Epidemiology and Risk Factors

Epidemiology

In the United States there are 28,000 to 30,300 newly diagnosed cases of pancreatic cancer and approximately an equal number of deaths per year from pancreatic cancer.[1,2] Ductal adenocarcinoma is the most common form of pancreatic cancer. The annual incidence rate for all types of pancreatic cancer is approximately 9 new cases per 100,000 people, ranking it 11th among cancers.[2] The peak incidence occurs in the seventh and eighth decades of life, with the average age at diagnosis being 60 to 65 years.[3] The incidence rate is slightly higher in men than women (relative risk 1.35), and 30% to 40% higher in black men.[3] This racial discrepancy persists in spite of similar cigarette smoking rates in black men (27%) and white men (25%).[2]

Survival in untreated patients with pancreatic cancer is poor. For all stages combined, the 1-year survival rate is 19% and the 5-year survival rate is 4%.[2] Survival is low because of the late development of clinical symptoms, such that 80% of pancreatic cancers are metastatic at the time of diagnosis. Surgical resection (when margin negative and node negative) offers the best possibility for cure in pancreatic cancer, with the 5-year survival rate improving to 40%, when performed at specialized, high-volume major medical institutions.[4] The perioperative mortality rate is considerably higher when resection is performed at low-volume institutions.[4,5]

In the United States the incidence rates of pancreatic cancer increased threefold between 1920 and 1978, an increase that has also been observed in other developed countries.[6] Since 1978, incidence rates for men and women have declined slightly and appear to have stabilized at the current levels. A portion of the increased incidence from 1920 to 1978 may have been attributable to more accurate disease diagnosis and less misclassification of the disease. Additionally, improved surveillance may partially account for the increased incidence. Many studies have found a relationship between certain environmental exposures and cases of pancreatic cancer, including personal cigarette smoking, environmental tobacco

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smoke, and chemical exposures.[7] Cigarette smoking in the United States and in other countries increased greatly in the first half of the 20th century, such that 40% of adult Americans smoked in 1965. A large portion of the increased incidence of pancreatic cancer is also likely attributable to increased smoking through the 1960s. By 1990 the prevalence of smoking had decreased to 25%, with modest declines again noted in 1999.[8] It remains to be seen if this will translate into lower pancreatic cancer incidence rates in the future.

Risk Factors

A number of demographic risk factors have been associated with the development of pancreatic cancer (Table 1). These include older age, black race, male sex, low socioeconomic status, and Ashkenazi Jewish heritage.[7] Host etiologic factors associated with an increased risk of pancreatic cancer include diabetes mellitus, pancreatitis, and prior cholecystectomy.[2,3,9–11] The association between diabetes, pancreatitis, and the development of pancreatic cancer is complex because pancreatic cancer, as it destroys pancreatic parenchyma, can itself cause diabetes and pancreatitis.

Environmental factors, particularly tobacco smoking, also play a significant role in the development of pancreatic cancer.[7] Smokers have a 2- to 3-fold increased risk of pancreatic cancer. It has been estimated that tobacco smoking contributes to the development of almost 30% of pancreatic cancers. Importantly, smoking cessation can reduce this risk. Indeed, Mulder et al[12] have estimated that moderate reduction in smoking in Europe could save almost 68,000 lives that would otherwise be lost to pancreatic cancer by the year 2020.

Other risk factors associated with the development of pancreatic cancer are a high-fat/cholesterol diet, cirrhosis, chronic pancreatitis, diabetes mellitus, and a history of cholecystectomy.[13,14] Everhardt and Wright[14] performed a metaanalysis of 20 epidemiologic studies on diabetes and pancreatic cancer. The pooled relative risk (RR) of pancreatic cancer in persons with diabetes for 5 years was double (RR 2.0; confidence interval [CI] 1.3 to 2.2) the risk of persons without diabetes. Gapstur et al[15] reported a correlation between elevated plasma glucose levels and pancreatic cancer risk, further suggesting that impaired glucose tolerance, insulin resistance, and hyperinsulinemia are involved in the cause of pancreatic cancer. In a recent cohort study of more than 160,000 health care professionals, Michaud et al[16] found that high body mass index (a measure of obesity), height, and reduced physical activity increased pancreatic cancer risk. Activity levels and weight were ascertained before

TABLE 1. Risk factors associated with cancer of the pancreas

Life-style factors
Cigarette smoking (dose-response relationship)
Lack of exercise
Race/ethnic factors
Black men
Native female Hawaiians
Ashkenazi Jewish heritage (may be due to BRCA2 gene found in some Jewish families)
Inherited predispositions:
Hereditary breast and ovarian cancer (BRCA2)
Familial atypical multiple mole melanoma
Peutz-Jehghers syndrome
HNPCC
Hereditary pancreatitis
Ataxia-telangiectasia
Medical conditions
Cirrhosis
Diabetes mellitus
Chronic pancreatitis
Dietary factors
High fat/cholesterol
Obesity
Nitrosamines in food
Occupational exposure to carcinogens
2-naphthylamine
Benzidine
Gasoline products
Polychlorinated biphenyls
Dry cleaning agents
DDT (dichloro-diphenyl-trichloroethane)
Selected high-risk occupations
Dry cleaning
Chemical plant work
Sawmills
Electrical equipment manufacturers
Mines
Metal working
Height
Relative risk 1.81; (CI 1.31 to 2.52) when comparing tallest and shortest height categories for men and women
Previous surgery
Cholecystectomy

pancreatic cancer detection. They found an excess risk of pancreatic cancer among obese men and women and a direct association between height and the risk of pancreatic cancer. An inverse relationship between moderate physical activity and pancreatic cancer was observed. Walking or hiking 1.5 hours or more per week was associated with a 50% reduction in pancreatic cancer. Physical activity had no effect on

pancreatic cancer risk for nonoverweight participants. Likewise body mass index had no effect if the participant was a moderate exerciser. For cigarette smoking, the strongest associations with pancreatic cancer were observed when the pack-years smoked were within the previous 15 years. These findings suggest that smoking cessation, weight loss, and exercise may all reduce pancreatic cancer risk. Factors that have been studied and appear to have no association with the development of pancreatic cancer include moderate alcohol intake, nonhereditary and acute pancreatitis, and coffee intake.

Genetic Predisposition

A growing body of research evidence now suggests that genetic predisposition plays a significant role in pancreatic cancer risk. Most case-control studies have found that patients with pancreatic cancer are more likely to have a relative with pancreatic cancer than are healthy control subjects.[17] Similarly, several cohort studies have shown an increased risk of developing pancreatic cancer among individuals who report a family history of pancreatic cancer. Tersmette et al[18] have shown that this risk may increase with the number of affected members in the family.[19] Segregation analyses suggest that aggregation of pancreatic cancer in families has a genetic rather than an environmental basis.[20] Klein et al[20] performed complex segregation analysis on 287 families ascertained through an index case diagnosed with pancreatic cancer at The Johns Hopkins Medical Institutions between January 1, 1994, and December 31, 1999. Nongenetic transmission models were rejected ($P < .0001$) and the most parsimonious model included autosomal dominant inheritance of a rare allele, (still to be identified). Klein et al[20] were further able to estimate that approximately 0.5% of the population carry this allele.

Inherited Syndromes

Although accounting for less than 10% of the familial aggregation of pancreatic cancer, at least 6 genetic syndromes caused by germline mutations in genes that are associated with an increased risk of pancreatic cancer have been identified. These are summarized in Table 2 and include the following:

1. Familial breast cancer with germline mutations in the *BRCA2* gene.[21,22] Carriers of germline *BRCA2* mutations have a 3.5- to 10-fold increased risk for development of pancreatic cancer. One in 6 (17%) patients with pancreatic cancer and a strong family history of

TABLE 2. Genetic disorders and germ-line genetic alterations associated with familial pancreatic cancer (high-risk syndromes)

Disorder	Gene (location)	Increased risk of pancreatic cancer
Hereditary breast and ovarian cancer	BRCA2 (13q12-q13)	×3.5 to 10
Familial atypical multiple mole melanoma syndrome	p16 (9p21)	×12-20
Peutz-Jeghers syndrome	STK11/LKB1 (19p13)	×130
Hereditary nonpolyposis colorectal cancer (Lynch II variant)	hMSH2, hMLH1, others	?
Hereditary pancreatitis	*PRSS1* (7q35)	×50
Ataxia-telangiectasia	ATM (11q22-23)	Rare

Modified from CJ Yeo, JL Cameron, Pancreatic cancer. Curr Prob Surg 1999;36:57–152, Table 5.

pancreatic cancer (at least 3 first-degree family members with pancreatic cancer) have recently been shown to have germline *BRCA2* mutations. This makes the *BRCA2* mutation the most common germline mutation in patients with hereditary pancreatic cancer. Of interest, not all patients with pancreatic cancer and a germline *BRCA2* mutation come from classical *BRCA2* families.[23] In fact, some have no family history of breast cancer.

2. Familial atypical multiple mole melanoma syndrome with germline mutations in the *p16* gene.[24,25] In addition to an increased risk of melanoma, carriers of germline *p16* mutations have a 12- to 20-fold increased risk for development of pancreatic cancer.
3. The Peutz-Jeghers syndrome is characterized by mucocutaneous melanocytic macules and hamartomatous polyps of the gastrointestinal tract. Patients with the Peutz-Jeghers syndrome have approximately a 130-fold increased risk for development of pancreatic cancer.[26]
4. Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome with germline mutations in one of the DNA mismatch repair genes (*hMSH1, hMSH2*, etc).[27,28] Of interest, the pancreatic carcinomas that arise in patients with HNPCC often have a distinct histologic appearance called "medullary histology" (marked by pushing borders, poor differentiation, and a syncytial growth pattern).
5. Hereditary pancreatitis with germline mutations in the *PRSS1* (cationic trypsinogen gene).[29] Patients with hereditary pancreatitis have development of severe pancreatitis at a young age (childhood and adolescence), may have pancreatic pseudocysts and diabetes, and have a 50-fold increased risk for development of pancreatic cancer.[30]
6. Ataxia-telangiectasia, an autosomal recessive inherited disorder, is characterized by cerebellar ataxia, oculocutaneous telangiectasias, and

cellular and humoral immune deficiencies.[31] The gene responsible, ATM, is associated with an increased risk of leukemia, lymphoma, and cancers of the breast, ovaries, biliary tract, stomach, and rarely, the pancreas.

A seventh syndrome, that of pancreatic cancer, pancreatic insufficiency and diabetes mellitus, has recently been described in a family (termed *Family X*), and the phenotype has been linked to chromosome 4q32-34.[32]

Data From The National Familial Pancreas Tumor Registry

Most of the cases in which there is a familial aggregation of pancreatic cancer are not related to these high risk inherited syndromes. The National Familial Pancreas Tumor Registry (NFPTR*) was established at Johns Hopkins, with the hope of identifying the causes for the aggregation of pancreatic cancer in families. To date, nearly 1000 families have enrolled in this registry. Early analyses of the kindreds enrolled in the NFPTR have shown that the risk of pancreas cancer is 18-fold greater in first-degree relatives of familial pancreatic cancer cases (at least 2 first-degree relatives with pancreatic cancer in the family), than it is in first-degree relatives of sporadic pancreatic cancer cases (families in which there has been only one member with pancreatic cancer).[18] In addition, the increased risk of pancreatic cancer in familial pancreatic cancer kindreds extended to second-degree relatives, as a significantly increased proportion of pancreatic cancer existed in second-degree relatives of familial cases compared with sporadic pancreatic cases (3.7% vs 0.6%, $P < .0001$).[18] When 3 or more first-degree family members in the familial kindred subset were affected, the risk of pancreatic cancer increased 57-fold, with an incidence rate of 301/100,000, compared with the reported U.S. incidence rate of 8.8/100,000.[18] Thus, although not fully defined, there is a clear familial predisposition to pancreatic cancer when 2 or more first-degree relatives are affected.

*NFPTR, c/o Kieran Brune, The Johns Hopkins University, Ross 632, 720 Rutland Avenue, Baltimore, MD 21205; 410-955-3502; email: kbrune@jhmi.edu.

Developmental Aspects

Recent advances in stem cell biology have not only generated hope regarding potential therapeutic applications, but also contributed considerable information regarding epithelial stem cells as potential precursors for human neoplasia. Although the location and identity of dedicated stem cells in adult pancreatic epithelium is currently an area of intensive investigation, recent studies have suggested that a precursor cell type may participate in generation of pancreatic ductal adenocarcinoma. During normal pancreatic development, undifferentiated precursor cells are responsible for generating mature ductal, acinar, and islet cell types. Recent studies have demonstrated that metaplastic and neoplastic ductal epithelium share features in common with embryonic pancreas, suggesting that further insight into factors regulating pancreatic development may be useful in identifying initiating events in pancreatic cancer.

