 1985 of more than 10,162 asymptomatic persons >40 years-of-age in Japan, using either ultrasonography alone, or CA19-9 plus elastase-1. In this project, only four (0.04%) cases of pancreatic cancer were detected, and only one was curably resected. Screening 4,506 outpatients with GI complaints or jaundice, discovered 85 (1.9%) patients with pancreatic cancer; 28 of whom had resectable disease. In addition, another 73 (1.6%) patients were diagnosed with other GI malignancies (Homma T and Tsuchiya R, Int J Pancreatol, Summer 1991;9:119-24).

DIAGNOSIS, PROGNOSIS AND DISEASE MONITORING

Currently, diagnosis of pancreatic cancer relies on physical examination to establish the presence of jaundice, and any abnormalities in the patient's abdominal area, including buildup of fluid (ascites); basic tests such as biopsy/pathologic examination of the tumor for cytogenetic abnormalities and alterations in proliferation; blood work; and visualization/imaging modalities. All these approaches suffer from limitations of various degrees.

In the future, diagnosis, prognosis and disease monitoring in pancreatic cancer is expected to be based on the recognition of mutations in oncogenes and tumor-suppressor genes, and aberrant regulation of key proteins such as cell receptors, and growth factors, among others. For instance, K-ras and telomerase activity have been used as molecular markers for the diagnosis of pancreatic carcinoma, whereas p53 and p16 may be a prognostic indicator of pancreatic cancer (Inoue S, etal, Hepatogastroenterology, Jul-Aug 2001;48(40):933-8).

Biopsy

Histologic or cytologic confirmation based on tissues/aspirates obtained by biopsy are necessary for a definitive diagnosis and staging of pancreatic cancer. Tissue or cell material may be obtained by percutaneous puncture as part of the preoperative workup, and by core-needle, incisional, and wedge biopsies, or by fine-needle aspiration (FNA) that can also be performed intraoperatively. Another sample option is pancreatic juice obtained by endoscopic retrograde cholangiopancreatography (ERCP), or by pancreatic duct puncture intraoperatively, or postoperatively from external drainage.

In FNA, a needle is inserted into the pancreas, usually under the guidance of computed tomography (CT) or ultrasound, to aspirate cells for cytologic evaluation. FNA has become the procedure of choice for biopsies of pancreatic lesions. However, this and other biopsy methods, such as ERCP cytology, are still hampered by a substantial false-negative rate. Endoscopic ultrasonography-guided FNA biopsy may play a valuable role in the evaluation of a pancreatic mass when results on other biopsy methods are negative. FNA biopsy is also suitable for tumor staging.

Both FNA and core biopsy, have a specificity in the 90% to 95% range. Sensitivity and diagnostic accuracy are high for both histologic and cytologic evaluations, with a low incidence of false-positives, resulting in a specificity of nearly 100%. However core biopsy is associated with significant complications in 5% to 20% of cases, including

hemorrhage, fistula formation, pancreatitis and even death (Ihse I, etal, World J Surg, Sep 1999;23(9):896-900).

Cytology from aspirates obtained by FNA is the most precise single technique for the diagnosis of PDAC. Also, because it is less traumatic and associated with fewer side effects, it is preferable to core biopsy that is restricted to those few cases in which the former does not yield sufficient information. FNA biopsy has essentially replaced tissue biopsy or frozen section examination of the pancreas. However, a significant number of cancer cases may still be overlooked, so a negative biopsy result does not always exclude malignancy.

Exploratory surgery may be the last resort when other approaches fail. In a less invasive approach to exploratory surgery, a laparoscope, a specialized telescope with a camera attached, is inserted into the patient's abdomen to view the pancreas and surrounding organs to determine the extent of the disease. With laparoscopy, patients who are not candidates for surgery can begin chemotherapy the next day, while those with resectable tumors can immediately proceed to a conventional operation.

When adequate specimens are not available to reach a cytologic diagnosis, the addition of K-ras mutational analysis may offer the best alternative strategy. The point mutation rate of the k-ras gene at codon 12 is reported to be as high as 90% in PDAC; no such mutations are seen in normal pancreatic tissues or other pancreatic disorders. Detection of codon-12 K-ras mutations was performed by the restriction fragment length polymorphism-polymerase chain reaction (PCR) method in 136 aspirates obtained by endosonography-guided FNA, from 33 patients with PDAC and 24 with other lesions. The effectiveness of conventional cytology, K-ras mutational analysis, and their combination, was established with respect to the definitive diagnosis. In patients in whom specimens were adequate (cytology=93% and mutational analysis=100%), the specificity of both techniques was 100%, whereas the sensitivity favored cytology (97% versus 73%). When inadequate samples were considered as misdiagnosed, a combination of both techniques reached the highest overall accuracy with cytology at 91%, mutational analysis at 84%, and the combination of both at 98% (Pellisé M, etal, Aliment Pharmacol Ther, May 2003;17(10):1299).

The incidence of K-ras mutations in IPMT is almost similar to those in PDAC. Among 20 resected IPMT specimens (6 carcinomas and 14 adenomas) and 7 PDAC, K-ras mutation were found in 16/20 (80%) of IPMT, 5/6 (83.3%) of carcinomas, 11/14 (78.5%) of adenomas, and in 7/7 (100%) of PDAC. Multiple different types of K-ras mutations in main tumors were recognized in 7/16 (43.8%) of IPMT, 3/5 (60%) of carcinomas, and 4/11 (36.4%) of adenomas, and in none of PDAC. K-ras mutations in peritumoral lesions, and lesions separated from the main tumors, were detected in 7/11 (63.6%) of IPMT, 1/3 (33.3%) of carcinomas, 6/8 (75%) of adenomas, and 4/5 (80%) of PDAC. In 5/7 (45.5%) IPMT, K-ras mutations were identical to mutations of the main tumors, and in the other 2 cases mutations were distinct from those of the main tumors. In PDAC, mutations were identical to those of the main tumors in 2 cases while they were distinct in the other 2 (Kitago M, etal, AACR02, Abs. 28).

In vitro Diagnostics

Currently, there are no *in vitro* tests specific for the diagnosis of pancreatic cancer. Blood, urine, and stool are routinely checked for abnormal levels of bilirubin and other substances. Bilirubin may reach high levels in patients with pancreatic cancer because of blockage of the common bile duct by the tumor.

The only *in vitro* test, specifically designed for pancreatic cancer, is CA19-9 (CA19.9), which has been approved for disease monitoring and not the diagnosis of pancreatic cancer. Other mucin-type markers such as CA50, CA242, CA195, DUPAN-2 (a precursor of CA19-9), SPAN-1, pancreatic oncofetal antigen (POA), and CAM 17.1/WGA, have also been evaluated in pancreatic cancer, albeit less widely. Combining of two or more tumor markers increases the sensitivity of accuracy in the diagnosis of pancreatic cancer. A "triplet" of tumor markers such as CA 19-9, CA 125 and CEA, in an "integrated" use with ultrasonographic evaluation of the lesion, may provide a more effective diagnostic approach (Cappelli G, etal, Tumori, Jan-Feb 1999;85(1 Suppl 1):S19-21).

Actually, CAM 17.1, developed by Diagnostic Products (DPC; Laos Angeles, CA), was approved in Europe in March 1997 as a screening test for pancreatic cancer. DPC had obtained exclusive worldwide marketing rights to the test through an agreement with the University of Liverpool, in the UK. However, the test did not prove very useful as a screening approach in pancreatic cancer.

The quest for novel diagnostic and prognostic markers continues. Investigators at Johns Hopkins University performed SELDI (surface enhanced laser desorption/ionization) ProteinChip, analysis on 180 serum samples to identify unique tumor markers associated with PDAC. A total of 60 specimens from patients with PDAC, other pancreatic diseases (pancreatitis, pancreatic cysts, cystadenomas, neuroendocrine tumors, IPMN, and ampullary adenomas) and healthy controls, were analyzed using IMAC-Cu ProteinChip surfaces, and read on a mass spectrometer. Analysis of the resulting proteomic profiles identified a set of 4 protein peaks that separated PDAC from normal controls with a sensitivity of 83% (48/59) and specificity of 85% (50/58). An independent set of protein peaks differentiated PDAC from pancreatitis with 70% sensitivity (41/58) and 85% specificity (22/26). Based on CA 19-9 levels available for a subgroup of the samples (27/60 disease controls, and 42/60 malignancies), CA19-9 provided a diagnostic sensitivity of 64% and specificity of 85% for PDAC. These findings illustrate the potential of the SELDI ProteinChip approach for the diagnosis of PDAC (Koopmann J, etal, AACR03, Abs. 5735). ProteinChip has been developed by Ciphergen Biosystems (Fremont, CA).

CA19-9 is a serological tumor marker test that is being used in the monitoring of patients with pancreatic cancer undergoing pancreatic resection and chemotherapy. It has a mean sensitivity of 81% (range=76% to 99%) and a mean specificity of 90% (range=69% to 93%). A decrease in CA 19-9 assay values correlates with a positive response to therapy. An increase in CA 19-9 values may indicate progressive disease, and thus aid the physician in assessing treatment regimens. Increased concentrations of CA19-9 are not, however, specific for PDAC. High levels can also be found in other GI malignancies, especially in advanced disease, and in various benign conditions, such as chronic and acute pancreatitis, cirrhosis, cholangitis and hepatocellular jaundice. Also, the specificity of CA 19-9 is particularly low in the presence of jaundice. For example in a recent prospective study using a cut-off value of 40 U/ml, the specificity was 92% in patients without jaundice but only 69% in those with jaundice. With a cut-off point of 100 U/ml, the respective specificities were 99% and 83%, respectively.

Exhibit 5
Estimated Age-adjusted Incidence Rates (per 100,000) by Age and Race in the USA (1966-2000)

Age Groups	All Races	African Americans
<50	0.9	1.5
20-54	2.6	4.3
<65	3.7	6.0
55-64	22.2	35.2
50+	38.1	55.0
65-74	49.0	70.6
65+	62.8	87.5
75+	78.0	106.0

Source: SEER incidence age-adjusted rates from 9 registries, 1996-2000

As a diagnostic test in pancreatic cancer, CA19-9 can complement radiologic procedures, especially in patients without jaundice. When used as a diagnostic aid, it is important to bear in mind that elevated level of CA19-9 are seen in only about 55% of patients with pancreatic tumors <3 cm in size. Also, because patients with certain benign diseases such as jaundice and pancreatitis may present with elevated levels of CA19-9, its utility is of limited value in the diagnosis of pancreatic cancer, particularly early forms of the disease. CA19-9 may also be of value as a prognostic marker.

In May 2003, the FDA approved for marketing in the USA a radioimmunoassay (RIA) test to measure serum CA 19-9 for monitoring of disease status in pancreatic cancer patients, developed by Fujirebio Diagnostics (FDI; Malvern, PA). This test is also available worldwide for this indication. FDI, a diagnostics company specializing in tumor-marker assays with an emphasis in oncology, was formerly known as Centocor Diagnostics, acquired by Fujirebio (Tokyo, Japan) in November 1998. In the USA, the exclusive distributor of FDI's CA 19-9 RIA is Polymedco (Cortlandt Manor, NY).

As of July 1, 1999, costs associated with CA-19.9 are being reimbursed when it is used to monitor response to treatment in patients with an established diagnosis of pancreatic cancer. Normal use of this test is once every 3 months during treatment.

CA50 is a tumor-associated gangliosidic antigen elevated in sera from patients with GI malignancies, including pancreatic cancer, and is also detected in benign lesions such as chronic pancreatitis.