As demonstrated in Fig 1, the embryonic pancreas is first apparent as dorsal and ventral buds of foregut endoderm, evident by day E9.5 in the mouse.[33] As suggested by Edlund[34] the complex events required for normal pancreatic development can be resolved to 3 essential components. First, foregut endoderm becomes patterned to form dorsal and ventral pancreatic buds. Second, cells undergo commitment to either endocrine or exocrine cell fates. Third, pancreatic morphogenesis occurs by way of extensive growth and branching. During this process, islet, acinar, and ductal cell types differentiate from common precursor cells within developing pancreatic epithelium. The molecular pathways regulating lineage commitment in the exocrine pancreas have recently been reviewed.[35] In particular, activation of the Notch signaling pathway is required for cells to avoid early endocrine differentiation, thereby reserving a pool of undifferentiated precursor cells required for both epithelial expansion and subsequent exocrine differentiation. Normal exocrine differentiation[36,37] also requires permissive signals provided by adjacent pancreatic mesenchyme, including laminin-1 and soluble follistatin.[38–40]

As previously reviewed,[35] differentiation of endocrine and exocrine pancreatic cell types is regulated by a hierarchy of lineage-specifying transcription factors. A summary of these factors is provided in Table 3. Among these, the homeodomain transcription factor *Pdx1* and the paired/homeodomain factor *Pax6* may have special relevance in the

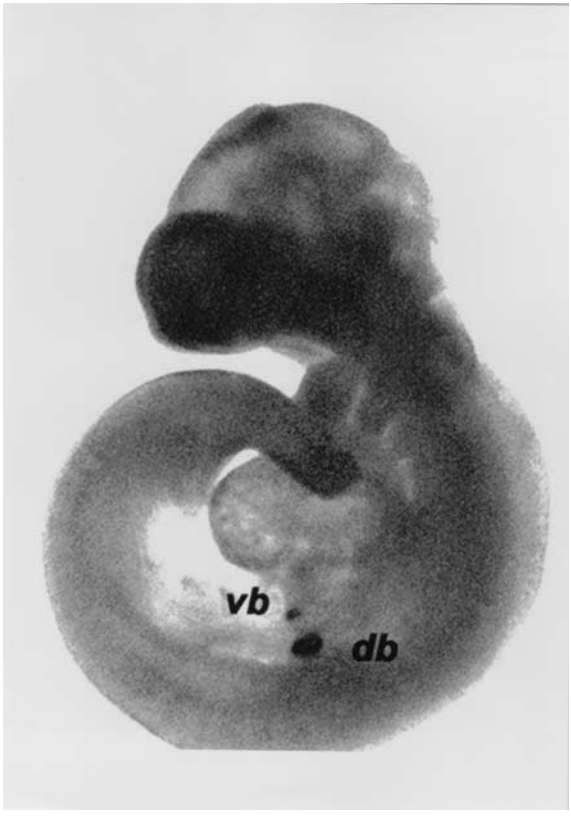

FIG 1. Initiation of pancreatic development in ventral and dorsal pancreatic bud. Day E9.5 mouse embryo stained for Pdx1 to visualize ventral *(vb)* and dorsal *(db)* pancreatic buds. At this stage, only small numbers of differentiated endocrine cell types are present, and Pdx1 is expressed in all undifferentiated precursor cells.

initiation of pancreatic ductal neoplasia. The mammalian *Pdx1* gene encodes a homeodomain transcription factor known to be required for normal pancreatic development. Homozygous deletions result in the successful specification of pancreatic mesenchyme and initial pancreatic bud formation, but severely aborted morphogenesis and failure to generate differentiated islet, acinar, or ductal cell types.[41]

Consistent with its required role in early pancreatic morphogenesis, analysis of *Pdx1* gene activation during murine pancreatic development confirms expression by multipotent stem cells within the embryonic ductal epithelium, which ultimately give rise to mature islet, acinar, and ductal elements. Beginning on day E8.5, *Pdx1* is expressed in a segment of prepatterned epithelium within the embryonic foregut and subse-

TABLE 3. Partial list of nuclear transcription factors required for normal pancreatic development. Developmental expression times indicated for mouse embryos

	Onset of expression	Where expressed	Null phenotype
Factors required for early morphogenesis			
Hlxb9	E8	Endoderm Notocord	Absence of dorsal epithelial bud Reduced β-cells, abnormal islets
Isl1	E9	Dorsal mesenchyme	Absence of dorsal mesenchyme
		Dorsal endoderm	Failure of dorsal bud outgrowth
		Differentiating and mature islet cells	Failure to differentiate islet cells
Pdx1	E8.5	Endoderm	Aborted morphogenesis Failure to generate differentiated cell types
Factors required for islet differentiation			
HNF6	E9.0	E9-E14.5: all epithelial cells Adult: Acinar and ductal cells	Absence of ngn3 Impaired endocrine differentiation Defect in ductal maturation
Ngn3	E9.5	Endocrine precursors	Absence of all endocrine cells
NeuroD/Beta2	E9.5	Differentiating and mature islet cells	Marked reduction in β-cells Abnormal islet migration, organization
Pax6	E9.5	Differentiating and mature islet cells	Absence of α-cells Marked reduction in other islet cells
Pax4	E9.5	Differentiating and mature β-cells	Absence of β- and δ-cells Increase in α-cells
Nkx2.2	E9.0	Differentiating and mature islet cells	Absence of β-cells Reduction in α- and PP-cells
Nkx6.1	E9.5	Differentiating and mature β-cells	Marked reduction in β-cells
Factors required for exocrine differentiation			
P48/PTF1	E10.5*	Differentiating and mature acinar cells	Absence of exocrine pancreas Displacement of islet cells to spleen

From Meszoely, Means, Scoggins, Leach. Developmental aspects of early pancreatic cancer. Cancer J 2001;7:242–50, Table 1.

quently in most epithelial cells within the dorsal and ventral pancreatic buds.[41] By day E17.5, however, most pancreatic duct and acinar cells have extinguished expression, and high levels of *Pdx1* expression subsequently become restricted to developing islets. Similarly, the paired/

homeodomain transcription factor *Pax6* is expressed by endocrine precursor cells within embryonic pancreatic epithelium, and deletions in this gene result in widespread reductions in endocrine cell types.[42] In adult ductal epithelium, however, *Pax6* expression is not observed. Thus both *Pdx1* and *Pax6* may be considered markers of a precursor cell type within ductal epithelium.

Using a murine model of pancreatic tumorigenesis, we have recently identified reactivation of *Pdx1* and *Pax6* expression as early events during the process of pancreatic neoplasia.[43,44] When the EGF receptor ligand transforming growth factor–α (TGF-α) is overexpressed in mouse pancreas, extensive acinar-to-ductal metaplasia is observed.[45] These mice subsequently accumulate increasingly dysplastic PanIN lesions, with eventual generation of malignant ductal adenocarcinoma.[46] Using in vivo reporter gene analysis in bitransgenic $Pdx1^{lacZ/+}$ /MT-TGF-α and $Pax6^{lacZ/+}$/MT-TGF-α mice, it is apparent that TGF-α-induced premalignant epithelium is characterized by widespread *Pdx1* gene activation and abnormal focal reactivation of *Pax6* expression, similar to the pattern in embryonic pancreas. In addition to providing markers for the embryonic nature of premalignant pancreatic epithelium, reactivation of developmental genes may contribute to malignant transformation of ductal epithelium, on the basis of observations that *Pax* genes may be oncogenic when expressed outside their normal developmental context.

In addition to these observations, we have recently identified reactivated Notch signaling as another feature of pancreatic neoplasia shared in common with embryonic pancreatic epithelium (Leach, unpublished data, 2002). Notch represents a highly conserved signaling pathway, often acting to maintain cells in an undifferentiated precursor state. In the embryonic pancreas, undifferentiated epithelium is characterized by widespread expression of the Notch target gene, *Hes1*, demonstrating activated Notch signaling. However, minimal Notch pathway activation is evident in adult exocrine pancreas. Using oligonucleotide microarray–based expression profiling of normal human pancreas and resected pancreatic cancer, we have observed up-regulated expression of a variety of Notch pathway components, including Notch receptors (eg, Notch-2, Notch3), Notch ligands (eg, delta, jagged-1), and Notch target genes (eg, Hes1, Hey4). Using immunohistochemical analysis of Hes1 protein expression, we have further confirmed Notch pathway activation not only in invasive pancreatic cancer, but also in metaplastic ductal epithelium and PanIN lesions. When activated ectopically in explant cultures of normal pancreas, Notch induces expansion of metaplastic ductal epithelium. These data suggest that a Notch signaling "module" is activated in

pancreatic cancer and that Notch pathway activation may represent an early event in pancreatic tumorigenesis.

These identified links between pancreatic development and pancreatic neoplasia will, it is hoped, contribute to new strategies for treatment and chemoprevention of this disease. With respect to Notch signaling, we are currently evaluating pharmacologic inhibition of Notch pathway activation as a means of preventing initiation of the metaplasia/neoplasia sequence in MT–TGF-α mice.

Pathology

There are many different types of "pancreatic cancer." This review, however, will focus on ductal adenocarcinomas because they account for the vast majority of malignant pancreatic neoplasms. We will also briefly discuss several rarer variants of pancreatic neoplasia.

Although ductal adenocarcinoma makes up less than 2% of new cancer cases in the United States, it is the fifth leading cause of cancer-related death.[47] Ductal adenocarcinoma is so virulent because most patients present with late-stage disease. Thus although surgical resection can produce survival rates of up to 40% for margin-negative, node-negative tumors, most patients with pancreatic adenocarcinoma actually are not eligible for surgery.[48] We therefore need new tests that can diagnose *early* pancreatic cancers or better yet the *precursors* to these cancers. Thus this article also discusses some of the *precursors* to infiltrating duct adenocarcinoma, including pancreatic intraepithelial neoplasias (PanINs),[49] mucinous cystic neoplasms (MCNs),[50] and intraductal papillary mucinous neoplasms (IPMNs).[51]

Ductal Adenocarcinoma

Infiltrating ductal adenocarcinoma accounts for almost three fourths of all malignant pancreatic neoplasms. These neoplasms are white-yellow, poorly defined, firm masses that often obstruct and dilate the distal common bile and pancreatic ducts. Microscopically, infiltrating ductal adenocarcinomas are composed of infiltrating epithelial cells forming glands of various shapes and sizes surrounded by dense, reactive, fibrous connective tissue (Fig 2,*A*). The nuclei of these cells can show marked pleomorphism (variation in size and shape), hyperchromasia (increased nuclear staining), loss of polarity, and prominent nucleoli. Most ductal adenocarcinomas infiltrate into perineural, lymphatic, and vascular spaces. In addition, lymph-node metastases are identified in most resected ductal adenocarcinomas.

There are several histologic variants of infiltrating ductal adenocarcinoma. One of these variants, the so-called *mucinous non-cystic adenocarcinoma* or *colloid carcinoma*, shows prominent extracellular mucin production. This finding is more common in infiltrating duct adenocarcinomas arising in association with either IPMNs or MCNs. (See below.) Thus the finding of a colloid carcinoma should alert the pathologist to

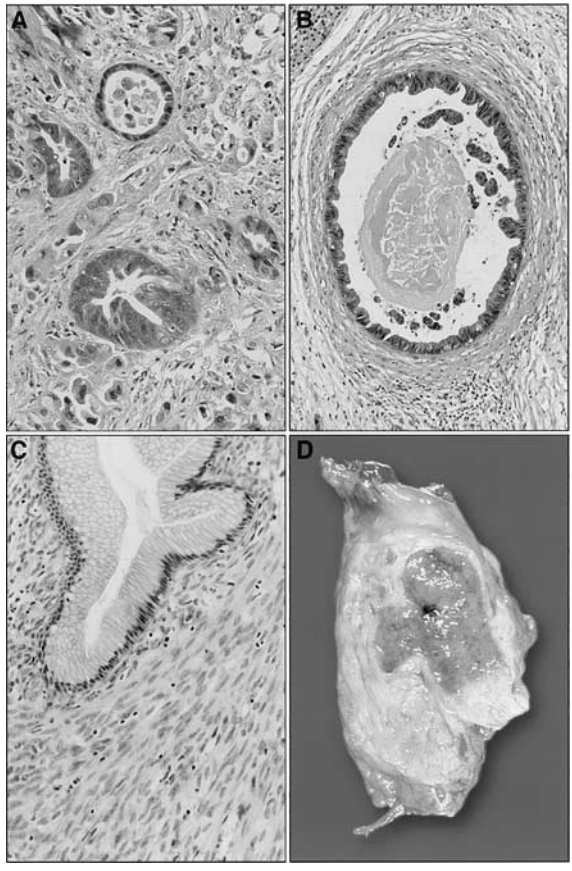

FIG 2. Four different types of neoplastic pancreatic disease. Microscopic appearance of infiltrating ductal adenocarcinoma **(A)**, microscopic appearance of PanIN-3 **(B)**, microscopic appearance of a mucinous cystadenoma **(C)**, and gross appearance of an intraductal papillary mucinous neoplasm with *in situ* carcinoma. Note the ovarian-like stroma in the mucinous cystadenoma **(C)**. The intraductal papillary mucinous neoplasm **(D)** fills the main pancreatic duct.

search for one of these precursor lesions. Such a precursor lesion is important to find because it may have an impact on the complete surgical excision of that neoplasm.[52]

Another important variant of infiltrating ductal adenocarcinoma is medullary carcinoma. These neoplasms are poorly differentiated adenocarcinomas with a syncytial growth pattern, pushing borders, and an associated lymphoid infiltrate. They may have a better outcome than conventional ductal adenocarcinomas. They may also signal an inherited susceptibility to development of pancreatic carcinoma, for example,

through HNPCC syndrome.[53] These carcinomas, unlike conventional ductal adenocarcinomas, often show a particular genetic trait called *microsatellite instability,* may harbor Epstein-Barr virus RNA, and usually are K-*ras* wild type.[53,54]

Molecular data such as these may form the basis for better diagnostic techniques, not only for medullary carcinomas but also for *all* ductal adenocarcinomas. For instance, we have demonstrated that mutant K-*ras* shed from carcinomas of the pancreas can be detected in duodenal fluid and in stool of patients with pancreatic carcinoma.[55] We have also recently developed an immunohistochemical assay for the *DPC4* protein, the product of a gene inactivated in approximately 55% of pancreatic ductal adenocarcinomas that may help establish the diagnosis of cancer in morphologically challenging lesions.[56,57] As our understanding of the molecular genetic alterations in carcinomas of the pancreas improves, the application of molecular biology will help in the analysis of histologically ambiguous lesions and in the detection of early pancreatic neoplasms.