CA195 is a circulating serum marker associated with pancreatic, colorectal and other GI malignancies. To establish the performance of CA195 as compared to that of carcinoembryonic antigen (CEA), expression of CA195 in serum was studied in patients with pancreatic cancer and with biliary or pancreatic benign disease, and results were compared with CEA expression. Overall sensitivity with CA195 was higher than that for CEA alone. Sensitivity increased with the simultaneous use of these two antigens, but the difference was statistically significant only for CEA. Specificity of CA195 calculated from all patients with benign diseases was lower than that of CEA. Using a cut-off value of 100 U/ml for CA195, the specificity of this antigen was higher than that of CEA. Results demonstrate that significant elevations of tumor antigen CA195 are relatively specific for pancreatic carcinoma, and that this antigen is superior to CEA for diagnosing pancreatic cancer by virtue of its higher sensitivity (Andicoechea A, etal, World J Surg 1999;23:227-32).

CA242 is a sialylated carbohydrate epitope expressed on the mucin antigen. CA242, situated on the same macromolecule as CA50, bears structural but no chemical similarities to CA19-9 and CA50. CA-242 antigen is shed from the tumor, and can be detected in serum from patients with carcinoma. The antigen is expressed mostly on pancreatic, and colon cancer, and adenocarcinoma of other organs. CA242 exhibits tumor specificity, with rare occurrences of normal tissue staining. It has been described as a marker for lung, GI, pancreatic and colorectal cancer. In one series, sensitivity of CA 242, CA 19-9 and CA 50 for pancreatic cancer were 41.3%, 54.3% and 47.8%, respectively. No significant improvement was achieved by combining CA 242 with CA 19-9 and/or CA

Exhibit 6
Molecular Markers Associated with Pancreatic Cancer

General Description*	Pancreatic Cancer Association
AKT2	
AKT2 is an oncogene that encodes a protein-serine/threonine kinase containing a pleckstrin homology domain characteristic of many signaling molecules, implicated in phosphatidylinositol-3-OH kinase signaling.	Amplification of AKT2 has been noted in approximately 10% of pancreatic cancer, in 2/18 cell lines and 1/10 primary tumor specimens. Overexpression of AKT2 contributes to the malignant phenotype of a subset of PDAC (Cheng JQ, etal, PNAS USA, 16 Apr 1996;93(8):3636-41). Overexpression of wild-type or constitutively active AKT (i.e., AKT1 or AKT2) resulted in elevated IGF-1r expression. Therefore, there is a link between AKT signaling and the regulation of IGF-1r expression. Active AKT promotes the invasiveness of pancreatic cancer cells through the upregulation of IGF-1r expression (Tanno S, etal, Cancer Res, 15 Jan 2001;61(2):589-93).
Activity-dependent neuroprotective protein (ADNP)	
The human ADNP gene is frequently amplified in many neoplasms, including breast, bladder, ovarian, pancreatic, head and neck, brain, and colon cancer. Sequence analysis suggests a transcription factor function. Downregulation of ADNP by antisense oligodeoxynucleotides upregulated p53, and reduced the viability of intestinal cancer cells by 90%. Thus, ADNP is implicated in maintaining cell survival, perhaps through modulation of p53 (Zamostiano R, etal, J Biol Chem, 5 Jan 2001;276(1):708-14).	
ADAMTS1/METH-1	
ADAMTS1/METH-1 are members of a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) family of genes. METH-1 protein is a potent inhibitor of angiogenesis and ADAMTS1 shows metalloproteinase function.	METH-1 was expressed in both pancreatic cancer and non-cancerous pancreas but its expression in pancreatic cancer tissue was significantly lower than that in noncancerous pancreas. METH-1 appears to be involved in progression of pancreatic cancer through local invasion and lymph node metastasis (Masui T, etal, Clin Can Res, Nov 2001;7:3437-43).
Annexin II	
Annexin II, a heterotetrameric molecule consisting of pairs of heavy (36 kDa) and light chains (11 kDa), is present in selected normal tissues and cells, and overexpressed in many cancers. Annexin II is secreted into the extracellular environment. There, it participates in plasminogen activation, cell adhesion, and tumor metastasis and invasion via interactions with specific proteases and extracellular matrix (ECM) proteins. Other activities of annexin II may include cell proliferation, differentiation, vesicular secretion, as well as cell-surface fibrinolysis, and migration. Annexin II has been implicated as an important mediator of cancer-cell survival, and as a potentiator of metastasis.	Expression of annexin II is enhanced in human pancreatic carcinoma cells and primary pancreatic cancer. Annexin II mRNA and protein levels in 5 established human PDAC cell lines, 3 primary pancreatic tumors and 1 metastatic tumor, were 5- to 15-fold higher in all 5 cell lines compared to normal pancreas. Significant elevations (2- to 8-fold) of annexin II expression were observed in the 3 primary pancreatic tumors and 1 metastatic tumor. Increased expression of annexin II was limited to proliferating PDAC. Annexin II expression localized with cells that express proliferating cell nuclear antigen (PCNA). In normal pancreas, annexin II is expressed in ductal and ductular cells but not in acinar or islet cells (Vishwanatha JK, etal, Carcinogenesis, Dec 1993;14(12):2575-9).
BCL2-associated X protein (BAX)	
BCL2-associated X protein (BAX) regulates apoptosis in cellular pathways involving both BCL2 and p53. Bax promotes apoptosis via heterodimerization with BCL2. Overexpressed Bax counters the death-repressor activity of Bcl-2.	Expression of several members of the Bcl-2 family proteins was investigated in 30 invasive PDAC and 23 IPMT, and in 6 cancer tissues and 7 pancreatic cancer cell lines. Bcl-2 was expressed in 23%, Bax in 53%, Bcl-X in 90%, and Mcl-1 in 90% of invasive PDAC. IPMT of Bax was expressed in 44% and Bcl-XL and Mcl-1 in 88%. Bcl-XL was the predominant form of the Bcl-X protein in both pancreatic cancer tissues and cell line. Both Bcl-XL and Mcl-1 protein levels were uniformly high in all cell lines. These results suggest that an imbalance between antiapoptosis proteins (such as Bcl-2, Bcl-XL, and Mcl-1) and proapoptotic proteins (such as Bax and Bcl-X) is involved in PDAC. Furthermore, predominantly high expressions of Bcl-XL and Mcl-1 in IPMT, may be involved in the carcinogenesis in IPMT (Miyamoto Y, etal, Oncology 1999;56(1):73-82).

— continued on next page

Cancer-associated Sm-like (CaSm)	
Cancer-associated Sm-like (CaSm) belongs to the Sm-like protein family and appears to be an important modulator of RNA biogenesis and function. CaSm mRNA is upregulated in pancreatic cancer and in several other cancer-derived cell lines, compared to their normal tissue cognates, including cell lines from cancer originating in liver, ovary, lung, pancreas, and kidney.	The CaSm oncogene is overexpressed in 87.5% of pancreatic tumor/normal tissue pairs (Schweinfest CW, etal, Cancer Res, 15 Jul 1997;57(14):2961-5). Antisense CaSm mRNA can alter the transformed phenotype of pancreatic cancer cells by reducing their ability to form large colonies in soft agar when compared to untransfected cells, suggesting that CaSm expression is required to maintain the transformed state.
CD44v6	
CD44v6 is a variant of CD44 that comprises a family of widely distributed cell adhesion molecules with multiple functions in cell-matrix interactions. The standard form of the CD44 (CD44H) protein is a transmembrane glycoprotein present in a wide variety of human lymphoid cells, epithelial cells and tumors. It also has many variant isoforms, which are generated by alternative splicing. CD44 can act as a growth agent and a tumor suppressor, depending largely on the isoform pattern of CD44 expressed in the cell, the cellular equipment with ERM protein members, the nature of the ECM, and other unknown factors.	Expression of the CD44 antigen and its associated isoforms has been detected in pancreatic cancer, and this cancer is characterized by a high, yet independent expression of CD44 and p53 protein. CD44 expression shows no correlation with the degree of tumor differentiation, while CD44v6 expression is higher in malignancies of higher histologic grade. Intraductal pancreatic cancer shares a similarity with invasive cancer with respect to CD44 and v6 expression, indicating that already at the stage of its intraductal growth, pancreatic cancer exhibits properties affecting its invasiveness and tendencies to metastasize (Tomaszewska R, etal, Pol J Pathol 1999;50(3):145-53).
Cyclooxygenase 2 (COX-2)/PTGS2	
Cyclooxygenase (COX) is the rate-limiting enzyme in prostaglandin synthesis. Two isoforms of COX, have been characterized. The isoform COX-1 is a constitutively expressed enzyme in most tissues, while COX-2 is the inducible form, whose synthesis can be upregulated by several cytokines, growth factors, and tumor promoters. Overexpression of COX-2 has been linked to carcinogenesis and tumor progression in many human malignancies, including colorectal, lung, breast, esophagus, bladder, pancreatic, and prostate neoplasms	Material from 36 pancreata (30 PDAC, 65 PanIN, and 30 normal pancreatic ducts) was analyzed for COX-2, and was found to be considerably heterogenous between and within examined samples. The overall average percentage of positive cells was 47.3% in PDAC, 36.3% in PanIN, and 19.2% in normal ducts. COX-2 was expressed in more than 20% of cells in 23 (77%) PDAC, 42 (65%) PanIN, and 12 (40%) normal ducts. Significant differences in COX-2 expression were demonstrable in PDAC and, PanIN versus normal ducts, and in PanIN 2/3 versus PanIN 1a/1b. In general, the pattern of COX-2 expression increased from normal to PanIN to PDAC (Maitra A, etal, Am J Clin Pathol, Aug 2002;118(2):194-201).
Cyclin D1 (CCND1)/bcl-1 oncogene	
Cyclin D1 is involved in both normal regulation of the cell cycle and neoplasia. In the G1 (resting) phase of the cell cycle, cyclin D1 together with its cyclin dependent kinase (cdk) partner, is responsible for transition to the S phase (DNA synthesis) by phosphorylating the product of the retinoblastoma gene (pRb), which then releases transcription factors important in the initiation of DNA replication. Amplification of the bcl-1 gene or overexpression of the cyclin D1 protein releases a cell from its normal controls and causes transformation to a malignant phenotype (Donnellan R and Chetty R, Mol Pathol, Feb 1998;51(1):1-7).	A cyclin D1mRNA transcript was present in all cultured human pancreatic cancer cell lines, and in normal as well as cancerous pancreatic tissues, although cyclin D1 mRNA levels were 2.1-fold higher in pancreatic cancer than in normal pancreas. MST of cancer patients with lower cyclin D1 levels (n=16) was 15.5 months whereas in patients with higher levels (n=16) was 6.5 months. These data indicate that cyclin D1 expression may not only serve as a predictor of postoperative survival of patients with pancreatic cancer, but may represent a target for treatment modalities that block cyclin D1 activity in the therapy of these patients (Kornmann M, etal, Oncology, Jul-Aug 1998;55(4):363-9). Suppression of cyclin D1 expression after stable transfection of a cyclin D1 antisense construct in PANC-1 and COLO-357 human pancreatic cancer cells resulted in a significant increase in sensitivity to cisplatin, the fluoropyrimidines 5-FU and 5-fluoro-2'-deoxyuridine, and mitoxantrone (Kornmann M, etal, Cancer Res, 15 Jul 1999;59(14):3505-11, and Kornmann M, etal, J Clin Invest, 15 Jan 1998;101(2):344-52).