Precursors to Ductal Adenocarcinoma

PanINs

Most ductal adenocarcinomas of the pancreas are believed to arise from PanINs. PanINs are lesions composed of mucin-producing epithelia with varying degrees of cytologic and architectural atypia that involve the *small ducts* of the pancreas (Fig 2,*B*). PanINs can be flat (PanIN-1A), papillary without atypia (PanIN-1B), papillary with atypia (PanIN-2), or may even meet histopathologic criteria for carcinoma in situ (PanIN-3).[49] We believe that just as there is progression from adenoma to adenoma with high-grade dysplasia to infiltrating adenocarcinoma in the colon, so too is there progression from PanIN-1 to PanIN-2 to PanIN-3 to infiltrating adenocarcinoma in the pancreas. The histologic criteria used to grade PanINs are explained in detail on the World Wide Web at *http:/pathology.jhu.edu/pancreas_panin*.

Several lines of evidence suggest that PanINs are the precursors to infiltrating ductal adenocarcinoma. First, PanINs are frequently found in pancreata with infiltrating cancers. Furthermore, higher-grade PanINs are more common in pancreata from patients with pancreatic cancer than they are in pancreata from patients without pancreatic cancer.[58] Second, some PanINs have been observed to progress to infiltrating cancer over time. Brat et al[59] reported 3 patients in whom infiltrating ductal adenocarcinomas developed months to years after PanINs were identified in their pancreata. Third, and most convincingly, PanINs display some of the

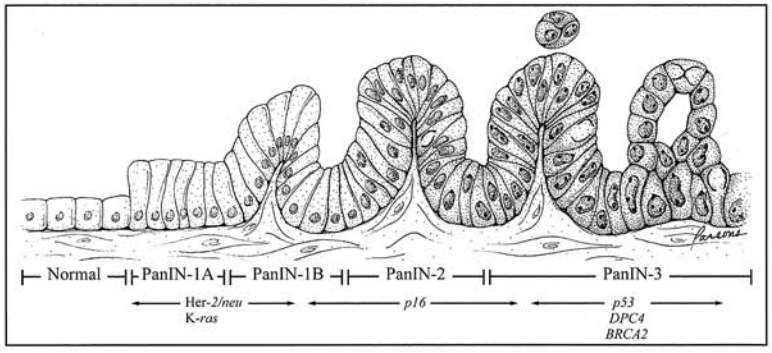

FIG 3. Histologic-genetic progression model of infiltrating pancreatic ductal adenocarcinoma from PanIN. Printed with permission from Wilentz RE et al. Cancer Res 2000;60:2002–6.

same genetic changes as infiltrating adenocarcinomas. For example, activating point mutations in codon 12 of the K-*ras* gene, common in infiltrating adenocarcinoma, have been demonstrated in both early and late PanINs. PanINs also harbor mutations in tumor-suppressor genes, namely *p16*, *p53*, *BRCA2,* and *DPC4*.[60] We recently showed that *p16* inactivation first occurs primarily in PanINs-2 and –3 and that *DPC4* first occurs very late in neoplastic progression, in PanINs-3.[61] This work on genetic alterations in PanINs has led us to formulate a histologic-genetic progression model for pancreatic duct adenocarcinoma (Fig 3).

Importantly, this progression model suggests that the molecular detection of precursor lesions and early cancers is indeed possible. For example, like mutant K-*ras* genes originating in infiltrating ductal adenocarcinomas, mutant K-*ras* genes shed from PanINs have also been identified in stool, duodenal fluid, and pancreatic juice samples.[62]

MCNs

Although much less common than PanINs, MCNs can also be precursors of infiltrating ductal adenocarcinoma of the pancreas. As with PanINs, MCNs progress through stages of increasing dysplasia, from mucinous cystadenoma to borderline mucinous cystic neoplasm to mucinous cystic neoplasm with in situ carcinoma, finally to reach the stage of invasive adenocarcinoma.

MCNs are composed of tall mucin-producing columnar cells (Fig 2,*C*). They are different from PanINs because they are *grossly visible* neoplasms that do *not* involve the native duct system of the pancreas. In addition, most MCNs have a dense ovarian-like stroma surrounding the

cysts of the neoplasm. Mucinous cystadenomas (the lowest grade lesions) contain a single layer of epithelium lacking significant atypia. In borderline mucinous cystic neoplasms, the epithelium may form papillae and complex architectural patterns. When an in situ carcinoma or an invasive carcinoma is present, then the diagnosis of a mucinous cystadenocarcinoma should be made.[50,63]

MCNs are heterogeneous neoplasms, and invasive adenocarcinoma can arise *focally* within an otherwise benign-appearing mucinous cystic neoplasm. Thus mucinous cystic neoplasms should be not only completely excised but also microscopically examined *in their entirety*. Although there have been reports of "metastasizing" mucinous cystic neoplasms that do not contain invasive carcinoma,[50,64] we believe that sampling error explains these results. When only *completely resected and completely histologically examined* mucinous cystic neoplasms are included in studies, no mucinous cystadenoma, borderline mucinous cystic neoplasm, or mucinous cystic neoplasm with in situ carcinoma has been reported to recur.[63]

Even though invasive adenocarcinomas arising in the setting of MCNs are rare, it is extremely important to differentiate them from ductal adenocarcinomas arising from PanINs. Studies show that patients with the former do significantly better. There is a long-term survival rate of approximately 50% for MCNs.[63]

IPMNs

IPMNs are mucinous, often villous, neoplasms of the pancreas that grow within the *larger native ducts* of the pancreas and that *do not contain ovarian-like stroma* (Fig 2,*D*). Like the other forms of incipient neoplasia in the pancreas, IPMNs can show varying degrees of atypia and can progress. "Intraductal papillary mucinous adenomas" are IPMNs without significant cytologic or architectural atypia. "Borderline IPMNs" show a moderate amount of dysplasia. "Intraductal papillary mucinous neoplasm with carcinoma in situ" is the designation given to those tumors in which the intraductal lesion displays severe cytologic and architectural atypia. The term *infiltrating adenocarcinoma arising in association with an IPMN* is reserved for those cases in which an invasive cancer is identified.[51] As with MCNs, IPMNs should be completely resected, submitted, and histologically examined because of the heterogeneity of these tumors.

IPMNs must be distinguished from both PanINs and MCNs. By definition, IPMNs are grossly visible lesions that involve the *larger* pancreatic ducts and ductules, whereas PanINs grow within the smaller

TABLE 4. PanINs, IPMNs, and MCNs

	PanINs	IPMNs	MCNs
Age	60s–70s	60s–70s	40s–50s
Sex	Both men and women	Both men and women	Mainly women
Location	Head-body-tail	Head	Body-tail
Native duct system involvement	Small ducts	Large ducts	No involvement
Endoscopy/radiology	No findings (lesions are microscopic)	Mucin oozing out of ampulla of Vater	Pancreatic tail mass
Ovarian stroma	No	No	Yes

ducts and are microscopic lesions. Mucinous cystic neoplasms are independent masses that do not grow within the native pancreatic duct system. In addition, IPMNs do not contain the ovarian stroma frequently seen in mucinous cystic tumors. IPMNs occur with approximately equal frequency among men and women, whereas almost all MCNs arise in women. In addition, unlike PanINs and MCNs, patients with IPMNs have a particular endoscopic feature—mucin oozing from the ampulla of Vater during endoscopy.[51] Table 4 summarizes the differences between PanINs, IPMNs, and MCNs.

Not surprisingly, recent genetic studies support a progression model for IPMNs. Activating point mutations in codon 12 of the K-*ras* oncogene occur in approximately 60% of IPMNs, and these mutations seem to occur more frequently in the more atypical areas of IPMNs.[65] Fujii et al[66] identified numerous allelic losses in IPMNs; again, these allelic losses occurred more frequently in the more atypical areas of the IPMNs. Iacobuzio-Donahue et al[67] recently studied *DPC4* expression in IPMNs and found that, in contrast to ductal adenocarcinomas and mucinous cystic neoplasms, virtually all IPMNs express *DPC4*.

As with mucinous cystic neoplasms, this progression model implies that noninvasive IPMNs follow benign courses. Indeed, current evidence supports this assertion. In contrast, invasive adenocarcinomas arising in the setting of an IPMN can recur and metastasize.[51]

Other Pancreatic Neoplasms

Pancreaticoblastoma

Pancreaticoblastoma is a neoplasm that occurs primarily in children younger than 15 years of age. Typically, these tumors contain nests of squamoid cells in a sea of uniform, undifferentiated cells. The survival rate for patients with resected pancreaticoblastomas is relatively good. Six

of 8 patients reviewed by Buchino et al[68] were alive at an average of 4 years after surgical resection.

Recently, Abraham et al[69] showed that pancreaticoblastomas are genetically different from other pancreatic neoplasms, including ductal adenocarcinomas. They found that, in contrast to most ductal adenocarcinomas, most pancreaticoblastomas had allelic loss on chromosome 11p and molecular alterations in the APC/β-catenin pathway. In addition, the changes usually found in ductal adenocarcinomas—K-*ras*, *p53*, and *DPC4* alterations—were absent in pancreaticoblastomas. These findings suggest that pancreaticoblastomas are more closely related to hepatoblastomas than they are to ductal adenocarcinomas of the pancreas. This information is useful because it may be used in conjunction with histologic study to separate pancreaticoblastomas (with their relatively good prognosis) from ductal adenocarcinomas.[69]

Acinar Cell Carcinoma

As the name implies, acinar cell carcinomas (ACCs) are malignant tumors with acinar differentiation. Microscopically, these tumors form clusters of cells around small, central lumina. Many patients with this tumor have a peculiar syndrome of subcutaneous fat necrosis, rash, peripheral eosinophilia, or polyarthralgias. The mean survival for patients with resected ACC of the pancreas is 18 months, slightly better than that for ductal adenocarcinoma.[70,71]

Abraham et al[72] have genetically characterized ACCs. These neoplasms appear to be more closely related to pancreaticoblastomas than to ductal adenocarcinomas. Approximately half of the ACCs studied had allelic loss on chromosome 11p, and alterations in the APC/β-catenin pathway were present in close to one fourth of the tumors. No *DPC4* or *p53* gene alterations were seen. These data underscore the distinct molecular pathways of ductal and nonductal pancreatic neoplasms.[72]

Solid-pseudopapillary Neoplasm

Solid-pseudopapillary neoplasms (SPNs) of the pancreas occur primarily in women in their third decade of life. They contain solid areas, cysts, pseudopapillae, hemorrhage, and necrosis. Most patients survive for many years after surgical resection; however, metastases do occur, and surgeons should try to remove these neoplasms completely.[73]

As with pancreaticoblastomas and acinar cell carcinomas, SPNs appear to be genetically distinct from ductal adenocarcinomas. Almost all SPNs harbor alterations in the APC/β-catenin pathway. The genes involved in

infiltrating duct adenocarcinoma, including the K-*ras* oncogene and the *p53* tumor-suppressor gene, are generally not affected in SPNs.[74]

Summary

Most pancreatic neoplasms arise from histologically recognizable precursors. The 3 most common precursors to invasive cancer—pancreatic intraepithelial neoplasias, mucinous cystic neoplasms, and intraductal papillary mucinous neoplasms—all contain tall columnar mucin-producing cells. In PanIn lesions, these cells line small native pancreatic ductules; in IPMNs, they form a villiform mass within large native pancreatic ducts. In contrast, MCNs contain de novo cysts not connected to the native pancreatic duct system.

The recognition of incipient neoplasia in the pancreas is important, because it provides the opportunity to identify a curable disease. For example, the complete removal of a mucinous cystadenoma can cure a patient of a neoplasm that, if not removed, can progress to an incurable infiltrating cancer. Similarly, the application of modern molecular screening tests has the potential to lead to the identification of early-stage, and therefore surgically treatable, ductal adenocarcinomas and their precursors.

Molecular biology may lead not only to the earlier diagnosis of pancreatic tumors but also to the more accurate diagnosis of these neoplasms. The molecular characterization of pancreaticoblastomas, acinar cell carcinomas, and solid-pseudopapillary neoplasms has revealed that there are separate genetic pathways of neoplastic progression for pancreatic ductal and nonductal neoplasms. Genetic changes may, in conjunction with histologic study, provide important information on tumor type and thus on patient treatment and prognosis.

Molecular Genetics

Over the last decade, significant advances have been made in our understanding of the molecular biology of pancreatic ductal adenocarcinoma. Many of our advances in the understanding of the molecular genetics have focused on events that occur in the development and early genetic progression of the disease, with pancreatic adenocarcinoma now considered one of the better characterized neoplasms at the genetic level. The genetic progression is associated with the accumulation of multiple mutations in various cancer-causing genes, which can be broadly divided into 3 categories: oncogenes, tumor-suppressor genes, and genomic maintenance gene.