— continued on next page

Cyclin-dependent kinase inhibitor 2A (CDKN2A)/p16	
CDKN2A (p16) is a tumor-suppressor gene that encodes a key checkpoint protein in the cell cycle. This protein functions as an inhibitor of cyclin-dependent kinase cdk4 and cdk6, blocking phosphorylation of pRb leading to G1 growth arrest acting as a molecular brake during a key step in the cell division process. When the gene is mutated, it loses its braking function and uncontrolled cell growth may result. Overexpression of p16 has been shown to block the transition through the G1/S phase of the cell cycle in an Rb-dependent fashion. The p16 gene is mutated in 46% of all cancers, making it the second most important gene in cancer, based upon its frequency of occurrence, after p53.	The p16 tumor-suppressor protein is inactivated in more than 90% of pancreatic tumors. The p16 locus of tumor tissue is nearly always altered in pancreatic cancer. Alterations of the p16 gene for the carcinogenesis in the pancreas involve different molecular mechanisms, including deletion, mutation or methylation. Germline mutations in p16 gene have been demonstrated to predispose to pancreatic cancer. In addition, the anticancer effects of exogenous p16 gene have been shown by introduction of it into human pancreatic tumor cell lines (Wang C, and Lu X, Zhongguo Yi Xue Ke Xue Yuan Xue Bao, Oct 2000;22(5):491-3).
Ecto-5'-nucleotidase (5'-NT)/CD73	
Ecto-5'-nucleotidase (5'-NT) is an extracellular enzyme which catalyzes the dephosphorylation of AMP and other nucleoside monophosphates, to corresponding nucleosides. It is anchored to the cell membrane through a glycosylphosphatidylinositol (GPI) linkage. As an enzyme that produces nucleosides, particularly adenosine, 5'-NT is thought to modulate neuronal signaling, vascular perfusion, drug metabolism and immune response. However, like other GPI-anchored proteins, 5'-NT also has cellular functions that are independent of its enzymatic activity. For instance, 5'-NT binds to the intracellular filament protein actin and ECM proteins laminin and fibronectin, suggesting a possible role for 5'-NT in cell adhesion.	Using a genome-wide gene expression profiling by cDNA microarray, 5'-NT was identified as one of the most highly upregulated genes in pancreatic cancer cells. All 11 pancreatic cancer cell lines for which gene expressions was compared to that of normal pancreatic cells by microarray, showed more than a 10-fold upregulation in 5'-NT expression. Levels of 5'-NT protein at the cell surface of these cell lines correlated well with its mRNA levels inside the cell. Also, compared to normal pancreatic tissue, 6/7 (86%) cancer samples showed 5'-NT overexpression; 3 samples had high degree upregulation (>15 fold), while upregulation in the other 3 was moderate (between 3- and 7-fold). 5'-NT may be an important factor in pancreatic tumor growth and metastasis and a potential molecular marker and drug target for pancreatic cancer (Han H, etal, AACR03, Abs. 5018).
Epidermal growth factor receptor (EGFr)	
EGFr is a multisided, multifunctional transmembrane glycoprotein with intrinsic tyrosine kinase activity. Upon ligand binding, the monomeric receptor undergoes dimerization resulting in kinase activation, and the phosphorylation of its own tyrosine residues (autophosphorylation), followed by activation of signal transducers. Deregulation of signaling as a result of EGFr gene amplification or rearrangement, has been implicated in the development of a number of neoplasms	In pancreatic cancer, mRNA expression has been shown to be enhanced compared with normal controls for a number of important tyrosine growth factor receptors, including EGFr (4-fold), suggesting that coexpression of EGFr and its ligands may contribute to the aggressiveness of human pancreatic cancer (Yamanaka Y, etal, Anticancer Res, May-Jun 1993;13(3): 565-9; Korc M, Surg Oncol Clin N Am, Jan 1998;7(1):25-41; Friess H, etal, Ann Surg, Dec 1999;230(6):767-74, discussion 774-5).
Epiregulin	
Epiregulin is a growth regulating peptide related to epidermal growth factor (EGF). The human epiregulin gene encodes a putative transmembrane precursor protein secreted as a soluble form that is biologically active by stimulating DNA synthesis. Epiregulin inhibits the growth of several epithelial tumor cells, and stimulates the growth of fibroblasts and various other cell types. Human epiregulin is expressed mainly on peripheral blood macrophages and the placenta in normal tissues; it is highest on epithelial tumor cell lines.	In 5 pancreatic cancer cell lines, and in PDAC and chronic pancreatitis (CP), epiregulin mRNA was present at high (MIA-PaCa-2 cells) or moderate (ASPC-1, CAPAN-1, and T3M4) levels in most cells, but was below detection levels in PANC-1 cells. All cell lines exhibited a dose-dependent increase in growth in response to recombinant human epiregulin. A moderate to intense epiregulin mRNA signal was present in most pancreatic cancer cells in PDAC. In contrast, only a weak (normal pancreas) to moderate (CP) signal was present in the ductal and acinar cells in CP. These findings suggest that epiregulin may contribute to the pathobiology of PDAC, and may also have a role in CP (Zhu Z, etal, Biochem Biophys Res Commun, 14 July 2000;273(3):1019-24).

— continued on next page

Fas/APO-1 and its ligand (FasL)	
Fas is a type I membrane receptor belonging to the tumor necrosis factor (TNF)/nerve growth factor (NGF) family. Fas activates apoptosis independently of bcl-2. Fas ligand (FasL) is a type II transmembrane protein. Upon binding to FasL, the Fas/FasL ligand complex induces apoptosis in target cells. Dysregulation of Fas and FasL expression is present in several malignant neoplasms. Also, expression of FasL by tumors may mediate their counterattack on cytotoxic lymphocytes.	Fas and FasL are expressed in most if not all pancreatic carcinoma cell lines. In 81 human primary pancreaticobiliary or ampullary ductal adenocarcinoma, Fas was expressed in 19% of patients with strong or intermediate intensity but with variable percentages of tumor-cell staining. FasL was expressed in 49% of patients, usually with diffuse expression but variable intensity. Fas expression was more common in women than men. In women, Fas expression was associated with strong HER2 expression (67% in Fas+ versus 18% of Fas-). Fas expression tended to be less common in blacks (Fas+ tumors=4%) than whites (Fas+ tumors=22%), and was associated with Stage IV disease at diagnosis (24% versus 0%). Neither Fas nor FasL expression was associated with survival, suggesting that their role, if any, in contributing to the aggressiveness of these tumors is complex (Pernick NL, etal, Pancreas, Oct 2002;25(3):E36-41).
Fibroblast growth factor receptor 4 (4FGFr4)	
FGFr4, one of four members of the fibroblast growth factor receptor (FGFr) family expressed in a variety of tissue types. It plays a key role in important physiologic processes among which is regulation of angiogenesis.	Fibroblast growth factor receptor 4 (FGFR4) is expressed in 50-70% of pancreatic carcinomas and a similar proportion of derived cell lines.
Heparanase	
Heparanase is an endo-β-D-glucuronidase that can cleave heparan sulfate. Heparan sulfate and heparan sulfate proteoglycans are important structural components of the ECM and the external cell surface, bind various molecules such as growth factors and cytokines, and modulate the biologic functions of binding proteins. Heparan sulfate proteoglycans are also important structural components of the basement membrane. Heparanase has been implicated in inflammation and tumor angiogenesis and metastasis, and it is upregulated in metastatic cancers. In addition to its other functions, heparanase may support tumor growth by releasing heparan sulfate-bound growth factors.	Heparanase plays a role both in chronic pancreatitis (CP), and in localized and metastatic pancreatic cancer. In PDAC, there is a significant inverse correlation between heparanase expression and postoperative survival. Heparanase expression was investigated in 8 human pancreatic cancer cell lines (AsPC-1, BxPC-3, Capan-1, CFPAC-1, HPAF-II, Hs766T, MIAPaCa-2 and PANC-1) and primary PDAC, as well as histologically normal pancreas, and the results were correlated with survival. All in all, 25 cases of CP and 50 cases of PDAC were evaluated for heparanase expression. All 8 pancreatic cancer cell lines expressed heparanase at different levels, with strong correlation of mRNA and protein expression, while no detectable heparanase expression was observed in normal pancreatic tissue. Heparanase expression was absent in 20% of CP samples, was moderate in 56% and strong in 24%, while it was absent in 24% of tumor specimens, moderate in 44%, and high in 32%, with a tendency of lower expression at metastatic sites. There was similar expression of heparanase mRNA and protein. Heparanase-expressing tumors had a tendency to metastasize to the lymph nodes, but results were not statistically significant. Survival analysis of 26 patients who underwent curative resection showed a significantly shorter survival of patients with heparanase-expressing tumors (MST=17 months), compared to those without such expression (MST=34 months). There was no correlation between stage and survival. Because heparanase expression was a much better prognostic factor for survival after curative resection than stage, it may prove valuable as a prognostic marker in PDAC (Rohloff J, etal, AACR02, Abs. 3544).
Hypoxia-inducible factor-1α (HIF-1α)	
The α subunit of the hypoxia inducible factor 1 (HIF-1α) is rapidly degraded by the proteasome under normal conditions, but is stabilized by hypoxia resulting in the transactivation of several proangiogenic genes. HIF-1α serves as a transcription factor that regulates gene expression involved in response to hypoxia and promotes angiogenesis. HIF-1α functions as a survival factor that is required for tumorigenesis in many types of malignancies, and is expressed in a majority of metastases and late-stage tumors. Tumors overexpressing HIF-1α are highly vascular and overproduce angiogenic peptides such as VEGF, which is also a transcriptional target for HIF-1α.	Constitutive expression of HIF-1α renders pancreatic cancer cells resistant to apoptosis induced by hypoxia and glucose deprivation. Dominant-negative HIF-1α reduces tumorigenicity of pancreatic cancer cells through suppression of glucose metabolism. Disruption of the HIF-1 pathway might be effective in the treatment of pancreatic cancer (Chen J, etal, Am J Pathol, Apr 2003;162(4):1283-91).

— *continued on next page*

Inhibitor of DNA binding 2 (Id2)	
Inhibitor of DNA binding 2 (Id2) belongs to the Id family of helix-loop-helix (HLH) proteins, which upon heterodimerization with basic HLH proteins prevent them from DNA binding. Proteins of the Id family act as negative regulatory transcriptional factors, and their expression correlates with cell proliferation and arrested differentiation in many cell lineages.	Id2 mRNA was expressed at significantly higher levels in pancreatic cancer in comparison to normal pancreas. Furthermore, there was abundant Id2 immunoreactivity in cancer cells within the pancreatic tumor mass. In PANC-1 pancreatic cancer cells, steady-state Id2 mRNA levels increased upon serum addition, and decreased after induction of differentiation with either sodium butyrate or 12-O-tetradecanoylphorbol-13-acetate. Inhibition of Id2 expression with Id2 antisense oligonucleotides inhibited the growth of these cells, whereas random and sense oligonucleotides were without effect. These findings suggest that Id2 may have a role in human pancreatic cancer (Kleeff J, etal, Cancer Res, 1 Sep 1998;58(17): 3769-72).
Insulin-like growth factor I (IGF-I) and its receptor (IGF-1r)	
The insulin-like growth factor (IGF) signal transduction system involves ligands, receptors, and binding proteins. It has been shown to be mitogenic for malignant cell lines of both epithelial and mesenchymal origin. There are two types of IGF, IGF-I and IGF-II, whose biological activity is regulated by six IGF binding proteins (IGFBP 1-6). The cancer-related functions of IGF-I and IGF-II are mediated exclusively through their interaction with the type 1 IGF receptor (IGF-1r).	IGF-I and its receptor (IGF-1r) are highly expressed in pancreatic cancer. IGF-Ir is an important growth factor receptor for cell proliferation and invasion of pancreatic cancer cells, and VPF/VEGF expression in ASPC-1. IGF-1r mediates different signaling pathways to execute its functions. Activation of Ras by IGF-1r is required for cell invasion. Src activation through IGF-1r is required for the cell proliferation, invasion, and also VPF/VEGF expression. Taken together, these two activities highlight the importance of IGF-1r in growth and invasiveness of the pancreatic cancer, and also point out the multiple signaling pathways channeled through this receptor (Zeng H, etal, Biochem Biophys Res Commun, 28 Feb 2003;302(1):46-55).
K-ras	
K-ras is a proto-oncogene belonging to the ras family of genes.	K-ras mutations are the most common oncogene mutations in pancreatic cancer, occurring in 75% to >95% of pancreatic cancer tissues (Minamoto T, etal, Cancer Detect Prev 2000;24(1):1-12, Sakorafas GH, etal, Cancer Treat Rev, Feb 2000;26(1):29-52, and Lohr M, etal, Int J Pancreatol, Apr 2000;27(2):93-103).
Kang ai 1 (KAI1)/CD82	
Kang ai (stands for anti-cancer in Chinese) 1 (KAI1) is an adhesion molecule implicated in the progression/metastasis of several different tumor types. KAI1 belongs to a structurally distinct family of membrane glycoproteins, the transmembrane 4 (TM4) superfamily, which function via cell-cell and cell ECM interactions, thereby potentially influencing the ability of cancer cells to invade tissues and to metastasize into lymph nodes and distant organs (Huang C, etal, AACR96, Abs. 512:74). There is a direct relationship between KAI1 genes and loss of p53 function leading to KAI1 downregulation (Mashimo T, etal, AACR98, Abs. 172:26).	KAI1 is downregulated in pancreatic cancer, enhancing its metastatic potential. KAI1 expression in lymph node and liver metastases was compared with primary pancreatic cancer to evaluate its influence on metastasis, in 14 primary pancreatic cancer samples in which no lymph-node metastases were present, and 25 primary pancreatic cancer samples with lymph-node metastases. In 20 of these cases, both types of samples came from the same patient. In addition, 11 liver metastases were available for KAI1 analysis. Increased steady-state levels of KAI1 mRNA were found in 33/39 (85%) primary pancreatic cancers in comparison with normal controls; mRNA levels were significantly higher in nonmetastasized tumors compared with tumors associated with lymph-node involvement or distant metastases. In lymph-node metastases KAI1 mRNA expression was lower than in the corresponding primary tumors. In 14/20 lymph-node metastases, there was no KAI1 mRNA expression, and in 6/20 lymph-node metastases only weak KAI1 mRNA levels were present in some cancer cells. Distant metastases were devoid of or exhibited low KAI1 mRNA levels compared to primary pancreatic cancer cells. These data support the hypothesis that KAI1 gene expression might influence the metastatic ability of pancreatic cancer cells *in vivo*. Reduction of KAI1 appears to promote cancer cell spread in lymph nodes and distant organs (Friess H, etal, Int J Cancer, 21 Aug 1998;79(4): 349-55). The overall survival rate of 15 patients with pancreatic cancer positive for KAI1/CD82 was significantly higher than that of 25 patients with pancreatic cancer with decreased KAI1/CD82 gene expression (Sho M, etal, Int J Cancer, 23 Oct 1998;79(5):509-16).

— continued on next page

Mesothelin	
Mesothelin is a cell-surface glycoprotein present on normal mesothelial cells and overexpressed in mesothelioma, ovarian, pancreatic and cervical cancer, and some squamous cell carcinomas. Mesothelin is not shed in substantial amounts into the blood stream.	The N-terminal fragment (megakaryocyte potentiating factor) of mesothelin has been isolated from the culture supernatant of human pancreatic tumor cell line HPC-Y5. Mesothelin expression was studied in malignant and benign pancreatic disease, including 18 PDAC resection specimens 8 pancreatic resection specimens served as controls, 7 of which showed PC and one a benign adenoma. Mesothelin expression was graded as absent (0% cells staining), weakly positive (<5%), moderately positive (5-29% and strongly positive (>30%). Out of the 18 cases of PDAC, 16 (89%) were strongly positive for mesothelin expression while 2 were weakly positive. In none of these cases the adjacent normal pancreas stain for mesothelin. Out of the 8 specimens from patients with benign pancreatic histology, 7 (88%) did not exhibit mesothelin expression while one case of PC showed <5 % mesothelin expression. These results show that mesothelin is highly expressed in the majority of PDAC and not in benign pancreatic disease. Given this expression pattern, mesothelin could serve as a marker in differentiating benign from malignant pancreatic tumors (Hassan R, etal, ASCO03, Abs. 1138).
Matrix metalloproteinase 14 (MMP14)	
MMP14 is a transmembrane protein, unlike other reported MMP that are secretory proteins and its products are potentially expressed on the cell surface (Takino T, etal, Gene, 3 Apr 1995 Apr 3;155(2):293-8).	In PDAC, MMP14 and MMP9 mRNA were seen at moderate levels both in cancer and in stromal cells. Antigens of MMP2, MM-9, and MMP14 immunolocalized to the neoplastic epithelium and to the stromal cells. Correlation to the clinical data showed that MMP14 expression had a strong statistical association with a poor patient outcome. Results suggest the importance of MMP14 for malignant growth and indicate that increased MMP14 mRNA expression by tumor cells in PDAC may have prognostic significance (Maatta M, etal, Clin Can Res, Jul 2000;6:2726-34).
Mammalian target of rapamycin (mTOR)/FRAP-p70s6K	
The mammalian target of rapamycin (mTOR) is involved in the translation of mRNA in eukaryotic cells regulated by amino acids. mTOR controls a myriad of downstream effectors, including RNA polymerase I, S6K1, 4E-BP1, and eEF2 kinase. In yeast, mTOR signals through Tap42p/α4 to regulate protein phosphatases. Through phosphorylation of Tap42p/α4, mTOR abrogates dephosphorylation of the downstream effectors by PP2 A and/or PP6, resulting in their increased phosphorylation (Kimball S, Prog Mol Subcell Biol 2001;26:155-84).	mTOR signaling regulates mitogenic responses to growth factors in eukaryotic cells. To examine whether inhibition of this pathway affects mitogen-induced proliferation and cell-cycle progression of human pancreatic cancer cells *in vitro*, quiescent BxPC3 and PANC-1 pancreatic cancer cells were treated with rapamycin. Rapamycin inhibited the phosphorylation of p70s6K, while inducing G1 cell-cycle arrest. In both cell lines, rapamycin inhibited serum-induced proliferation without affecting apoptosis. Akt phosphorylation was not affected, indicating FRAP/mTOR specificity of rapamycin. mTOR signaling appears to be necessary for G1-to-S phase progression, and proliferation in pancreatic cancer cells (Shah S, etal, J Surg Res, 15 May 2001; 97(2):123-30).
MET	
Met, a proto-oncogene, is a transmembrane protein tyrosine kinase receptor for hepatocyte growth factor/scatter factor (HGF/SF). A number of c-Met activating mutations, many of which located in the tyrosine kinase domain, have been detected in various solid tumors, and have been implicated in tumor invasion and metastasis. Stimulation of c-Met via HGF/SF results in many biological and biochemical effects in the cell. Activation of c-Met signaling can lead to scattering, angiogenesis, proliferation, enhanced cell motility, invasion, and eventual metastasis. Mutated or overexpressed c-Met in malignant cells, is an important therapeutic target (Ma PC, etal, Cancer Metastasis Rev, Dec 2003;22(4):309-25).	In a panel of 10 pancreatic cancer cell lines, expression of HGF/SF was absent in all cell lines, but c-Met was overexpressed at varying levels in 9 out of 10. These results suggest that pancreatic cancer cells respond to HGF/SF through a paracrine signaling pathway. To test the responsiveness of these cells lines to HGF/SF, transwell migration assays were performed using CFPAC-1 (c-Met positive) and MiaPaCa-2 (c-Met negative) cells. Media containing HGF/SF had greater than a 3-fold effect on CFPAC-1 migration, while MiaPaCa-2 was not significantly affected. Analysis of the gene expression profiles of HGF/SF-treated CFPAC-1 and MiaPaCa-2 cells produced 22 genes specifically upregulated in CFPAC-1 (c-Met positive) cells. These genes represent potential mediators of HGF/SF signaling and include K-ras, ROCK1, Lyn, TRAF5 and ARNO all of which have been shown to play roles in cell motility and migration. In addition, HGF/SF-treated CFPAC-1 cells showed significant downregulation

— continued on next page

	of 42 genes. Interestingly, several of the downregulated genes are known negative regulators of cell migration such as APC and the tight junction protein, ZO-1. These data elucidates cell response downstream of HGF/SF and c-Met and may provide new targets for drug development in pancreatic and other tumors (Warner SL, etal, AACR02, Abs. LB42).
Mucin 4 (MUC4)/SMC	
Sialomucin complex (SMC)/MUC4 is a large, heterodimeric glycoprotein complex composed of two subunits, a heavily glycosylated mucin extracellular domain ASGP-1, stably associated with the N-glycosylated transmembrane subunit ASGP-2. The transmembrane subunit ASGP-2 has two EGF-like domains, one of which acts as an intramembrane ligand for the receptor tyrosine kinase ErbB2/HER2/neu, which has been strongly implicated in cancer progression.	Human MUC4 is frequently aberrant by expressed in PDAC, while remaining undetectable in normal pancreas (Andrianifahanana M, etal, AACR02, Abs. 5622).
Multiple endocrine neoplasia I (MEN1)	
Multiple endocrine neoplasia I (MEN1) is a tumor-suppressor gene. Defects in MEN1 are the cause of familial multiple endocrine neoplasia.	In 11 non-MEN1 malignant tumors of the endocrine pancreas, 9 nonfunctioning tumors, and 2 glucagonoma, loss of heterogeneity (LOH) of at least one informative locus on 11q13 was found in 70% of tumors. Also, 3 tumors displayed somatic mutations of the MEN1 gene together with LOH on 11q13, whereas the corresponding germ line DNA was normal. These findings support the hypothesis that MEN1 gene mutations contribute to the tumorigenesis of nonfamilial, malignant endocrine pancreatic tumors (Hessman O, etal, Cancer Res, 1 Feb 1998;58(3):377-9).
Nuclear factor κB (NFκB)	
The nuclear factor κB (NFκB) protein, encoded by two genes in the human genome, plays a role in almost every aspect of cell regulation, including immune cell activation, proliferation, apoptosis, stress responses, differentiation and oncogenic transformation. Constitutive activation of NFκB has been described in a many solid tumors. This activation appears to support cancer-cell survival and reduce sensitivity to cytotoxic drugs. Also, certain chemotherapeutics induce this transcription factor themselves and through this mechanism lower their cytotoxic potential. Inhibition of NFκB by various means has been shown to enhance the sensitivity to antineoplastic- or radiation-induced apoptosis *in vitro* and *in vivo*. Furthermore, suppression of NFκB results in attenuation of cancer cachexia and metastasis in some mouse tumor models (Arlt A, and Schafer H, Int J Clin Pharmacol Ther, Aug 2002;40(8):336-47).	Transcription factor NFkB is activated constitutively in human PDAC, and human pancreatic cancer cell lines, but not in normal pancreatic tissues or in immortalized/nontumorigenic pancreatic epithelial cells, suggesting that NFκB plays a critical role in development of PDAC. Inhibition of NFκB signaling can suppress the angiogenic potential and metastasis of pancreatic cancer. Therefore, the NFκB signaling pathway is a potential target for anticancer agents (Fujioka S, etal, Clin Cancer Res. 2003 Jan;9(1):346-54). The combination of pharmacologic inhibition of NFκB by established anti-inflammatory drugs together with certain anticancer agents may be of great benefit for the treatment of pancreatic cancer.
Pancpin	
Pancpin is a pancreas-specific serine protease (serpin). Serpins serve many functions in normal biologic processes, which are often usurped by cancer cells thus allowing progression of tumors by increasing the growth and metastatic potential of the neoplasia. Serpins regulate many physiological functions such as fibrinolysis, angiogenesis, apoptosis, inflammation, and metastasis. Changes in serpin expression can be associated with the development and progression of certain types of cancer.	When Pancpin was stably introduced into the pancreatic cancer cell lines MiaPaCa-2 and PANC-1. It inhibited the ability of these cells to form colonies in soft agar and to form tumors in xenograft models. Also, reintroduction of pancpin directly reduced cellular proliferation, and the cancer cells' ability to invade through a reconstituted basement membrane. In the PANC-1 transfected cell line mRNA expression of ≥3-fold was noted for the cellular proteases matrix metalloprotease-2 (MMP2), MMP9, and urokinase-type plasminogen activator (uPA). Downregulation of approximately ≥8-fold was seen in the mRNA for claudin-4 and endothelial PAS1. Increased mRNA expression of over 4-fold was seen for prostacyclin-stimulating factor, MSG-related protein 1, and Id3 (Olsen CE, etal, AACR02, Abs. 1883).