Oncogenes

Oncogenes are derived from normal cellular genes called *protooncogenes.* When activated by mutations or amplifications, these genes possess transforming properties. The names, locations, and mutational frequencies of oncogenes commonly mutated in pancreatic cancer are shown in Table 5.

K-ras

Roughly 95% of pancreatic ductal cancers have an activating point mutation in the K-*ras* oncogene.[75,76] The K-*ras* gene product functions as a GTPase. Mutations in K-*ras* impair the intrinsic GTPase activity, resulting in a protein that is constitutively active in signal transduction. Most of these mutations change codon 12 from glycine (normal or wild type) to aspartic acid or valine. Mutations in codons 13 and 61, as well as novel dinucleotide mutations, occur less frequently.[75,77] The localization of most K-*ras* mutations to a single codon makes them relatively easy to detect and therefore attractive targets for sensitive and specific diagnostic and screening tests in duodenal fluid,[78] pancreatic juice,[79] and stool.[80]

Gene Amplification

Amplified gene segments, or amplicons are usually large and it can be difficult to identify the target gene or genes within the amplicon. *AKT2* on chromosome 19q[81]and *MYB* on chromosome 6q[82] are amplified in 10% to 20% of pancreatic cancers and are therefore candidate oncogenes.

TABLE 5. Commonly mutated genes in pancreatic ductal adenocarcinoma

Gene name	Chromosomal location	Mutational frequency
Oncogenes		
K-*ras*	12p	95%
AKT2	19q	10%–20%
MYB	6q	10%
Tumor-suppressor genes		
p16/RB1	9p/13q	95%
p53	17p	75%
DPC4	18q	55%
LKB1/SKT11	19p	5%
MKK4	17p	4%
ALK4	12q	2%
Genome maintenance genes		
BRCA2	13q	7%–10%
MSI^{+}*/TGFBR2*	3p	3%
MLH1	3p	3%

Modified from Su GH, Kern SE. Molecular genetics of ductal pancreatic neoplasia. Curr Opin Gastroenterol 2000;16:419–25.

Tumor-suppressor Genes

Tumor-suppressor genes normally function to restrain cell proliferation. Loss of function of these genes occurs as a result of mutations, deletions, chromosomal rearrangements, methylation, or mitotic recombination. For most tumor suppressor genes, biallelic inactivation is required to cause loss of function, which results in abnormally increased or unregulated cell proliferation. The names, locations, and mutational frequencies of tumor-suppressor genes commonly mutated in pancreatic cancer are shown in Table 1.

Rb1/p16/CDK4

P16 is the most commonly mutated tumor-suppressor gene in pancreatic cancer. It is located on chromosome 9p and is inactivated in 95% of pancreatic ductal carcinomas. The *p16* protein functions as an inhibitor of the cyclinD-cyclin-dependent kinase 4 (cdk4) kinase complexes. This in turn inhibits the phosphorylation of a number of growth and regulatory proteins, including Rb, leading to failure of cell cycle control and unchecked proliferation.[83,84]

P53

P53 is located on chromosome 17p and is mutated in approximately 75% of pancreatic ductal carcinomas.[85,86] The *p53* gene encodes for a tumor-suppressor protein that functions as a short-lived transcription

factor, crucial in cell cycle regulation and apoptosis, and it is activated and stabilized in response to a wide variety of genotoxic cellular stresses.[85] The *p53* regulates many downstream genes, including *p21, mdm-2* (which inhibits *p53* in a negative feedback loop), various *PIGs* (*p53*-induced genes), and *14-3-3σ*.[85]

TGF-β/DPC4

The *DPC4* gene *(SMAD4* or *MADH4)* is a tumor-suppressor gene located on chromosome 18q and mutated or homozygously deleted in approximately 55% of pancreatic ductal cancers.[87] *DPC4* is a member of the *SMAD* gene family, functioning as both a transcriptional activator and repressor. Its activity is dependent on its ability to bind specific DNA sequences: the Smad-binding elements.[88] Mutations that interfere with the DNA-binding function or the ability of DCP4 to react to ligand-mediated signaling contribute to tumorigenesis.

ACVR1B (ALK4, activin receptor type 1B)

DPC4 is known to mediate signals initiated by TGF-β, as well as other TGF-β superfamily ligands including BMP and activin. Mutational surveys of such non-TGF-β receptors including *ALK1 (TSR-1), ALK2 (ActR-1), ALK3 (BMPR-1A)*, and *ALK6 (BMPR-1B)* have been negative. Recently, a 2% incidence of novel somatic mutations of *ALK4 (ACVR1B)*, located on chromosome 12q13, was described, providing the first evidence from human tumors to support ACVR1B as a tumor-suppressor gene.[89]

MKK4

MKK4 (mitogen-activated protein kinase kinase 4), located on chromosome 17p, is genetically inactivated at low frequency in many sporadic cancer types but has been best demonstrated in breast and pancreatic cancers.[90,91] *MKK4* plays a central role in the stress and cytokine-induced signal transduction pathway involving mitogen-activated protein kinase proteins. Somatic mutations in *MKK4* have been identified in 4% of sporadic pancreatic cancers, 6% of sporadic biliary cancers, and 14% of breast cancer cell lines.[90]

STK11/LKB1

The *STK11/LKB1* protein is ubiquitously expressed in all tissues and is believed to function in cell cycle arrest. Germline mutations of *STK11/LKB1* cause the Peutz-Jeghers syndrome, an autosomal dominant disorder best known for gastrointestinal polyps and mucocutaneous melanotic macule. Patients with Peutz-Jeghers syndrome are at a high risk for

development of colon, other gastrointestinal tract (ie, duodenal), and pancreatic cancers. A germline mutation associated with loss of heterozygosity was observed in a pancreatic cancer that developed in a patient with Peutz-Jeghers syndrome, and somatic genetic alterations in STK11 have been identified in 4% of sporadic pancreatic cancer cases.[92]

Genome-maintenance Genes

Genome-maintenance genes encode for proteins that correct many errors that normally occur when DNA is replicated. When these mismatch repair genes are dysfunctional, errors in DNA replication are not repaired. There are at least 2 types of DNA replication checkpoints that are important for maintaining chromosome stability. The first is the spindle checkpoint, which ensures that the chromatids do not separate until they are properly aligned along the mitotic spindle. The second is the DNA-damage checkpoint, a G2-M checkpoint, which prevents cells with DNA damage from entering mitosis. The names, locations, and mutational frequencies of genome-maintainence genes commonly mutated in pancreatic cancer are shown in Table 5.

Microsatellite and Chromosomal Instability

In replication error–positive tumors tumors, mutations accumulate in normal simple repeated sequences located throughout the genome, known as "CA" repeats. This leads to well-defined molecular phenotype called "microsatellite instability" (MSI). Approximately 3% of pancreatic carcinomas display MSI. These tumors have a distinct phenotypic and genotypic profile.[93] The Mlh1 mismatch repair protein is not expressed in some of the MSI cases.[77]

BRCA2

The BRCA2 gene, located on chromosome 13q, is inactivated in approximately 7% to 10% of pancreatic cancers. Interestingly, the BRCA2 mutation usually is an inherited germline mutation, as opposed to the acquired somatic mutations commonly seen in p16, p53, and DPC4.[94,95] The BRCA2 protein may function as a genomic maintenance gene by preventing DNA strand breaks that occur during normal cell cycle division. Biallelic inactivation of BRCA2 is a late event, with the remaining wild-type allele being lost well after the progression of neoplasia in patients with germline BRCA2 mutations. This likely explains why BRCA2 carriers do not have an increased frequency of precursor lesions in the pancreas and why the age of onset of pancreatic cancer in BRCA2 carriers is not reduced.

Gene Expression Profiling

The development of global gene and protein expression methods has resulted in a virtual explosion of information in the study of human cancers. The use of these various techniques in the study of pancreatic ductal adenocarcinoma exemplifies this phenomenon. Compared with only 5 years ago, we are now aware of hundreds of genes with potential importance in the biologic study of pancreatic cancer.

Four technologies have revolutionized our ability to study gene or protein expression in pancreatic duct adenocarcinoma. These include serial analysis of gene expression (SAGE), cDNA microarrays, oligonucleotide arrays, and proteomics. Methods such as SAGE, cDNA microarrays or oligonucleotide arrays allow for the detection of total mRNA expression in samples of interest. In proteomic methods, small amounts of protein are directly applied to biochips coated with specific chemical matrices (hydrophobic, cationic, anionic, normal phase, etc) and analyzed by mass spectrometry to obtain a protein "fingerprint" of a sample.

Gene expression profiling has advanced our understanding of pancreatic ductal adenocarcinoma. Recently, greater than 100 novel markers of pancreatic cancer have been identified, most of which have never been reported for this tumor type (Table 6). For example, oligonucleotide arrays were used to identify genes differentially expressed in resected pancreatic cancer tissues and pancreatic cancer cell lines compared with normal pancreas and gastrointestinal mucosa.[96] With this approach, 97 gene fragments were identified that were expressed at least 5-fold or greater in pancreatic cancer samples as compared with normal tissues. Of these 97 genes identified, 69 genes have not previously been reported in association with pancreatic cancer. Thus these 97 genes represent novel markers of pancreatic cancer, each with the potential for development into pancreatic cancer screening tests or targets for novel therapeutic modalities.

Novel markers of pancreatic cancer have also been identified by SAGE.[97–99] Comparisons of SAGE libraries derived from pancreatic ductal adenocarcinomas to SAGE libraries derived from normal pancreatic duct epithelium have identified mesothelin, prostate stem cell antigen (PSCA) and S100A4 as highly expressed in pancreatic cancers. The tag for mesothelin was present in 7 of 8 pancreatic cancer cell line SAGE libraries, but not in 2 SAGE libraries derived from normal duct epithelial

TABLE 6. Some genes confirmed as overexpressed in pancreatic cancer

Gene	Method of validation*	Previously reported in pancreatic cancer	Reference
Mesothelin	IHC, IS, RT-PCR	No	97
PSCA	IHC, RT-PCR	No	98
S100A4	IHC, RT-PCR	No	99
Claudin 4	RT-PCR	No	102
14-3-3 sigma	IHC, RT-PCR	No	101
Trop2	IHC	Yes	101
Fibronectin	IHC	Yes	101
Transglutaminase II	IHC	Yes	101
S100A10	IHC	No	101
Ron	IHC	No	101
Cytokeratin 19	IHC	Yes	101
cdc2	IHC	No	101
Fascin	IHC	No	96
Heat shock protein 47	IHC	No	96
Pleckstrin	IS	No	96
Topoisomerase II alpha	IHC	No	96
Gamma synuclein†	IHC	No	101

*IHC, immunohistochemistry; RT-PCR, reverse transcriptase-polymerase chain reaction; IS, in situ hybridization.

†Occasional overexpression (<20% of cases studied).

cells. PSCA and S100A4 were also identified by use of this approach.[98,99] Immunohistochemical validation of mesothelin expression revealed 60 of 60 ductal adenocarcinomas were strongly immunoreactive for mesothelin protein,[97] whereas PSCA protein was detected in 36 of 60 ductal adenocarcinomas.[98] Interestingly, overexpression of S100A4 mRNA in pancreatic cancer cell lines was strongly associated with hypomethylation of the first intron of this gene in 90% of pancreas cancer cell lines analyzed.[99]

As an alternative approach, ProteinChip SELDI technology has been used to screen for differentially expressed proteins in pancreatic juice samples from patients both with and without pancreatic duct adenocarcinoma.[100] A 16.5-kDa protein peak was identified in 10/15 (67%) patients with pancreatic adenocarcinoma, but in only 1/7 (17%) patients with other pancreatic diseases. This protein was identified as hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein-1 (HIP/PAP-1), a protein released from pancreatic acini during acute pancreatitis and overexpressed in hepatocellular carcinoma. The quantification of HIP/PAP-1 amounts in pancreatic juice and serum samples by enzyme-linked immunosorbent assay confirmed the significantly elevated amounts of this protein in the samples from patients with pancreatic adenocarcinoma. Furthermore, patients with pancreatic juice HIP/PAP-1 levels $\simeq$20

μg/mL were found to be 21.9 times more likely to have pancreatic adenocarcinoma than patients with levels less than 20 μg/mL.

Gene expression profiling has also provided novel insight into the complex biology of pancreatic cancer. In collaboration with Brown at Stanford, we have used cDNA microarrays to analyze samples of infiltrating pancreatic duct adenocarcinoma, pancreatic cancer cell lines, and normal pancreatic tissues.[101] With hierarchical clustering, one large cluster was identified that contained cDNAs whose expression was increased in both pancreatic cancer cell lines and tumor tissues as compared with normal pancreas. Genes included in this pancreatic cancer–specific cluster spanned a variety of classes of gene function and were characterized by those involved in cell membrane junctions, cell/matrix interactions, cytoskeletal assembly, cell cycle regulation, transcription factors, calcium homeostasis, and proteolytic processing. Similar type genes were also identified by Ryu et al[102] in SAGE comparisons of pancreatic cancer cell lines, normal pancreatic duct cells, and pancreatic cancer tissues. Forty-nine genes were identified as over-expressed by the cancer cell lines or tissues as compared with normal cells and included secretory, cell-surface, transmembrane, and tight junction protein coding genes, possibly corresponding to altered cellular attachments and resulting in aberrant cell-cell interactions.