— *continued on next page*

Phosphatidylinositol 3`kinase (PI3K)	
Phosphatidylinositol 3`kinase (PI3K) plays a central role in cellular proliferation, neovascularization, viability, and senescence. Because PI3K-mediated activation of the cell-survival kinase PKB/Akt, and negative regulation of PI3K signaling by the tumor suppressor PTEN, are key regulatory events in tumorigenesis, it has been postulated that PI3K promotes development of cancer (Sasaki T, etal, Nature, 24 Aug 2000, 24;406(6798):897-902).	Cross signaling between PI3K and NFκB enhances the antitumor effect of TNF-α in human pancreatic cancer cells. Because TNF-α and certain chemotherapeutic agents activate both apoptosis and NFκB-dependent antiapoptotic genes, they may neutralize their own antitumor effects. The cell-signaling mechanisms for such chemoresistance have not been fully elucidated but may involve PI3K. PI3K inhibition significantly enhanced the antiproliferative and proapoptotic effects of TNF-α in cell lines, and Ly294002, a PI3K inhibitor, also blocked TNF-α-induced Akt activation but failed to alter cytoplasmic IκBα degradation or subsequent NFκB nuclear translocation. NFκB-dependent gene expression, however, was ultimately suppressed by Ly294002, suggesting that PI3k-dependent activation of NFκB is IκBα independent. PI3K inhibition can block NFκB-dependent gene expression regardless of cytoplasmic IκBα/NFκB activation. Because it also regulates the antitumor effects of TNF-α, PI3K may in part determine NFκB-induced chemoresistance in human pancreatic cancer (Shah SA, etal, J Gastrointest Surg, Nov-Dec 2001;5(6):603-13).
Prostate stem cell antigen (PSCA)	
PSCA is a small lipid-modified protein located on the surface of prostate cancer cells. It is expressed during normal prostatic development most highly on cell populations undergoing rapid proliferation. The level of PSCA goes up during cancer progression and is very high in metastatic lesions in the bone marrow. MAb reactive with human PSCA can block establishment of prostate cancer xenografts in immunodeficient mice and block metastasis from established tumors.	Comparing SAGE libraries derived from PDAC with those derived from nonneoplastic tissues, it was discovered that PSCA was expressed in 4/6 pancreatic cancer SAGE libraries, but not in the libraries derived from normal pancreatic ductal cells. Therefore, PSCA is a novel tumor marker for pancreatic carcinoma with potential diagnostic and therapeutic implications (Argani P, etal, Can Res, 1 June 2001;61:4320-4).
Protease M/kallikrein 6 (KLK6)	
Kallikreins are proteolytic enzymes that constitute a subfamily of serine proteases. KLK6 gene, a member of the human kallikrein gene family, encodes for a secreted protease, human kallikrein 6 (hK6), also known as zyme/protease M/neurosin. X-ray data provide support for the characterization of hk6 as a degradative protease with structural features more similar to trypsin than the regulatory kallikreins (Bernett MJ, etal, J Biol Chem, 5 Jul 2002;277(27):24562-70).	The protease M gene is present in both normal and malignant pancreatic tissues. However, expression of protease M at the mRNA level differ in these two tissue types. Protease M was not expressed in normal pancreatic tissue and islet tumor of the pancreas, while expression was noted in PDAC. Pancreatic tumor cell lines also differed in their expression of protease M. Cell line SU 86.86 lacked protease M expression, while the HPAF-11 cell line, which was derived from an adenocarcinoma, demonstrated high levels of expression. HPAF-11 exhibited a 91.6 ± 0.5 invasion index compared to 60.7 ± 2.0 in SU 86.86, which lacked protease M expression. Transfection of protease M into SU 86.86 increased its invasion index to 94 ± 1.4. This data suggests that expression of protease M in pancreatic cancer is associated with increased invasion. It may also be an important biomaker for monitoring and staging PDAC because of its total lack of expression in islet tumors of the pancreas (Yan-Sanders Y, AACR02, Abs. 2650).
Protein gene product 9.5 (PGP9.5)	
PGP9.5 is a neurospecific peptide that removes ubiquitin from ubiquitinated proteins and prevents them from targeted degradation by proteasomes. It belongs to a family of ubiquitin carboxyl-terminal hydrolases that play important roles in nonlysosomal proteolytic pathway (Kon Y, etal, Mol Reprod Dev, Dec 1999;54(4):333-41).	PGP9.5 expression was evaluated using immunohistochemistry in 69 resected PDAC and also in normal pancreatic tissue. A significant negative correlation was found between overexpression of PGP9.5 and postoperative survival. Multivariate analysis also suggested that PGP9.5 along with tumor stage and extrapancreatic plexus invasion to be strong predictors of outcome. This suggests that PGP9.5 expression may be used as a marker for predicting outcome of pancreatic cancer patients treated by resection (Tezel E, etal, Clin Cancer Res 2000 Dec;6(12):4764-7).

— continued on next page

Protein tyrosine phosphatase type IVA, member 1 (PTP4A1)/PRL-1	
PRL-1 encodes an enzyme involved in cellular growth of the liver and in the differentiation of intestines and other tissues; it is also expressed in mitogen-stimulated fibroblasts. Expression of PRL-1 is associated with cell proliferation and differentiation attributed to its ability to regulate protein tyrosine phosphorylation and dephosphorylation of certain substrates.	Overexpression of the PRL-1 gene was confirmed in 9 pancreatic cancer cell lines when compared to normal pancreas (Farnsworth AL, etal, AACR03, Abs. 957).
Ras homolog gene family member C (rhoC)	
Ras homolog gene family member C (rhoC) belongs to the Rho GTPase family that regulates numerous cellular functions, most notably cytoskeletal organization in response to extracellular factors. RhoC is a regulator of cytoskeletal structure and cell movement, and it itself is regulated by cell adhesion and growth factor receptors. When overexpressed, RhoC enhances metastasis, whereas a dominant-negative Rho inhibits metastasis. Analysis of the phenotype of cells expressing dominant-negative Rho or RhoC indicates that RhoC is important in tumor cell invasion (Clark EA, etal, Nature, 3 Aug 2000;406(6795):532-5).	Expressions of the rhoA, B and C genes were examined by RT-PCR in 33 cases of PDAC, and mutations of the K-ras, rhoA, B and C genes, correlated with rho gene expression, were studied in the same series of tumor tissues. Expression levels of the rhoC gene were significantly higher in tumors than in nonmalignant cells. Metastatic lesions overexpressed the rhoC gene compared with primary tumors. Higher expression of the rhoC gene was noted in carcinoma tissues with perineural invasion and lymph-node metastasis than in tumors without these manifestations. Overexpression of the rhoC gene significantly correlated with poorer prognosis in patients with PDAC. In contrast, there was no significant relationship with clinicopathologic findings with the expression levels of rhoA and B. No mutations were found in the rhoA, B or C gene sequences examined. K-ras gene mutation, detected in 27/33 (81.8%) cases, did not affect expression levels in any rho gene. Therefore, elevated expression of the rhoC gene may be involved in the progression of pancreatic carcinoma independent of K-ras gene activation (Suwa H, etal, Br J Cancer 1998; 77(1):147-52).
ROCK-1	
GTPase Rho and one of its effector molecules, ROCK, regulate cytoskeleton and actomyosin contractility, and play a crucial role in cell adhesion and motility. Elevated activity of ROCK-1, one of the isomers of ROCK kinases, leads to an increase in the activity of invasion and metastasis of cancer cell lines.	Expression of ROCK-1 in two cancer cell lines and 31 human pancreatic tissues (21 pancreatic cancers and 10 histologically normal tissues) was detected in 18/21(85.7%) pancreatic cancer tissues but not in normal pancreatic tissues. ROCK-1 may contribute to pancreatic cancer cell invasion and/or metastasis by facilitating cancer cell migration (Pancreas, Apr 2002;24(3):251-7).
Smad-related proteins Smad6 and Smad7	
Smad6 and Smad7 genes are members of the Smad family, involved in the transforming growth factor-β1 (TGF-β1) signaling pathway. Overexpression of a dominant-negative Smad3 mutant or Smad7, both of which impair Smad-mediated signal transduction, inhibits TGF-β1-dependent apoptosis (Topper JN, etal, PNAS USA, 19 Aug 1997;94(17):9314-9).	Alterations in growth inhibitory pathways such as Smad4 mutations, and Smad6 and Smad7 overexpression, are often encountered in pancreatic cancer (Ozawa F, etal, Teratog Carcinog Mutagen 2001;21(1):27-44).
Smad4/Dpc4/MADH4	
SMAD4 is a tumor suppressor in the TGF-β signaling pathway. TGF-β is an extracellular ligand that binds to a heterodimeric receptor, initiating signals that regulate growth, differentiation, and apoptosis. Many cancers harbor defects in TGF-β signaling and are resistant to TGF-β-mediated growth suppression.	SMAD4 is genetically inactivated in approximately 55% of all PDAC, and gene transcription mediated by Dpc4 is a critical tumor-suppressive pathway in PDAC. Most PDAC exhibit allelic loss of chromosome 18. Detailed analysis revealed a consensus region of homozygous deletion at 18q21.1 in one third of PDAC. The DPC4/Smad4 gene, located in this region, was inactivated by intragenic mutations in another 20% of PDAC. Dpc4 protein was shown to mediate TGF-b-stimulated gene transcription through sequence-specific binding to DNA. Eleven mutant Dpc4 proteins, identified in human carcinomas, were all found to be impaired in their ability to regulate gene transcription. A functional grouping of the mutant proteins could be made in those that were deficient in DNA binding, those that had impaired nuclear translocation, and those that had affected their transcription activation domain (Schutte M. Ann Oncol. 1999;10 Suppl 4:56-9). Smad4 protein status was characterized in 249 PDAC resected from patients who underwent pancreaticoduo-