Gene expression profiling of pancreatic cancer has also provided new insights into the process of tumor invasion. Using principal component analysis of SAGE data derived from pancreatic cancer cell lines and cancer tissues, Ryu et al[103] identified a large cluster of "invasion-associated" genes of infiltrating pancreatic cancer. This cluster of genes was expressed in surgically resected pancreatic cancer tissues, but not in normal pancreas tissue or cultured pancreatic cancer cell lines. The genes identified within this "invasion-associated" cluster were believed to reflect the cellular components of the host stromal response seen in the presence of infiltrating carcinoma.

Because the spatial localization of gene expression was not determined for these invasion-associated tags, their cellular origin within the primary tumor remained unclear, as well as their role in the invasive process. Subsequently, in situ hybridization was used to evaluate 12 of these invasion-associated genes in samples of pancreatic cancer tissue.[104] Detectable expression of 8 of these genes localized to 1 of 4 distinct compartments of the invasive tumors, that is the neoplastic epithelium, angioendothelium, juxtatumoral stroma (those stromal cells immediately adjacent to the invasive neoplastic epithelium) or the panstromal com-

partment (all stromal tissue within the host response). Expression of the remaining 4 genes localized to 2 or more compartments.

These findings indicate that a highly organized and coordinated process of tumor invasion exists in the pancreas. Furthermore, although the gene expression compartments were distinct, the data suggest that potential lines of communication between different compartments may exist. For example, α-2 macroglobulin expression was detected within the juxtatumoral stroma, whereas the receptor for this gene product, α-2 macroglobulin receptor, was expressed by the neoplastic epithelium. Thus the host-stromal response, and the juxtatumoral stroma in particular, may play an active role in the invasive process.

In summary, our initial attempts to study pancreatic cancer by use of global gene expression methods have revealed a wealth of information. Clearly, pancreatic adenocarcinomas are complex tumors, as evidenced by the wide range of cellular functions represented by the genes identified. These findings not only provide novel insight into the biologic study of pancreatic cancer, but also serve to generate new hypotheses for study.

Screening and Early Detection

Current Approaches for Detecting Pancreatic Cancer

The vast majority of pancreatic cancers are diagnosed at a late, incurable stage. However, early detection of small, resectable cancers may improve the outcome of this deadly disease.[105] The optimal approach for early detection of pancreatic cancer is unknown and still under study. An ideal approach would use an imaging test along with molecular markers of neoplastic disease to diagnose a benign, dysplastic precancerous lesion or an early, localized cancer. Ongoing research at Johns Hopkins related to early detection of pancreatic neoplasia in patients with Peutz-Jeghers syndrome and at-risk relatives from familial pancreatic cancer kindreds will provide additional data on the potential benefits of a screening program with this approach. Currently, 2 imaging modalities for screening and early detection include magnetic resonance imaging (MRI)/magnetic resonance cholangiopancreatography, and endoscopic ultrasonography (EUS). Standard radiologic imaging tests such as transabdominal ultrasonography, magnetic resonance imaging, and spiral (helical) computed tomography (CT) scanning used in the community are not sufficiently sensitive for detecting small early cancers, even in patients with symptoms. However, CT imaging continues to improve with the development of multidetector technique and 3-dimensional reconstruction, which allow improved resolution.[106] For early detection, EUS may be the imaging modality of choice because it detects smaller pancreatic lesions than those detected with thin section dual phase spiral CT.[107,108] The accuracy of diagnosis of pancreatic cancer approaches 100% for EUS and 92% for dual-phase CT.[109] Furthermore, EUS can readily discriminate between solid and cystic lesions (unlike CT scanning) and provide a cytologic diagnosis of minute lesions as small as 2 to 5 mm not visualized by CT scanning, ultrasonography, or magnetic resonance imaging. Fine-needle aspiration (FNA) performed during an EUS procedure also help to establish a diagnosis of malignancy with an accuracy of 90%. Endoscopic retrograde cholangiopancreatography (ERCP) is less likely to detect small tumors, because earlier lesions are associated with minimal effects on the pancreatico-biliary tree. Other investigative tools for the detection of pancreatic cancers include positron

emission tomography (PET) and intraductal ultrasound scanning. PET may be helpful for identifying occult metastases.[110] Intraductal ultrasonography may be performed as an extension of an ERCP procedure to detect small pancreatic tumors and IPMNs.[111]

CA19-9, the only commercially available, widely used tumor marker for pancreatic cancer, can be valuable for monitoring the therapeutic response of patients with pancreatic cancer that have elevated serum CA19-9 levels.[112] But CA19-9 is of limited value as a screening marker, because approximately 10% to 15% of individuals do not secrete CA19-9 because of their Lewis antigen status. In addition, CA19-9 levels may be within the normal range while the cancer is small and asymptomatic and can be elevated in benign biliary or pancreatic conditions.

Current approaches to survey at-risk individuals use EUS of the pancreas, multidetector CT with thin sections of the pancreas, and serum CA 19-9 measurements. ERCP, EUS-FNA, and other investigations may be performed if abnormalities are found on EUS. Recently, Brentnall et al[113] reported their experience with screening of high-risk families. Of 14 patients from 3 families surveyed primarily by EUS, 7 were found to have EUS and ERCP abnormalities suggestive of pancreatic ductal lesions. Pathologic analysis of pancreatectomy resection specimens revealed high-grade PanIN, but no cancers. Because total removal of the pancreas (total pancreatectomy) is a major surgical procedure that results in both endocrine and exocrine insufficiency (requiring insulin and enzyme replacement), its application to patients for prophylaxis of pancreatic cancer requires careful consideration and counseling.

Developing Biomarkers for Early Detection

Biomarkers of pancreatic cancer are needed for both early diagnosis of individuals with symptoms whose initial workup fails to yield a diagnosis and for use as a screening test to permit the early detection of pancreatic cancer in symptom-free individuals at high risk for development of the disease. Ideally, a screening test should be easy to perform, such as serum testing for PSA levels to identify prostate cancer or testing for fecal occult blood to screen for colorectal neoplasias. The potential high concentration of DNA and proteins, and the relative lack of other normal constituents like in the serum, make pancreatic juice a potentially optimal specimen to use when screening patients at high risk for pancreatic cancer. Such a practice would be analogous to using sputum to screen for lung cancer[114] or nipple aspirates to screen for breast cancer.[115] Furthermore, pancreatic juice can be collected during routine upper gastrointestinal endoscopy after secretin stimulation, without the need for ERCP. Even when

pancreatic cancer is suspected, imaging tests sometimes fail to identify a pancreatic mass. In this setting, molecular markers could facilitate early diagnosis by aiding in the interpretation of inconclusive cytology specimens obtained by sampling the pancreatic duct or from fine-needle aspirates obtained during EUS.

Biomarkers can be divided into 3 biochemical targets: DNA-based, RNA-based, and protein-based. DNA-based techniques aim to detect cancer-specific DNA alterations. The diagnostic potential of both DNA- and RNA-based markers has improved with the use of quantitative polymerase chain reaction (PCR). Pancreatic cancer DNA can be detected in pancreatic juice samples, in fine-needle aspirates of the pancreas, in the serum, and in the stool of affected patients. For example, mutant K-*ras* is found in the pancreatic juice or pancreatic duct brushings and in stool of many patients with pancreatic cancer. However, it is not a specific marker, being found among symptom-free smokers and in pancreatic juice from patients with chronic pancreatitis without cancer. In addition, mutant K-*ras* in plasma is observed late in the natural history of pancreatic cancer, correlating with advanced disease.[116,117] Of the DNA-based abnormalities described in pancreatic cancer, DNA methylation abnormalities may be particularly suitable for use in early detection strategies. First, numerous aberrant methylation events occur during carcinogenesis (eg, methylation of *hMLH1* and *p16*).[118] Second, aberrant methylation can be detected in secondary sources with the very sensitive methylation-specific PCR technique. Third, aberrant methylation patterns of affected genes are typically consistent across cancers from a number of different patients and thus can be targeted for diagnostic tests.[119] Methylation-specific PCR has successfully been used to identify methylated DNA in blood, saliva, prostatic fluid, and sputum of patients with cancers. Pancreatic carcinomas harbor aberrant methylation of a number of cancer-related genes (*ppENK, p16*, TSLC1, and others).[120,121] Of these, methylation of *ppENK* occurs in more than 90% of pancreatic cancers and is not seen in normal pancreas, suggesting that it may be a valuable molecular marker of pancreatic cancer.

As with detection of pancreatic cancer DNA, detection of pancreatic cancer mRNA, may be less useful for serum-based diagnosis and may be more applicable for the analysis of pancreatic juice or fine-needle aspirates. The main RNA-based marker investigated to date has been hTERT. About 90% of cancers express the telomerase hTERT subunit, and $<$90% of patients with pancreatic cancer have detectable telomerase activity in their pancreatic juice.[122] The detection of telomerase enzymatic activity or the hTERT subunit may be helpful in differentiating

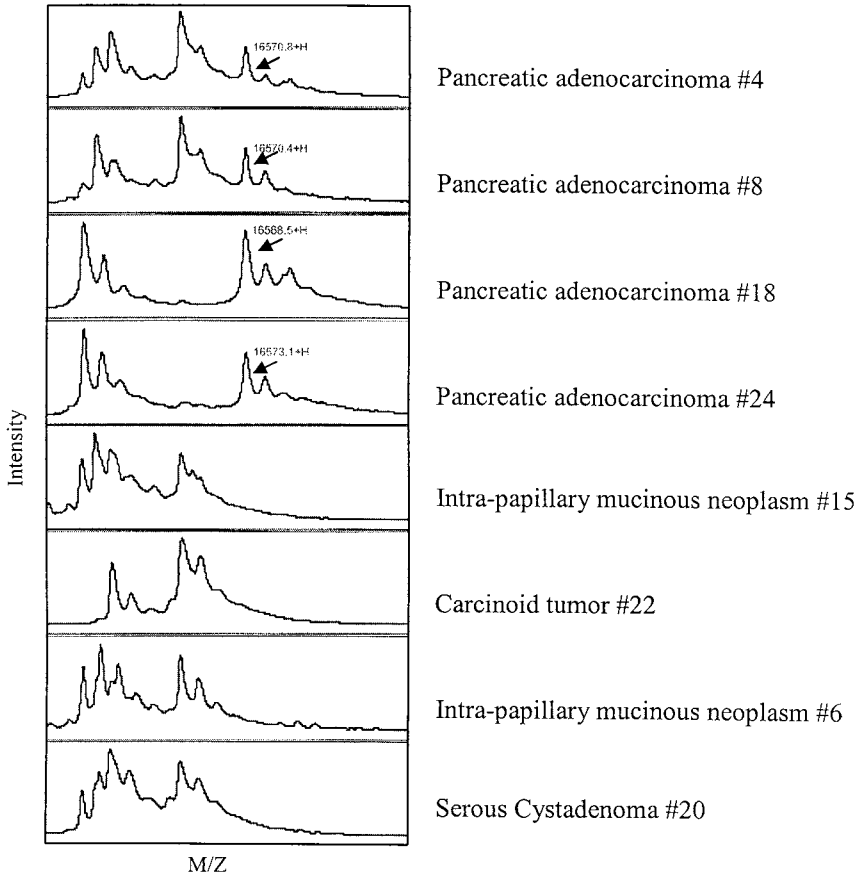

FIG 4. Representative spectrum examples of SELDI analysis of pancreatic juice samples bound to IMAC-3 cupper ProteinChip array. A peak of <16,570 Da (*arrow*) was present in the 4 pancreatic juice samples from patients with pancreatic adenocarcinoma (*PC4, PC8, PC18, PC24*) but absent in the 4 patients with other pancreatic diseases [*IPMN*; islet cell tumor (*ICT*); serous cystadenoma (*SC*)].

pancreatic cancer from benign pancreatic disease. However, because telomerase is expressed in inflammatory cells, it may not be sufficiently specific for use as a cancer-screening marker. Several recent studies of gene expression profiling by cDNA and oligonucleiotide microarrays and SAGE, which permit the expression levels of tens of thousands of genes to be monitored simultaneously and rapidly, have generated a long list of genes that are overexpressed at the RNA level in pancreatic cancers compared with normal pancreas.[123,124] Mesothelin, expressed at the RNA and protein level in almost all pancreatic cancers but not in normal

pancreas, is one of several such overexpressed genes likely to be tested as a marker of pancreatic cancer.[125]

Protein-based markers ultimately may have the most application for pancreatic cancer diagnostics. The ultimate goal of such a marker would be a "PSA-test" for pancreatic cancer. In addition to the use of genes overexpressed at the RNA level to identify new protein markers, large-scale analysis of proteins in biologic fluids or cells is an attractive approach for identifying cancer markers. "Proteomics" is the term now used for profiling proteins in biologic samples. One such proteomics technique is SELDI (surface-enhanced laser desorption ionization mass spectrometry), which analyzes protein profiles of samples applied to protein chips.[126] SELDI profiling of pancreatic juice led to the identification of markedly elevated HIP/PAP levels in pancreatic juice samples from patients with pancreatic cancer compared with patients with other pancreatic diseases (Fig 4).[126] Several investigators have shown that bioinformatic analysis of SELDI mass spectrometry spectra of serum can accurately predict the presence of ovarian and prostate cancer compared with benign disease.[127,128]

The recent advances in proteomics, gene expression analysis and DNA methylation suggest that molecular markers of pancreatic cancer may soon prove useful in the early detection and diagnosis of this disease. As additional advances are made, their most valuble application would appear to be screening of populations at high risk for pancreatic cancer.