— continued on next page

	denectomy at Johns Hopkins between 1990 and 1997, as well as the SMAD4 gene status of 56 of 249 (22%) pancreatic carcinomas. Patients with PDAC with Smad4 protein expression lived significantly longer with an unadjusted MST of 19.2 months, compared with 14.7 months for those lacking Smad4 protein expression. This survival benefit persisted after adjustment for prognostic factors including tumor size, margins, lymph-node status, pathologic stage, blood loss, and use of adjuvant chemoradiotherapy. The relative hazard of mortality for cancers lacking Smad4 after adjusting for other prognostic factors was 1.36 (Tascilar M, etal, Clin Cancer Res, Dec 2001;7(12):4115-21). DPC4/smad4 is also an important target gene promoting tumorigenesis of nonfunctioning neuroendocrine pancreatic carcinomas (Bartsch D, etal, Oncogene, 8 Apr 1999;18(14):2367-71).
Sonic hedgehog homolog (SHH)	
Sonic hedgehog, a secreted hedgehog ligand, is a human homolog of the Drosophila segment polarity gene hedgehog, cloned by investigators at Harvard University (Marigo V, etal, Genomics, 1 Jul 1995;28(1):44-51). Analyses of human tumors reveal mutations in various components of the sonic hedgehog signaling pathway that appear to activate this pathway, as inferred by the increased expression of the transcription factor, Gli-1. Interestingly, a proportion of the human tumors and most of those arising in mouse models continue to express the normal patched (ptc) allele, suggesting the involvement of additional molecular events in the transformation of the haploinsufficient cells (Wetmore C, Curr Opin Genet Dev, Feb 2003;13(1):34-42).	Sonic hedgehog is abnormally expressed in PDAC, and its precursor lesions, PanIN. Pancreata of Pdx-Shh mice in which Shh is misexpressed in the pancreatic endoderm, develop abnormal tubular structures, a phenocopy of human PanIN-1 and -2. These PanIN-like lesions also contain mutations in K-ras and overexpress HER-2/neu, genetic mutations found early in the progression of human pancreatic cancer. Furthermore, hedgehog signaling remains active in cell lines established from primary and metastatic PDAC. Inhibition of hedgehog signaling by cyclopamine induced apoptosis and blocked proliferation in a subset of the pancreatic cancer cell lines both *in vitro* and *in vivo*. Therefore, that this pathway may play an early and critical role in the genesis of PDAC and that maintenance of hedgehog signaling is important for aberrant proliferation and tumorigenesis (Thayer SP, etal, Nature, 23 Oct;425(6960):851-856).
Survivin	
Survivin is a member of the inhibitor of apoptosis (IAP) protein family. In cells, survivin binds specifically to the effector cell death proteases, caspases-3 and -7, but not to the initiator protease caspase 8, and complexes with caspase-9. Expression of survivin is cell-cycle dependent and developmentally regulated. Survivin plays important roles in both cell proliferation and cell death. Overexpression of survivin results in accelerated S phase shift, resistance to G1 arrest, and activated Cdk2/Cyclin E complex leading to Rb phosphorylation. Survivin is normally expressed during fetal development but not in normal adult tissues. However, high levels of survivin expression are detected in many human cancers. Based on its structure, its apoptotic action appears to involve attachment to the mitotic spindle (Verdecia MA, etal, Nature Structural Biology, July 2000;7:602-8). Its 3-dimensional structure implies that it accomplishes its task by setting the stage for the assembly of the mitotic spindle. If it is torn down, the cell is unable to pull itself apart and dies.	Expression of survivin in 4 pancreatic carcinoma cell lines, 56 human pancreatic tissues (normal=5, chronic pancreatitis=12, PDAC=26), and 16 lesions from 13 IPMT, was investigated to establish its association with tumor apoptosis and/or tumorigenesis. Survivin expression was found in tumor cells but not in non neoplastic pancreatic tissues. Survivin expression was observed in 20/26 cases of PDAC (76.9%) and in 9/16 IPMT lesions that ranged from adenoma to invasive (56.3%) tumors. Survivin was more frequently expressed in malignant tumors than in benign tumors. In PDAC, high levels of survivin expression were associated significantly with a reduction in the apoptotic index of tumor cells. Therefore, it appears that expression of survivin may be upregulated during an early step of tumorigenesis, and during the development of cancer by reducing cancer-cell apoptosis (Satoh K, etal, Cancer, 15 Jul 2001;92(2):271-8).
Synuclein-γ (SNCG)	
Synucleins are small conserved proteins associated with cancer and neurodegenerative diseases. Synuclein-γ, a member of synuclein family, localizes to centrosomes and poles of the spindle of mitotic cells.	When expression of synuclein-γ mRNA was investigated in 12 pancreatic cancer cell lines, including ASPC1, MDAPanc28, Capan1, Capan2, PANC-1, HS766T, MDAPanc3, MDAPanc48, Colo-357, MiaPaca2, CFPac1 and BxPC3, it was found to be overexpressed in 11/12. The positive rates were 67% (8/12) in pancreatic cancer cell lines and 69% (22/ 32) in tissue samples of pancreatic tumors. Adjacent normal pancreatic tissues did not express synuclein-γ, suggesting that it may be used as a marker of pancreatic cancer (Zhongkui Li, etal, AACR03, Abs. 2890).

— *continued on next page*

Thrombospondin-1 (TSP-1)	
Thrombospodin-1, an ECM glycoprotein, is an inhibitor of angiogenesis. It is upregulated by wild-type p53. Low TSP-1 expression may be associated with tumor neovascularity and mutant p53 expression. TSP-1 in the ECM binds to cell surface receptors including integrin $\alpha v\beta 3$, an interaction which may interfere with the endothelial cells' ability to form new blood vessels. TSP-1's interaction with its cellular receptors plays a role in modulating cell migration, cell adhesion, platelet aggregation, blood coagulation, cell growth, angiogenesis, tumor invasion and metastasis, and malarial cytoadhesion (JNCI, 5 Feb 1997, 89(3): 219-27). Urokinase plasminogen activator receptor (uPAr) also plays a crucial role in the regulation of tumor-cell adhesion and TSP-1-mediated tumor-cell invasion (Albo D, etal, J Surg Res, Apr 1999;82(2):331-338). In malignant tumors, high concentrations of TSP-1 work as an angiogenic agonist.	TSP-1, in a receptor-mediated process that involves the activation of TGF-β1, upregulates PAI-1 expression in pancreatic cancer without affecting uPA production (Albo D, etal, J Gastrointest Surg, Jul 1999;3(4):411-417). When TSP-1 expression and the correlation between TSP-1 expression pattern and clinicopathologic features were examined in 77 cases of invasive PDAC, TSP-1 immunoreactivity was detected in the cancer stroma. Diffusely positive and focally positive patterns of TSP-1 were found in 33/77 (42.9%) and 40/77 (51.9%) cases, respectively. TSP-1 diffuse expression was significantly correlated with lymph-node metastasis, neural invasion and TNM stage. Based on univariate analysis, significant parameters were histologic differentiation, lymphatic invasion, venous invasion, neural invasion, TNM stage and TSP-1 expression, suggesting that TSP-1 plays important roles in pancreatic cancer cell growth and metastasis, and that stromal TSP-1 immunoreactivity is a good prognostic marker of pancreatic cancer (Tobita K, etal, Int J Oncol, Dec 2002;21(6):1189-95). Expression of TSP-1 in pancreatic carcinoma correlates with microvessel density (MVD). Among 98 pancreatic carcinomas analyzed with respect to TSP-1 immunoreactivity and its correlation to intratumoral MVD, 87 tumors showed strong TSP-1 immunoreactivity, 9 carcinomas were only weakly positive, and 2 lesions were negative for TSP-1. TSP-1 immunoreactivity was detected in the ECM, mostly at the invasion front of the tumor. High levels of TSP-1 mRNA were observed in 3/7 pancreatic carcinomas. The mean MVD in pancreatic carcinoma was 38.8 vessels per mm^2. MVD was higher in tumors with a high expression of TSP-1, and the correlation between TSP-1 immunoreactivity and MVD was highly significant. As a modulator of angiogenesis, TSP-1 is strongly expressed in most PDAC and likely contributes to the extensive neovascularization and spread of this highly aggressive tumor (Kasper HU, etal, Virchows Arch, Feb 2001;438(2):116-20).
Transforming growth factor β (TGF-β)	
As the most potent immunosuppressor known, transforming growth factor β (TGF-β) plays a key role in advanced cancer, by regulating metastasis, angiogenesis, and tumor-cell proliferation. Three mammalian TGF-β isoforms have been identified (TGF-β1, β2, and β3), each encoded by different genetic locus. Many cancers, including pancreatic cancer, harbor defects in TGF-β signaling and are resistant to TGF-β-mediated growth suppression.	PDAC overexpresses TGF-β. Human pancreatic cancer cells are resistant to TGF-β-mediated growth inhibition, and frequently harbor Smad4 mutations, overexpress inhibitory Smad6 and Smad7, and underexpress the type I TGF-β receptor (TGFBr1). The action of TGF-β is dependent on its ability to bind to TGFBr1, which heterodimerizes with TGFBr1 thereby activating downstream signaling. TGF-β acts in *vivo* to enhance gene products that promote tumor growth and metastasis of pancreatic cancer cells, and raise the possibility that soluble TGFr2 may ultimately have a therapeutic benefit in PDAC (Rowland-Goldsmith MA, etal, AACR02, Abs. 5267).
Transmembrane 4 superfamily, member 5 (TM4SF5)	
TM4SF5, a cell-surface protein and a member of the transmembrane 4 superfamily, is found on many different cell types in many organisms. A major characteristic of this family is their ability to form cell-surface complexes with other molecules participatingin cell adhesion, either to the ECM or to other cells, and with molecules required for signaling.	TM4SF5 was previously identified in a large-scale screening for differentially expressed genes in pancreatic cancer. It is highly homologous to the tumor-associated antigen L6. TM4SF5 was overexpressed in pancreatic cancer tissues as compared to both normal pancreas, and in CP tissues, and was detected at high levels in other tumor tissues. TM4SF5 may be useful as a pancreatic cancer diagnostic in the clinical setting (Muller-Pillasch F etal, Gene, 16 Feb 1998;208(1):25-30).
Transmembrane Serine Protease TMPPRSS3	
TMPRSS3 is a novel membrane-bound serine protease overexpressed in cancer. It may play a role in metastasis formation and tumor invasion (Wallrapp C, etal, Can Res, 15 May 2000; (60)2602-06).	TMPRSS3 mRNA is strongly expressed in a subset of pancreatic cancer, and in various other cancer tissues, and its expression correlates with the metastatic potential of the clonal SUIT-2 pancreatic cancer cell line.

— *continued on next page*

Trefoil factor 2 (TTF2)/human spasmolytic polypeptide (hSP)	
Trefoil factor 2 (TFF2) is a polypeptide that inhibits GI motility and gastric acid secretion. It is involved in mucin production and cell growth. TFF2 protein is expressed in digestive cancers but not in normal pancreas.	When expression of TFF2 was investigated in PDAC, ampullary carcinomas, mucin-producing tumors, serous cystadenomas and islet cell tumors of the pancreas, it was found to be expressed in 23% of PDAC. It was more frequently detected in cases of early-stage or histologically low grade PDAC than in cases of late-stage or histologically high grade carcinomas; hSP expression was detected in 92% of mucin-producing tumors, but was not detected in serous cystadenoma, or islet cell tumors. Patients with hSP protein expression had a better prognosis than those without hSP expression. Immunohistochemical hSP expression was related to differentiation and a better prognosis in PDAC. Furthermore, hSP protein is related to the pathogenesis and clinical characteristics of mucin-producing tumors of the pancreas (Ohshio G, etal, Dig Dis Sci, Apr 2000;45(4):659-64).
Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)	
Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family of death signal transduction proteins with a mechanism of cell death similar to that of Fas and FasL system. It is the ligand for the newly discovered DR4 and DR5 receptors. TRAIL is expressed as a type II membrane protein (memTRAIL), and signals apoptosis via the death domain-containing receptors TRAIL-R1 and -2. Apoptosis regulated by Fas receptor/FasL and DR4, DR5/TRAIL plays a major role in tumor escape and elimination mechanisms. Soluble recombinant derivatives of TRAIL (sTRAIL) are considered novel tumor therapeutics because of their selective apoptosis-inducing activity in a variety of human tumors but not in normal cells.	The effect of therapeutic agents such as gemcitabine on the susceptibility to TRAIL-induced apoptosis was studied in pancreatic cell lines. PDAC expressed high levels of apoptosis-inducing receptors and ligands, which demonstrated differential susceptibility to cell death induced by TRAIL, despite expressing intact receptors and signaling machinery. The observation that treatment with commonly used therapeutic agents did not augment their susceptibility to apoptosis, may be explained by the fact that they expressed differentially high levels of decoy receptors, as well as molecules known as inhibitors of apoptosis. Pancreatic carcinoma cells may have developed different mechanisms to evade the immune system, such as expression of nonfunctional receptors, decoy receptors, and molecules that block cell death, such as bcl2 and bcl-xL, or expression of apoptosis-inducing ligands, such as TRAIL, may induce cell death of immune cells. Treatment of malignant tumors by recombinant TRAIL might apply to some but not all pancreatic tumors because of their differential resistance to TRAIL-induced cell death (Ibrahim SM, etal, Pancreas, Jul 2001;23(1):72-9).
Tyrosine kinase receptors trkA (NTrK1), trkB (NTrK2), and trkC (NTrK3)	
Tyrosine kinase receptors (trk) are a family of receptors comprising a tyrosine kinase domain (which phosphorylates proteins on tyrosine residues), a hormone binding domain, and a carboxyl terminal segment with multiple tyrosines for autophosphorylation. Aberrant expression of trk receptors has been observed in clinical carcinoma specimens and cell lines. Neurotrophin receptor-linked trk (trkA, trkB, and trkC) have been implicated in cancer progression (Miknyoczki SJ, etal, Int J Cancer, 5 May 1999;81(3):417-27, Ruggeri BA, etal, Curr Med Chem, Sep 1999;6(9):845-57).	Aberrant expression of the Trk receptors (Trk A, B, and C), enhanced tumor stromal expression of neurotrophins in primary PDAC specimens, and human PDAC-derived cell lines. A dose-dependent biologic response of PDAC cells (*in vitro* invasiveness) to selective neurotrophins, has also been demonstrated (Miknyoczki, S J, etal., Int J Cancer 1999; 81:417-427). Expression of trk is a biologic marker for cell proliferation and perineural invasion in PDAC. A comparative study between several clinicopathologic factors and trk receptors in 28 surgically resected PDAC specimens, revealed a significant correlation between increased expression of trkA and cancer proliferation, as well as trkC and cancer invasion, including venous and perineural invasion. This novel mechanism in PDAC progression was mediated via an interaction of neurotrophins (NGF, BDNF, NT-3), trk tyrosine kinase receptors (trkA, B, C) and p75NGFR (Sakamoto Y, etal, Oncol Rep, May-Jun 2001;8(3):477-84).
Wnt1	
Wnt1 is a member of the Wnt family of secreted-type glycoproteins that play key roles in carcinogenesis and embryogenesis.	Wnt1 mRNA was relatively highly expressed in BxPC-3 cells in pancreatic cancer (Katoh M, Int J Oncol, Jan 2003;22(1):209-12).

**This information has been garnered from NEW MEDICINE's Oncology KnowledgeBASE (nm\|OK), a subscription-based resource residing at www.nmok.net. Additional information regarding these markers may be found in the Targets in Oncology module of nm\|OK.*

50. Therefore, CA 242 serum assay does not seem to improve diagnostic accuracy for pancreatic cancer compared to CA 19-9 and CA 50 (Ventrucci M, etal, Clin Chem Lab & Med, Mar 1998;36(3):179-84).

Carbonic anhydrase 3 (CAR-3) antigen is a carcinoma-associated marker, which is expressed on a mucin-like molecule. Serum levels of CAR-3 were measured in patients with various diseases of the GI tract, including pancreatic cancer. Cut-off levels were calculated on the basis of a 90% specificity. At a cut-off level of 6.15 U/l, the sensitivity of CAR-3 was 62.3% as compared to CA 19.9 at 77%, CA195 at 75.4% and CEA at 24.5%. There were significant differences in the differential diagnosis of pancreatic cancer and other GI diseases. There was no association either between serum levels of tumor markers and tumor stage, or between short- and long-term survivors. In a follow-up evaluation, CT scanning was superior to serologic tests. Among tumor markers, CAR-3 achieved a sensitivity of 62.5% while CA19.9 sensitivity was 83.3% and CA195 75%. CAR-3 is shed in the circulating stream in much lower proportions than that observed for antigen expression at immunohistochemistry (Giulianotti P et al, Ann Ital Chir, May-Jun 1997;68(3): 307-13).

PaCa-Ag1, developed at SUNY Downstate Medical School (Brooklyn, NY), is identified by MAb 3C4 against a 43 kD glycoprotein. PaCa-Ag1 may be a candidate antigen and specific tumor marker for pancreatic cancer. Immunofluoresnce localized the PaCa-Ag1 on the cell surface of all rat and human pancreatic cancer cells and cell lines including BMRPA1.TUC-3, Mia PaCa-2, BxPC-3, CAPAN 1 and CAPAN 2. PaCa-Ag1 antigen was not expressed on normal rat and human pancreas, white blood cells, or other normal and cancerous human tissues. *In vitro* studies, designed to assess if the PaCa-Ag1 is a marker of early stage disease, examined the timing of expression of PaCa-Ag1 during the gradual transformation over 12 passages of normal BMRPA1 cells into the malignantly transformed cell line BMRPA1.NNK, following incubation with a direct nicotine derivative and tobacco smoke carcinogen. Analysis of MAb 3C4 in cells from each of the 12 successive passages showed the presence of PaCa-Ag1 in approximately 60% of cells of the second passage, long before they began showing the characteristic transformed (spindle cells) and neoplastic morphology of uninhibited growth, formation of foci, colonies, and tumors. Analysis by cytofluorometry of the successive passages showed progressive increases in the fluorescence intensity for PaCa-Ag1 expression from passage 3 to passage 8, as well as in the number of cells that expressed the 3C4 antigen. Preliminary results also indicate that the PaCa-Ag1 glycoprotein is being shed from the cell surface into the circulation. These findings suggest that PaCa-Ag1 may serve as a valuable early pancreatic cancer-specific tumor marker (Hannan R, etal, AACR03, Abs. 1850).

Molecular beacons are oligonucleotide probes that can detect the presence of specific nucleic acids in homogeneous solutions. They represent a new type of biosensor for the detection of RNA within living cells. A beacon consists of a short piece of single-stranded DNA (ssDNA) in the shape of a hairpin loop, which is a probe sequence complementary to a target nucleic acid molecule. The ssDNA is synthesized to match a region on a specific mRNA that is unique to the gene or where a mutation is known to occur. Annealing the complementary arm sequences on the ends of the probe sequence forms the stem. A fluorescent moiety is attached to the end of one arm, and a quenching moiety to the end of the other arm. The fluorescence of the beacon is quenched, or suppressed, until it binds to a complementary target mRNA, which causes the hairpin to open up and the beacon to illuminate.

The Public Health Research Institute (PHRI; Warren, NJ), the owner of the molecular beacon technology, offers nonexclusive worldwide licenses in a variety of fields for a variety of uses. A license under PHRI's patents includes rights to six different patents and patent applications and further improvements to the technology. Numerous programs are using molecular beacon technology for the detection of a variety of infectious diseases. A detection approach based on molecular beacon technology, developed by bioMerieux (Marcy-l'Etoile, France), was launched in Europe, in July 2002, to monitor viral loads in HIV-infected patients.

Molecular beacons may eventually help locate intracellular molecular markers that signal the development of cancer or other diseases. When used for gene detection in living cells, however, the conventional molecular beacon design causes a lot of false-positive signals. An improved version of this nanoscale gene-detection tool has been developed by Gang Bao, PhD, at Emory University (Atlanta, GA) and the Georgia Institute of Technology. In his presentation at the 225th National Meeting of the American Chemical Society in New Orleans on March 26, 2003, Dr. Bao explained that using a pair of molecular beacons with fluorescence resonance energy transfer (FRET) dyes, to create a "dual-FRET" molecular beacon, reduces the incidence of false-positive signals. In his design, the FRET signal does not occur until both donor and acceptor beacons are bound to adjacent sites on the same target mRNA, resulting in energy transfer between the two dye molecules. This approach was applied in the detection of pancreatic cancer, to assess its value in the diagnosis of early stage disease by designing a molecular beacon to detect a specific genetic mutation in the K-ras gene that is present in 80% to100% of pancreatic cancers.

In vivo Diagnostics

In vivo diagnostics include both invasive and noninvasive approaches. Among the most commonly used radiologic techniques for imaging the pancreas are ultrasound, computed tomography (CT), magnetic resonance (MR)

imaging, and endoscopic retrograde cholangiopancreatography (ERCP). However, despite all these available technologies, objective radiographic assessment of pancreatic cancer is difficult and generally unreliable. Individual imaging techniques suffer from poor sensitivity for small masses (<2 cm), and cannot easily differentiate tumor tissue from pancreatic masses associated with chronic pancreatitis. Also, no radiologic examination is very sensitive at visualizing small metastases in the lymph nodes and peritoneum, or on the surface of the liver, making it difficult to establish with certainty whether a tumor is resectable. Functional imaging using positron emission tomography with fluorodeoxyglucose (FDG-PET) may be helpful in this regard, especially if the images are fused with those of CT or MR (Hanbidge AE, Can J Gastroenterol, Feb 2002;16(2):101-105).

Endoscopic retrograde cholangiopancreatography (ERCP) involves the insertion of a thin, lighted tube (endoscope) into the small intestine through the mouth and stomach. A smaller tube or catheter is passed through the endoscope and into the bile and pancreatic ducts of the patient who is lightly sedated. Dye is injected into the ducts and x-ray images of the ducts are obtained to detect tumors. A plastic or metal stent can be placed across the obstructed bile duct during ERCP to help relieve any obstructions causing jaundice. ECRP may also be performed via a small abdominal incision.

ERCP has been the gold standard of pancreatic cancer diagnosis. However, the role of ERCP is expected to become smaller as other approaches such helical CT, MRCP and endoscopic ultrasound gain prominence in the evaluation of patients presenting with documented or suspected pancreatic cancer. ERCP may eventually be reserved for the study of jaundiced patients with no mass demonstrable on conventional noninvasive studies. It may also be useful in establishing the site of obstruction and differentiating focal pancreatitis from tumor.

Endoscopic ultrasound (EUS), or endosonography, combines endoscopy with ultrasound for the transgastric and transduodenal imaging of the entire pancreas. EUS, commonly performed on an outpatient basis, uses a high frequency ultrasound transducer housed on the tip of a flexible endoscope. The tip is placed in the duodenum or stomach next to the biliary tree and pancreas, to image the gut wall, retroperitoneum and adjacent organs, vessels, and lymph nodes. In pancreatic cancer, EUS is superior to CT scan, abdominal ultrasound, ERCP, or angiography in detecting tumors <3 cm in size.

There are two types of EUS transducers, radial sector or linear array. Radial EUS provides high-resolution images while linear array EUS processors are equipped with color Doppler to allow for accurate identification of vascular structures and aid in vascular staging of pancreatic tumors. FNA guided with linear array EUS may also detect inaccessible pancreatic masses not found by surgical biopsy or laparoscopy with a very low incidence of complications.