Clinicopathologic Staging

A number of schemes for the staging of pancreatic carcinoma have been proposed in the past. The newest version of the American Joint Committee on Cancer Cancer Staging Manual was published in 2002.[129] Because only a minority of patients with pancreatic cancer undergo surgical resection, a single TNM classification must apply to both clinical and pathologic staging (Table 7). The definitions of TNM have changed from past versions, with specific changes made to the T classification and to the definition of stage III disease. It is also important to note that the extent of resection (R_0 = complete resection; R_1 = grossly negative but positive microscopic margins of resection; R_2 = grossly and microscopically positive margins of resection) is not a part of the TNM staging system, but is of great prognostic significance. Another commonly used staging

TABLE 7. Definitions

Primary tumor (T)			
Tx	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
Tis	Carcinoma in situ		
T1	Tumor limited to pancreas and 2 cm or less in greatest dimension		
T2	Tumor limited to pancreas and more than 2 cm in greatest dimension		
T3	Tumor extends beyond pancreas but does not involve celiac axis or superior mesenteric artery		
T4	Tumor invades the celiac or the superior mesenteric artery (unresectable primary tumor)		
Regional lymph nodes (N)			
Nx	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastases		
N1	Regional lymph node metastases		
Distant metastases (M)			
Mx	Distant metastases cannot be assessed		
M0	No distant metastases		
M1	Distant metastases		
Stage Grouping			
0	Tis	N0	M0
IA	T1	N0	M0
IB	T2	N0	M0
IIA	T3	N0	M0
IIB	T1 to T3	N1	M0
III	T4	Any N	M0
IV	Any T	Any N	M1

From Exocrine pancreas. AJCC cancer staging manual. 6th ed. New York: Springer; 2002. p. 157–163).

TABLE 8. UICC staging of pancreatic cancer

Stage grouping	T	N	M	5-Year survival rate
Stage I	T_1 or T_2	N_0	M_0	20%–40%
Stage II	T_3	N_0	M_0	10%–25%
Stage III	Any T	N_1	M_0	10%–15%
Stage IV	Any T	Any N	M_1	0%–8%

T, Tumor; *N*, lymph nodes; *M*, distant metastasis; T_1, limited to pancreas; T_2, extension directly to duodenum, bile duct or peripancreatic tissues; T_3, extension directly to stomach, spleen, colon or adjacent large vessels; N_0, no lymph node metastases; N_1, lymph node metastases; M_0, no distant metastasis; M_1, distant metastasis.
From UICC. TNM classification of malignant tumors. 4th ed. New York: Springer-Verlag; 1987.

TABLE 9. Japan Pancreas Society stage classification

Stage grouping	T	S	RP	PV	N	M	5-Year survival rate
Stage I	T_1	S_0	RP_0	PV_0	N_0	M_0	35%–45%
Stage II	T_2	S_1	RP_1	PV_1	N_1	M_0	15%–25%
Stage III	T_3	S_2	RP_2	PV_2	N_2	M_0	5%–15%
Stage IV	T_4	S_3	RP_3	PV_3	N_3	M_1	0%–10%

Tumor (T): T_1 = 0 to 2 cm; T_2 = 2.1 to 4 cm; T_3 = 4.1 to 6 cm; T_4 = >6.1 cm.
Serosal invasion (S); Retroperitoneal invasion (RP); Portal venous invasion (PV): 0 = absence of invasion; 1 = suspected invasion; 2 = definite invasion; 3 = severe invasion. Lymph nodes (N): N_0 = no metastasis; N_1 = primary lymph node group metastasis; N_2 = secondary lymph node group metastasis; N_3 = tertiary lymph node group metastasis.
Distant metastasis (M): M_0 = no distant metastasis; M_1 = distant metastasis.
From Japan Pancreas Society. General rules for surgical and pathological studies on cancer of the pancreas. 3rd ed. Tokyo: Kanehara; 1987.

system involves the Union Internationale Contre Le Cancer (UICC) system, which is also based on TMN factors (Table 8).[130]

A more complex stage classification system has been proposed by the Japan Pancreas Society (Table 9), adding other factors to the classification such as serosal invasion (S factor), retroperitoneal invasion (RP factor) and invasion of the portal venous systems (PV factor).[131] This system has been overly cumbersome and difficult to apply, and it has gained only limited use.

Diagnosis and Staging

Clinical Presentation

Most patients with pancreatic cancer are initially seen with the development of jaundice, which usually occurs as a result of the neoplasm, arising in the head of the pancreas, obstructing the intrapancreatic portion of the common bile duct. Accompanying the jaundice often are dark urine, light stools, weight loss, abdominal pain, and pruritus. Weakness and anorexia may also be present.

At times, pancreatic cancer may present in an unusual manner. New-onset diabetes may be the first clinical feature in approximately 10% of patients.[132] Occasionally acute pancreatitis may be the first signal of a pancreatic neoplasm, related to partial obstruction of the pancreatic duct. Such a presentation is being reported frequently in patients with IPMN.[133] It is important to consider the diagnosis of pancreatic neoplasia in these uncommon patients diagnosed with pancreatitis, particularly when there is no obvious cause for the acute pancreatitis (such as gallstones or alcohol abuse).

Other symptoms found in a small percentage of patients include nausea and vomiting related to gastroduodenal obstruction. Mechanical obstruction of the proximal duodenum by right-sided neoplasms, or at the ligament of Treitz by cancers of the midbody of the pancreas are often later findings of pancreatic cancer and suggest relatively advanced disease.

The most common physical finding at initial presentation is jaundice. Often, patients with deep jaundice will exhibit cutaneous signs of scratching, related to the pruritis. Hepatomegaly and a palpable gallbladder may also be found. Physical findings in patients with disseminated cancer may include palpable hepatic metastases, left supraclavicular adenopathy (Virchow's node), periumbilical lymphadenopathy (Sister Mary Joseph's nodes), and the finding of drop metastases in the pelvis encircling the perirectal region (Blumer's shelf). Patients with advanced disease may also exhibit cachexia and muscle wasting.

Patients with cancer of the head of the pancreas typically have laboratory study results marked by elevated serum total bilirubin, alkaline phosphatase and γ-glutamyl transpeptidase, with mild elevations of the hepatic aminotransferases. Hepatitis serologic study result are often assessed as part of the workup for jaundice, and they are typically

TABLE 10. The combination of CA19-9 and imaging tests for diagnosis of pancreatic cancer

	Positive predictive value		
	Without CA 19-9	**CA 19-9 >40 U/mL**	**CA19-9 >100 U/mL**
Abdominal ultrasonography	62%	100%	100%
Abdominal CT	71%	89%	100%
ERCP	62%	80%	100%

From Ritts R et al. Pancreas 1994;9:707–16.

negative. Normochromic anemia and hypoalbuminemia may reflect the chronic nature of the neoplastic process and its nutritional sequelae. In patients with localized cancer of the body and tail of the pancreas, standard laboratory values are usually normal. For these tumors, when liver function test abnormalities do occur, they typically indicate diffuse metastatic disease with involvement of the liver or porta hepatis. It is uncommon for patients with standard ductal adenocarcinoma of the pancreas to have either hyperamylasemia or hyperlipasemia. Patients with IPMN and associated cancer may, however, have elevations of either amylase or lipase. In patients with deep jaundice, the coagulation parameters should be checked, because prolonged exclusion of the bile from the gastrointestinal tract leads to a malabsorption of the fat-soluble vitamins and decreased hepatic production of vitamin K–dependent clotting factors. This can result in prologation of the prothrombin time.

Many different tumor markers have been studied in an attempt to facilitate an early diagnosis of pancreatic cancer. One of the most pressing needs for this disease is a specific and sensitive early tumor marker. At present there are no accurate and reliable serum markers that can be used for the diagnosis of pancreatic cancer. The best of the commercially available markers is the carbohydrate antigen 19-9 (CA19-9).[134–136] CA19-9 is a Lewis blood group–related mucin that has been extensively studied in the diagnosis, prognosis, and monitoring of pancreatic cancer. With an upper limit of normal of 37 units/mL, CA19-9 only approaches an 80% accuracy in identifying patients with pancreatic cancer. The combined use of CA19-9 and either ultrasonography, CT scanning, or ERCP improves the diagnostic accuracy of each individual test, to the point that accuracy can approach, but not achieve, 100% (Table 10). Unfortunately, CA19-9 has not been proven to be useful as an independent test for pancreatic cancer, and it has not proven sufficiently accurate to identify early potentially curable tumors. However, CA19-9 has been correlated both with prognosis and with tumor recurrence. In general, higher CA19-9 values before surgery indicate an increasing size of the

primary tumor and an increasing rate of unresectability. Additionally, CA19-9 has been used to monitor combined modality treatments, typically neoadjuvant chemoradiation treatment or postoperative combined modality therapies. In general, it has been true that increasing levels of CA19-9 reflect progression of disease, whereas stable or declining levels of CA19-9 are associated with a stable tumor burden, absence of tumor recurrence by imaging studies, and an improved prognosis.[136,137]

As has been discussed in previous sections of this monograph, one hope for the future involves new developments in the area of early detection, by use of molecular strategies. With gene expression data[138] and data from other molecular strategies,[139,140] it can be anticipated that earlier detection of pancreatic cancer will one day be possible.

Diagnostic Imaging

Currently, state-of-the-art CT scanning of the pancreas uses multidetector CT acquisition,[141] a technology introduced in the late 1990s. Before this, high-quality spiral (or helical) CT was the preferred noninvasive imaging modality for the diagnosis and staging of pancreatic cancer.[142] Multidetector CT incorporates dual-phase imaging in both the arterial and venous phases of enhancement. Water is used as the oral contrast agent of choice. Nonionic contrast medium (120 mL) is administered via a peripheral intravenous catheter at a rate of 3 mL/sec. Slices through the pancreas are obtained every 1.25 mm, with all images being acquired during one 20-second breath hold. For visualizing the study on film, 3- to 5-mm slices are printed on film. However, the 1.25-mm slices are reviewed at a 3-dimensional workstation, by use of a software platform (we use Siemens Virtuoso, Siemens, Iselin, NJ). The addition of 3-dimensional viewing of the data sets improves the detection, staging, and surgical planning.[141] Adenocarcinoma of the pancreas typically appears as a low-density (hypodense) mass within the pancreas (Figs 5–7), often best seen on the venous phase of enhancement. The tumor may obstruct the common bile duct or the pancreatic duct, resulting in ductal dilatation in the proximal biliary tree or left side of the pancreas. Tumor encasement of the major peripancreatic vascular structures can be seen as narrowing of the celiac axis vessels, superior mesenteric artery or vein, or splenic artery or vein. CT scanning also has the ability to detect lymph node enlargement and hepatic metastases (although a pathologic diagnosis cannot be obtained from imaging alone).

Recent advances in hardware and software have allowed MRI to improve its ability to diagnose and stage pancreatic cancer. These recent advances include high-resolution imaging, fast imaging, volume acquisi-

FIG 5. Late arterial phase of spiral CT scan, with contrast used as oral agent. The kidneys and aorta are contrast enhanced, as is the inferior vena cava. Dilated bile ducts are seen in the liver, and the gallbladder is distended. A large (5 cm) hypodense mass is seen in the head of the pancreas, and the superior mesenteric vein (SMV) is not seen. Additional caudal images confirmed occlusion of the SMV, with numerous mesenteric venous collaterals. This tumor was deemed unresectable, on the basis of the advanced local disease.

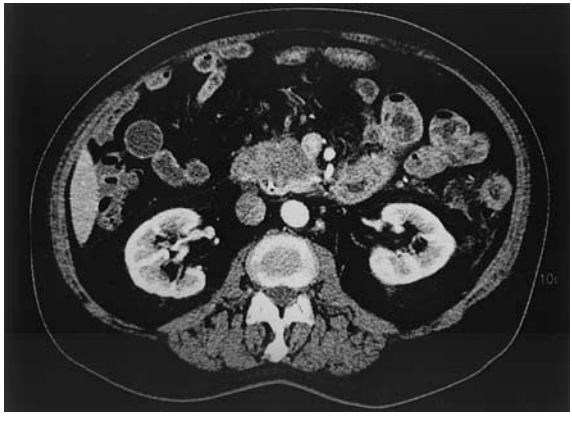

FIG 6. Arterial phase of multidetector CT scan, with water used as oral agent. The kidneys and aorta are contrast enhanced. A 3-cm hypodense tumor mass is seen in the pancreatic uncinate process, anterior to the aorta and inferior vena cava. The tumor abuts the right lateral aspect of the superior mesenteric vein. The superior mesenteric artery is contrast-enhanced, patent and not approached by tumor. This tumor was resected via pancreaticoduodenectomy, with negative resection margins.

tions, magnetic resonance cholangiopancreatography, and functional imaging.[143,144] Sequences with high temporal resolution are used for the detection and staging of pancreatic cancer. Arterial and venous phase