In order to assess the value of ultrasound in enhancing the outcome of standard laparoscopy in the staging of pancreatic cancer, a prospective evaluation of 90 patients with pancreatic tumors undergoing laparoscopy and laparoscopic ultrasound was performed over a 27-month period. The ultrasound laparoscope was equipped with an articulated curved and linear array transducer (6 to 10 MHz). All patients underwent rigorous laparoscopic examination to collect clinical, surgical, and pathologic data. Among the 90 patients examined, 64 had tumors in the head, 19 in the body, and 3 in the tail of the pancreas; 4 patients had ampullary tumors. Ultrasound laparoscopy in these patients imaged the primary tumor (98%), portal vein (97%), superior mesenteric vein (94%), hepatic artery (93%), and superior mesenteric artery (93%), and was particularly helpful in determining venous involvement (42%) and arterial involvement (38%) by the tumor. This resulted in a change in surgical treatment for 13/90 (14%) of the patients in whom standard laparoscopic examination was equivocal. In this trial, ultrasound laparoscopy was useful in evaluating the primary tumor and peripancreatic vascular anatomy, and accurately determined resectability. Therefore, supplementing standard with ultrasoumd laparoscopy improves assessment and preoperative staging of pancreatic cancer (Minnard EA, etal, Ann Surg, Aug 1998;228(2):182-7).

Computed tomography (CT) scans are used to determine the location and extent of pancreatic cancer. The specificity of conventional (incremental) CT scanning for pancreatic tumor detection is 70% to 85%, and the sensitivity ranges from 67% to 97%. However, helical (spiral) CT has demonstrated a sensitivity in excess of 90%. Helical CT represents a significant technical improvement over conventional CT, and is routinely used in the evaluation of patients with suspected pancreatic cancer. CT is an accurate and cost-effective method as a first test in diagnosing pancreatic cancer, to determine the respectability of the tumor, with a positive predictive value of nearly 100%. Most false positives occur in patients with chronic pancreatitis.

Ultrasound imaging of the abdomen exploits the differences in the reflection of sound waves between tumor and normal tissue. The specificity of the ultrasonography is 50% to 70%. In a retrospective study comparing ultrasonography and ERCP of the pancreas, the accuracy rate of ultrasound was 93.8% and of ERCP 62.5% (Feinberg SB, etal, J Clin Ultrasound, Apr 1977;5(2):96-100).

Magnetic resonance (MR) imaging is vying with CT as to which approach is superior in the setting of pancreatic cancer. Generally, MR imaging is considered a secondary modality after CT. MR imaging may be indicated in selected patients, particularly those in whom major blood vessels are compressed or invaded by cancer. MR cholangiopancre-

Exhibit 7
Staging of Pancreatic Cancer

UICCC TNM Staging	Description
TX	Primary tumor is not assessable
T0	No evidence of primary tumor
Tis	Tumor *in situ*
T1	Tumor is confined to the pancreas
T1a	Tumor is ≤2 cm
T1b	Tumor is >2 cm
T2	Tumor has spread to organs immediately adjacent to pancreas, such as the bile duct or the duodenum
T3	Tumor has spread to nearby organs such as the colon, stomach or spleen
Lymphatic System Staging	
NX	Regional lymph nodes are not assessible
N0	Cancer has not extended to nearby lymph nodes
N1	Regional lymph node metastasis
Metastasis Staging	
MX	It cannot be determined if there is distant metastasis
M0	No distant metastasis
M1	Distant metastasis

Source: International Union Against Cancer (UICC)

atography (MRCP) is considered a noninvasive alternative to ERCP. MRCP images the pancreatic ducts, the prime target of PDAC. In addition to being safer than ERCP, because it is noninvasive, it can delineate the ductal system upstream of complete obstructions. When conventional MR imaging is added to MRCP in the evaluation of pancreatic lesions, sensitivity and specificity improve further. Dual-phase helical CT and MR imaging have similar accuracies for detecting and staging PDAC. The specificity of MR imaging is 80% to 90%.

MRCP has all but replaced percutaneous transhepatic cholangiography (PTC), an invasive procedure in which a thin needle is inserted through the skin and into the liver. A dye is injected through the needle and x-rays are taken to visualize blockage of the bile ducts.

Fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) is a biochemically based functional imaging modality that distinguishes malignant from benign disease based on the higher metabolic activity of malignant tumors. FDG-PET detects tumor-associated alterations of glucose metabolism with very high sensitivity, and can effectively differentiate pancreatic cancer from benign lesions with high accuracy. However, a relatively wide overlap exists between semiquantitative uptake values obtained in cancer and those in inflammatory lesions. False-positive results arise from high FDG accumulation in active, chronic, and autoimmune pancreatitis sometimes mimicking pancreatic cancer with a shape of focal uptake. Furthermore, FDG-PET lacks the anatomical accuracy of CT or MR imaging, confusing a hot spot associated with a malignant disorder for intestinal or vascular activity, and vice versa. Combining biochemical detection by FDG-PET with the anatomical accuracy of CT may also avoid the difficulties inherent to each procedure and improve diagnostic accuracy. FDG PET may also be feasible in the differential diagnosis of cystic pancreatic lesions, such as IPMT.

Newly developed high resolution PET scanners can detect small pancreatic cancers, up to 7 mm in diameter, making it possible to identify resectable and potentially curable disease. FDG PET also is useful and cost-effective in the preoperative staging of pancreatic cancer because an unexpected distant metastasis can be detected by whole-body PET in about 40% of the cases, which results in avoidance of unnecessary surgical procedures. FDG PET is also useful in evaluating effects of treatment, patient monitoring after surgical intervention, and detection of recurrent pancreatic cancer (Higashi T, etal, Ann Nucl Med, Jun 2003; 17(4):261-79).

When values of different diagnostic modalities in pancreatic cancer were analyzed in 22 patients with focal pancreatic lesions, CA 19-9 was elevated in 4/5 malignant cases, for a sensitivity of 80%, and in 4/15 of benign cases for a specificity of 73%. CT/ultrasound resulted in a sensitivity of100%, but a specificity of 47% to 50%. ERCP was specific but not sensitive enough and the rate of unsuccessful investigations was relatively high at 4/22. The diagnostic value of FDG-PET was found to be superior to other diagnostic modalities, with a sensitivity of 100% and a specificity of 88%. FDG-PET should be the next step in the diagnostic strategy, in cases of focal pancreatic hypoechogenic/hypodense lesions detected by CT or ultrasound, suspected of malignancy (Papos M, etal, Orv Hetil, 26 May 2002;143(21 Suppl 3):1283-6, and Clin Nucl Med, Mar 2002;27(3):197-201).

Exhibit 8
Estimated Incidence by Stage of Pancreatic Cancer in the USA in 2003 and 5-year Survival

AJCC Staging[1]	AJCC Staging Description[1]	UICCC TNM Staging Equivalent	Estimated Incidence at First Diagnosis[2]		Estimated 5-year Survival[2]	
			(#)	(%)	(#)	(%)
Stage 0		Tis, N0, M0				
Stage I	The tumor is limited to the pancreas itself and has not spread to other organs	T1, N0, M0; T2, N0, M0	3,070	10.0	510	16.6
Stage II	The tumor has spread to nearby organs such as the duodenum and/or bile duct, but has not spread to the lymph nodes	T3, N0, M0	8,596	28.0	584	6.8
Stage III	The tumor has spread to the lymph nodes near the pancreas and may or may not have spread to nearby organs	T1, N1, M0; T2, N1, M0; T3, N1, M0	19,034	62.0	304	1.6
Stage IVa	The tumor has metastasized to organs near the pancreas such as the stomach, spleen and/or colon but not to distant organs like the liver or lungs	T1, N1, M0; T2, N1, M0; T3, N1, M0				
Stage IVb	Metastases present in distant organs such as the liver and lungs	Any T, any N, M1				
Total			30,700	100.0	1,398	4.5

[1] *American Joint Committee on Cancer*
[2] *SEER 1992-1999*

OctreoScan [(In-111-DTPA-D-Phe1)-octreotide], an In-111-labelled octreotide derivative, marketed by Mallinckrodt (St. Louis, MO), is a radioisotope-based, somatostatin-receptor imaging agent used in the detection of neuroendocrine tumors, including insulinomas of the pancreas. Somatostatin is a 14-amino-acid peptide hormone found on many cells of neuroendocrine origin. It acts as a neurotransmitter in the CNS. Hormonally, when it binds to cells, it inhibits the release of growth hormone, insulin, glucagon, and gastrin. Somatostatin receptors are found on the surface of human tumor cells, including those with amine precursor uptake and decarboxylation (APUD) properties such as endocrine pancreatic tumors. Ocreotide is an analog of somatostatin (Sandostatin; Novartis) that has been approved as treatment of carcinoid syndrome.

STAGING OF PANCREATIC CANCER

Because fewer than 20% of patients with pancreatic tumors are considered candidates for surgical intervention, staging of pancreatic cancer based on the standard TNM system (Exhibit 7), carried out during surgery, is not as common as it is with other malignancies. A more practical approach involves classifying tumors as confined to the pancreas (Stage I), extended to local structures but not the lymph nodes (Stage II), and having spread to the lymph nodes and distant sites (Stage III/IV).

Currently, patients who may benefit from surgery are identified by a rigorous diagnostic workup. Major advances in the diagnosis and staging of pancreatic cancer have reduced unnecessary surgeries from being performed in 80% of patients to <10%.

Incidence by Stage

Approximately 90% of pancreatic cancer has spread locally or distally at the time of diagnosis (Exhibit 8). Stage I disease is treatable successfully by surgery. In addition, surgery may be curative in another 10% of the cases involving Stage II or III disease.

5-Year Survival by Stage

The overall one-year survival rate of patients with cancer of the pancreas is 19%, and <5% of patients survive >5 years. However, there are several reports of better outcomes. The estimates presented on Exhibit 8 are based on SEER data collected between 1992 and 1999, with patients then followed to 2000. According to SEER, 5-year survival rates improved somewhat, from 2.6% in the 1974-1976 period to 4.4% in the 1992-1999 period.

Note: *The next three issues of FUTURE ONCOLOGY will cover current treatment approaches for pancreatic cancer, including protocols and results from combination trials of approved chemotherapeutics, and provide a comprehensive review of over 60 novel agents in preclinical and clinical development for the treatment of this devastating disease.*

INDEX OF COMPANIES & INSTITUTIONS

FUTURE ONCOLOGY

PUBLISHED BY **NEW MEDICINE, INC.**

PUBLISHER AND EDITOR:	**Katie Siafaca**
ASSOCIATE EDITOR:	**Tanya MacLean**
VICE PRESIDENT-OPERATIONS:	**Beth Schweitzer**
ASSISTANT EDITOR:	**Adele Simon**
ASSISTANT EDITOR:	**Amber De Volger Sabbatini**
CIRCULATION:	**Amish Kalyani**
DESIGN & PRODUCTION:	**Jill Burch**

NEW MEDICINE, INC. MAILING ADDRESS:

P.O. Box 909 ■ Lake Forest, CA 92609
Tel: 949. 830. 0448 ■ Fax: 949. 830. 0887
e-mail: info@newmedinc.com
www:http://www.newmedinc.com
www:http://www.oncologyknowledgebase.com
www:http://www.nmok.net

SUBSCRIPTION INFORMATION:

- FUTURE ONCOLOGY (ISSN 1082-331X) is published as 12 issues (several double issues) per subscription period, with a free index listing companies/institutions and subjects covered.
- A one-volume subscription to the electronic version is US $840 and to a hard copy version, sent first class to U.S. addresses, is US $1040, and sent air mail to foreign addresses, US $1,100.
- Volumes V2, V3, V4, V5 and V6 are $2,400 (U.S.) and $2,460 (outside the U.S.), either electronic or hard copy versions.
- Volumes V5 and V6 are $1,400 (U.S.) and $1,460 (outside the U.S.).
- Additional subscriptions sent to the same location are $390 each.
- Payment must accompany your order; checks must be drawn on a U.S. bank. (A purchase order number is acceptable; however, the subscription will not begin until payment is received.) Make checks payable to NEW MEDICINE. Payment may also be made by AMERICAN EXPRESS, VISA or MASTERCARD and wire transfer; please call 949. 830. 0448.