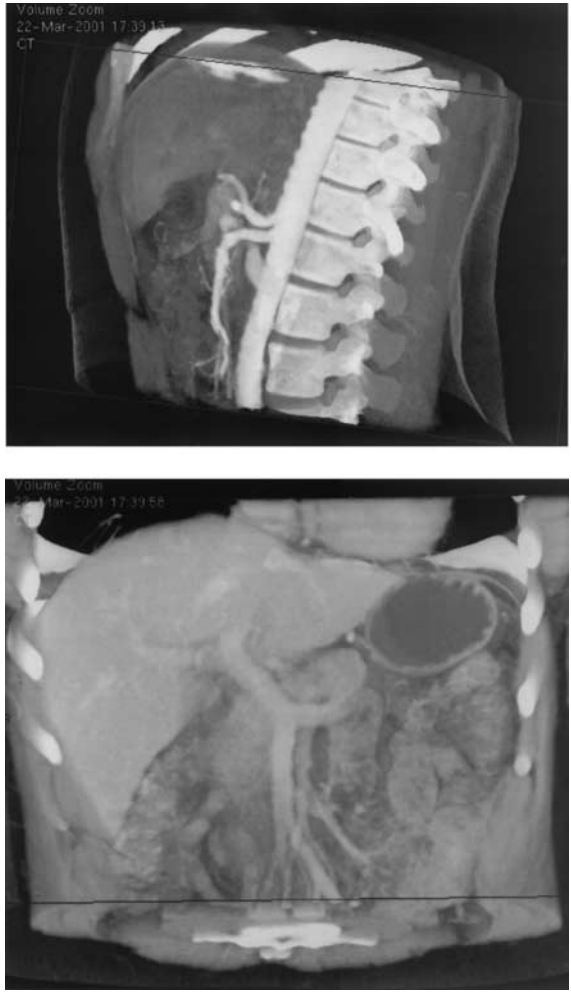

FIG 7. Multidetector CT images from patient with small cancer in head of pancreas. **(Top)** Sagital 3-dimensional reconstruction shows normal aorta, celiac axis, and superior mesenteric artery. **(Bottom)** Coronal 3-dimensional reconstruction shows normal liver, gastric fundus, portal vein, as well as intact superior mesenteric artery and vein.

studies are used to evaluate arterial and venous patency. Because most pancreatic cancers have sparse vascularity and dense cellularity, the tumors appear with low-signal intensity on T_1-weighted fat-suppressed images, and diminished enhancement on dynamic contrast-enhanced images (Figs 8 9).[144] For vascular assessment and tumor detection, optimally performed MRI and multidetector CT acquisition appear to

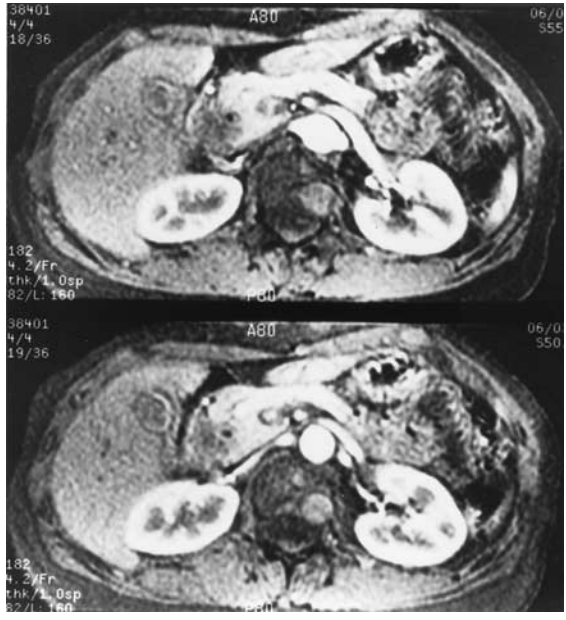

FIG 8. Two T_1-weighted MR images with contrast enhancement with gadolinium. A mass in the head of the pancreas appears as a hypointense area. (From Yeo CJ, Cameron JL. Pancreatic Cancer. Curr Prob Surg 1999;36:57–152, Fig 17).

yield similar results. There appears to be no advantage to obtaining both modern MRI and CT studies in patients with suspected pancreatic cancer.

ERCP allows direct imaging of the pancreatic duct, the site of origin of most pancreatic cancers. The sensitivity of ERCP for the diagnosis of pancreatic cancer is quite high, with the finding of long, irregular stricture in an otherwise normal pancreatic duct being virtually pathognomonic in the appropriate clinical setting (Fig 10). However, inaccuracy can arise when evaluation of short isolated pancreatic duct strictures occurring in patients with underlying chronic pancreatitis or pancreatic trauma is attempted. Although there is no question that ERCP is reliable in confirming the clinical suspicion of pancreatic cancer, it is rarely necessary and should not be routinely used. With the current sophistication of CT scanning and MRI, the routine practice of diagnostic ERCP is unsupported.

EUS is a recently developed and now established technique for obtaining images of the pancreas.[145–150] The close proximity of the transducer to the region of interest permits the use of higher ultrasound frequencies than do transcutaneous techniques, thereby improving image

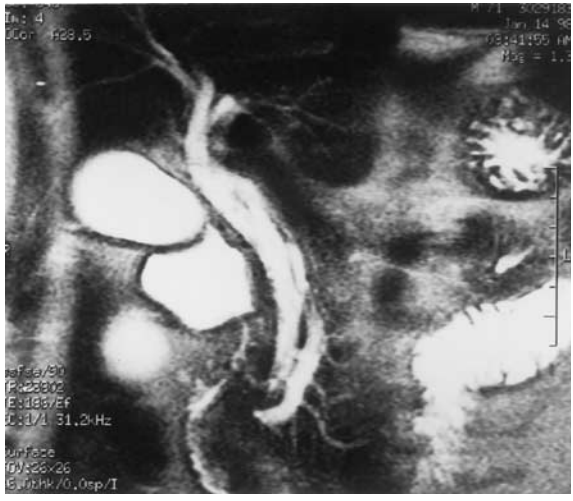

FIG 9. Single shot, spin echo MRI-cholangiopancreatogram in patient with obstructive jaundice. Both the common bile duct and the pancreatic duct are dilated, and a hypointense area of tumor is apparent in the periampullary region. (From Yeo CJ, Cameron JL. Pancreatic Cancer. Curr Prob Surg 1999;36:57–152, Figure 18).

resolution (Fig 11). Several studies have evaluated EUS in distinguishing benign from malignant pancreatic masses. Baron et al[151] looked at EUS in 105 patients and identified a sensitivity of 95% and a specificity of 88% when EUS-guided FNA was applied. Mertz et al[152] compared EUS, CT, and PET in identifying pancreatic carcinoma in 35 patients, 31 of whom had confirmed cancer. For tumor identification, EUS was more sensitive (93%) and specific (75%) than CT (53% and 25%, respectively) or PET (87% and 50%, respectively).

EUS-FNA appears to offer some advantage over other techniques in cases where a tissue diagnosis of pancreatic cancer is required before treatment. (Of note, unless protocol-based neoadjuvant chemotherapy or chemoradiation therapy is planned, in most patients with a presumed resectable tumor seen by imaging, such a tissue diagnosis is not necessary). Gress et al[153] evaluated EUS-FNA in 102 patients with suspected pancreatic cancer and previously-negative CT- or ERCP-guided cytologic examinations. The sensitivity for EUS-FNA cytologic study in this difficult setting was 93%, the specificity was 83%, and the complication rate was only 3%. Although these results clearly demonstrate that EUS-FNA is able to accurately yield a tissue diagnosis of pancreatic cancer (when CT- and ERCP-guided cytologic evaluation has failed), it must be stressed that patients with resectable lesions suspicious

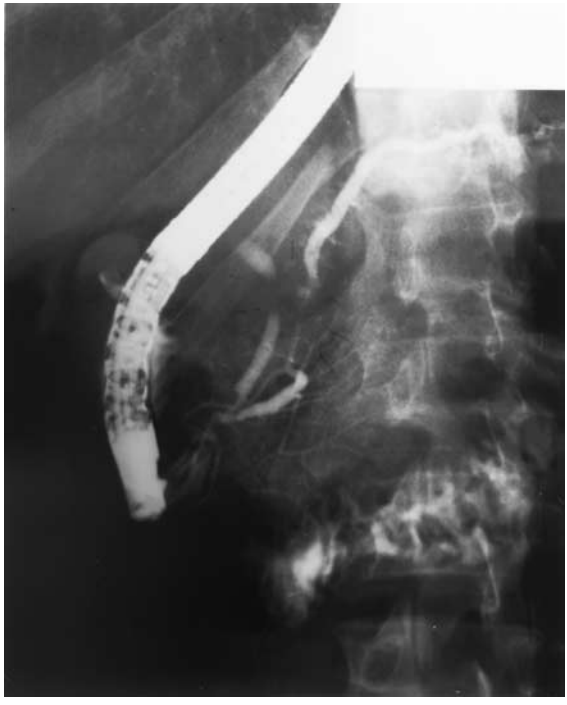

FIG 10. ERCP in patient with obstructive jaundice reveals classic "double-duct" sign. There is evidence of tumor at the genu of the common bile duct and pancreatic duct. (From Yeo CJ, Cameron JL. Pancreatic Cancer. Curr Prob Surg 1999;36:57–152, Figure 19).

for pancreatic cancer do not require such a tissue diagnosis before surgical resection.

Although CT, MRI, and EUS remain the mainstays of obtaining images of patients with suspected pancreatic cancer, the newer technique of PET may play an increasing role in the future.[154,155] PET uses the increased metabolism of glucose by pancreatic cancer cells as the basis of imaging. Current PET scanning for pancreatic cancer uses fluorine-18 (a positron-emitting tracer) as a glucose-like substrate in vivo. Fluorine-18 has a two hour physical half-life. When labeled to flurodeoxyglucose (FDG), it is rapidly taken up by malignant tumor cells. FDG-PET has been reported to be highly sensitive and specific for pancreatic cancer, with results that surpass CT: sensitivity (PET vs CT) 92% vs 65%, specificity 85% vs 62%.[156] Importantly, FDG localizes not only at tumor sites, but at sites of inflammation and infection. One way to differentiate benign from malignant lesions by PET uses a semiquantitative index called the "standard uptake value" (SUV). The SUV is an index derived from the

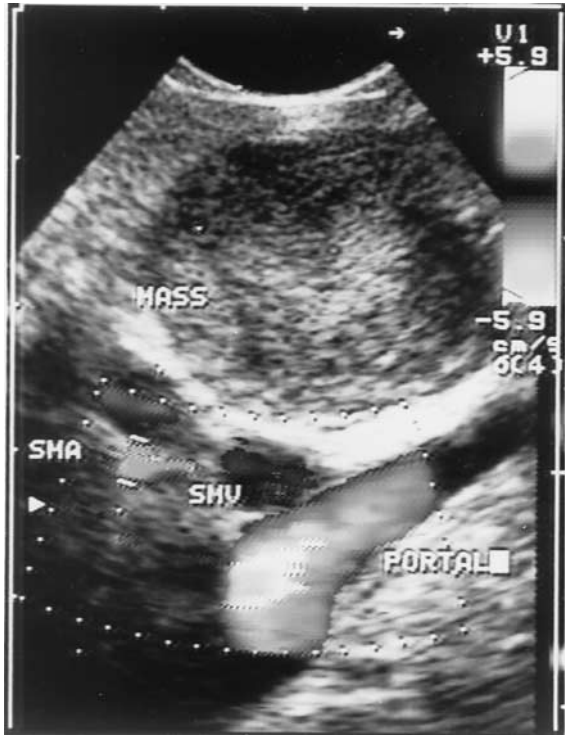

FIG 11. EUS image with linear array echoendoscope, revealing a mass in the head of the pancreas with no vascular invasion of the superior mesenteric artery *(SMA),* superior mesenteric vein *(SMV),* or portal vein *(portal).* (From Yeo CJ, Cameron JL. Pancreatic Cancer. Curr Prob Surg 1999;36:57–152, Figure 24).

decay-corrected dose, corrected for the patient's body weight. Generally, lesions with an SUV greater than 2.5 correlate with malignancy. FDG-PET has been shown, in patients with pancreatic cancer, to give information relevant to prognosis (based on high vs low SUV), and to add to the diagnostic accuracy of CT and ERCP in detecting tumor dissemination.[155] Unfortunately, currently Medicare does not reimburse for PET imaging in patients with pancreatic cancer, thus its use has been limited.

Histopathologic Diagnosis

The use of pancreatic biopsy (percutaneous or endoscopic) in the diagnostic workup of a patient with a suspected pancreatic cancer has advocates, as well as vocal opponents. Although percutaneous biopsy is generally safe, serious complications such as hemorrhage, pancreatitis,

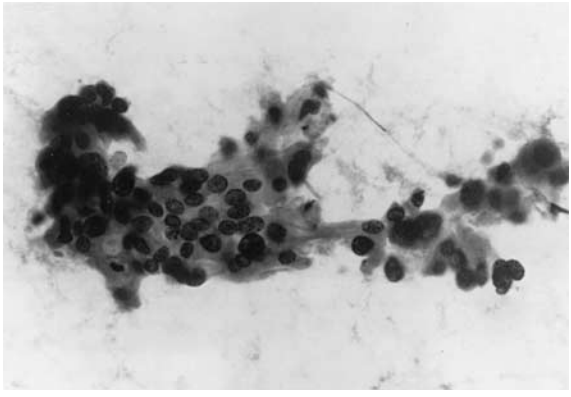

FIG 12. FNA of pancreatic adenocarcinoma: cluster of malignant cells with coarse nuclear chromatin, variable nuclear size and shape, and disorderly nuclear crowding. (Original magnification × 400.) (From Tsiotos GG, Sarr MG. Diagnosis and clinical staging of pancreatic cancer. In: Howard JM, Idezuki Y, Ihse I, Prinz RA, editors. Surgical diseases of the pancreas. 3rd ed. Baltimore: Williams and Wilkins; 1998. p. 504, Fig 52.5).

fistula, abscess, and death have been reported. Additionally, there have been reports of tumor seeding along the subcutaneous tract of the needle, and concerns regarding tumor dissemination by the act of capsular puncture of the neoplasm. Numerous studies have evaluated the results of percutaneous core biopsy or FNA cytologic study in the assessment of patients with pancreatic masses. Although a diagnosis of cancer may be highly specific (approaching 99%), the sensitivity is generally lower, ranging from 50% to 70%. This is because, although a histologic diagnosis of adenocarcinoma is quite reliable, malignancy cannot be excluded with certainty when the pathologist cannot find malignant cells in the specimen (Fig 12). At this time, pancreatic biopsy has no role in the evaluation of a patient at good risk who is an operative candidate with a clinically resectable pancreatic mass. A positive biopsy result for cancer would lead to the recommendation for exploration, and a negative biopsy result would not preclude operative exploration and resection. As noted previously, there is a role for pancreatic biopsy (or biopsy of distant metastases in liver or subcutaneous lymph nodes) in patients at poor risk in whom a major pancreatic resection is not possible, because they may be candidates for palliative chemoradiation therapy. Additionally, some form of tissue diagnosis to document adenocarcinoma is mandatory in patients who are undergoing consideration for preoperative neoadjuvant protocols. Further, biopsy may be considered in patients whose clinical presentation and imaging study results are not suggestive of pancreatic

adenocarcinoma, but rather of more uncommon entities such as pancreatic lymphoma, pancreatic islet cell tumor, etc. In these situations, the diagnosis of lymphoma would preclude surgical exploration and allow for treatment via chemotherapy protocols, and a diagnosis of islet cell carcinoma might warrant testing for humoral mediators and aggressive surgical therapy for tumor debulking.

Laparoscopy

The role of diagnostic staging laparoscopy in patients with pancreatic cancer remains controversial.[157] Proponents believe that laparoscopy can identify a substantial number of unresectable patients (with advanced disease) and therefore recommend that it be applied to all patients. Opponents believe that the costs of such a practice (money, patient risk, resources) outweigh the benefit derived by the small number of patients for whom diagnostic laparoscopy is useful. The rationale for the use of laparoscopy comes from data that indicate that between 20% and 40% of patients staged by other modalities (CT, MRI, EUS) will be determined to have unanticipated peritoneal or small liver metastases by laparoscopy. However, part of the rationale for using laparoscopy involves a presumed equivalence of nonoperative palliation with operative palliation in patients with pancreatic cancer. Thus routine laparoscopic staging only makes sense if the percentage of patients discovered to have disseminated or unresectable disease remains high (20% to 40%) in the era of current multidetector CT, and if patients who are deemed to be unresectable and spared laparotomy can be optimally palliated without operation. Diagnostic staging laparoscopy can be performed with minimal morbidity and mortality on an outpatient basis, by use of a 30-degree angled laparoscope and evaluating the entire peritoneal surface, the pericolic gutters, the hemidiaphragms, the pelvis, as well as the surfaces of the liver. Specimens of peritoneal or omental nodules or liver implants undergo biopsy under direct vision. Enlarged lymph nodes can also be sampled successfully via biopsy or by fine-needle aspiration. There are varying degrees of expertise in the application of laparoscopy, with some highly experienced groups performing a more extensive laparoscopic evaluation including examination of the hilus of the liver and mesenteric and celiac vessels, and adding the use of laparoscopic ultrasonography for the detection of nonsurface hepatic metastases, vascular invasion, or deep-seated lymphadenopathy.[158–163]

Patients diagnosed with obstructive jaundice caused by tumors in the head of the pancreas typically have only a 15% to 20% incidence of unexpected intraperitoneal metastases after modern staging studies. In

contrast, patients with cancer of the body and tail of the pancreas have unexpected peritoneal metastases in up to 50% of patients. On the basis of these data, staging laparoscopy appears to be best supported for patients with cancer of the body or tail of the pancreas. In these patients the primary tumor does not typically cause biliary or gastric outlet obstruction, and therefore patients do not routinely require palliation of biliary or gastric obstruction. Thus in this group of patients laparoscopy can spare the patient an unnecessary laparotomy because there is little role for operative palliation. However, the role of routine preoperative staging laparoscopy is controversial in patients with localized right-sided tumor by modern imaging, who present with obstructive jaundice, symptoms of gastric outlet obstruction, and tumor-related abdominal and back pain. Many surgeons believe that such patients are best managed via resection if possible, or operative palliation to include biliary-enteric bypass, gastrojejunostomy and alcohol celiac nerve block. Preoperative staging laparoscopy would serve no purpose in such a setting.

A recent report by Barriero et al[157] underscores this practice. A retrospective review of 188 patients with pancreatic or periampullary malignancy who underwent both high-quality CT and laparotomy over a 3-year period was performed. The overall respectability rate for all periampullary cancers was 67%, compared with only 18% for left-sided pancreatic cancers. After patients undergoing operative palliation were excluded, a nontherapeutic laparotomy would have been avoided by the use of diagnostic laparoscopy in only 2% of patients with periampullary tumors. In contrast, for left-sided pancreatic tumors, 53% of patients would have benefited from laparoscopy, and 35% of patients could have avoided an unnecessary laparotomy.

Palliative Intervention

Nonoperative Palliation

Nonoperative management is appropriate in patients with pancreatic cancer who are determined to have distant metastases, unresectable local disease, or disseminated intraabdominal tumor, or in patients with acute or chronic debilitating diseases that make anesthesia and surgery prohibitive. The exception to these indications for nonoperative management are those patients with symptomatic upper gastrointestinal obstruction (from tumors that obstruct the duodenal C loop or the ligament of Treitz), where nonoperative palliation may not be reliable, and where gastrojejunostomy may be appropriate. In patients who are to be managed without operation, a tissue diagnosis can be obtained via biopsy of distant metastases or local disease. As noted previously, preoperative biopsy is avoided in patients with localized, apparently resectable tumors, because the pathologic information gained by such an invasive procedure does not influence the decision to explore the patient.

Biliary Obstruction

Jaundice is present in most patients with pancreatic cancer. If untreated, obstructive jaundice can result in progressive liver dysfunction, liver failure, and early death. Furthermore, the pruritus associated with obstructive jaundice can be symptomatically debilitating and rarely responds to medications. Biliary decompression can now be achieved either by endoscopic or percutaneous transhepatic techniques in nearly all patients who are not candidates for surgical intervention.

The technique of endoscopic biliary stent insertion for palliation of malignant obstructive jaundice was first described in 1980.[164] The method and equipment are now well standardized, and a technical success rate exceeding 90% should be expected from those performing such endoscopic stenting regularly. Typically, after the endoscopic visual inspection, deep biliary cannulation is attempted with or without the assistance of a guide wire or sphincterotome. Occasionally, precut sphincterotomy with a needle knife may be necessary. Once deep biliary cannulation has been accomplished, a guide wire is manipulated above the malignant stricture (Fig 13) and a 7F or 10F plastic endoprosthesis is secured in position by being pushed over the guide wire (Fig 14). After stent placement, serial liver function tests are performed to confirm a

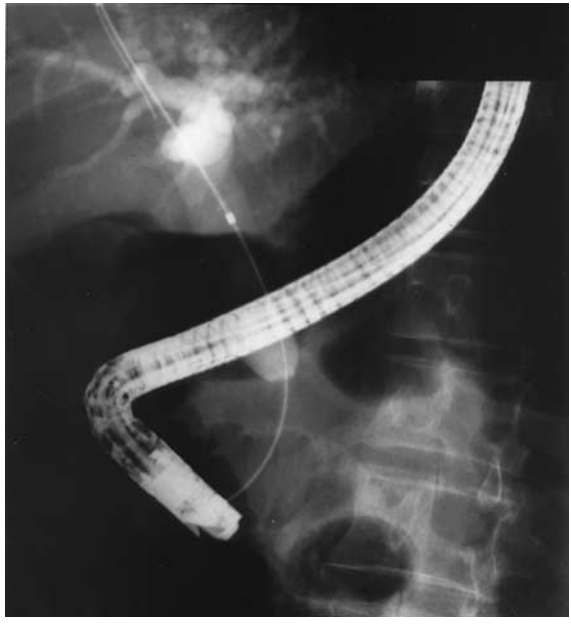

FIG 13. Image obtained at ERCP, with guide wire passed cephalad through distal common bile duct obstruction caused by cancer in head of pancreas. (From Yeo CJ, Cameron JL. Pancreatic Cancer. Curr Prob Surg 1999;36:57–152, Figure 26).

decline in the serum bilirubin. Complications of endoprosthesis placement can be categorized as early or late complications. Early complications include cholangitis, pancreatitis, bleeding, and bile duct or duodenal perforation. Late complications include stent occlusion, cholecystitis, delayed duodenal perforation and stent migration. The major late complication remains stent occlusion, which may occur anywhere from days to many months after placement. The occurrence of plastic stent occlusion, with its principal complication of cholangitis and recurrent jaundice, has led most endoscopists to favor planned stent removal and replacement. Although the optimal time interval for stent exchange has not been determined, in practice this has typically been scheduled at 3- to 6-month intervals. A report concerning stents 10F to 11.5F in size noted occlusion rates of 4.2% and 10.8% at 3 and 6 months, respectively, suggesting that an exchange interval of up to 6 months may be appropriate.[165] Metallic expandable endoprostheses have been developed by a number of manufacturers, all first deployed through the percutaneous transhepatic route, but with recent modifications to allow endoscopic placement. Once fully deployed, metallic endoprostheses become embedded in the wall of the

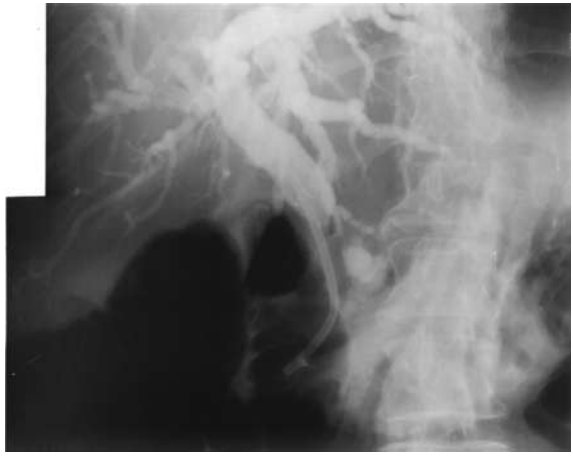

FIG 14. Image obtained immediately after successful ERCP. A 10F plastic endoprosthesis has been placed across a malignant distal bile duct obstruction. A markedly dilated intrahepatic biliary tree is visualized. (From Yeo CJ, Cameron JL. Pancreatic Cancer. Curr Prob Surg 1999;36:57–152, Figure 27).

bile duct and should be considered permanent (although they can be removed at surgery), effectively eliminating the problem of stent migration, and reducing the incidence of stent occlusion. However, tumor ingrowth remains a problem with metallic endoprostheses, causing late stent occlusion. The typical remedy for occlusion of a metallic stent is the insertion of a second metallic or plastic endoprosthesis through the existing lumen of the occluded stent, to allow adequate biliary drainage.

The use of a percutaneous transhepatic biliary drainage technique was first reported in 1974 as an extension of diagnostic transhepatic cholangiography, allowing bile diversion into the duodenum in patients with biliary obstruction.[166] The techniques of transhepatic cholangiography and percutaneous biliary drainage are now well standardized and widely disseminated.[167] Diagnostic cholangiography first defines the site of bile duct obstruction (Fig 15) and serves as a road map for biliary drainage. In most cases, biliary drainage with an internal-external catheter serves as the initial management, with passage of the drainage catheter through the obstruction into the duodenum possible in more than 90% of patients. Initial stiff drainage catheters can subsequently be exchanged for larger diameter, softer catheters. Subsequent management involves maintenance of internal-external drainage catheters (Fig 16), or the percutaneous placement of a totally indwelling endoprosthesis. Overall, percutaneous transhepatic biliary drainage can be successfully performed in approxi-

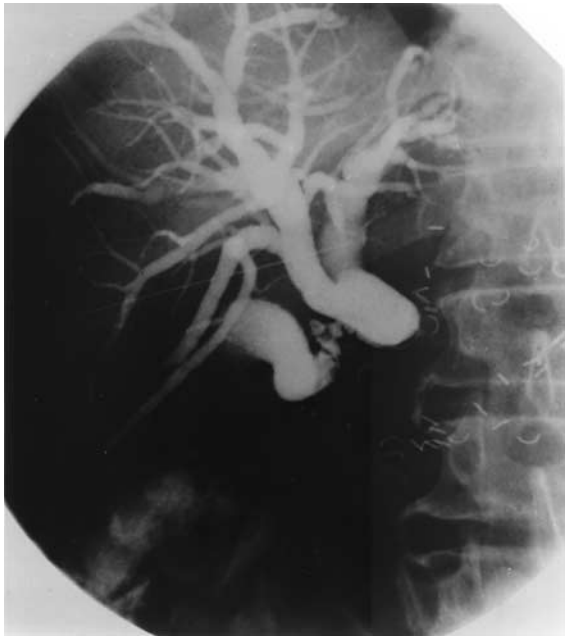

FIG 15. Percutaneous transhepatic cholangiogram in patient with obstructive jaundice. There is a malignant obstruction of the biliary tree at the level of the junction of the common hepatic duct and cystic duct. Staples are visible from a remote laparotomy for peptic ulcer disease. (From Yeo CJ, Cameron JL. Pancreatic Cancer. Curr Prob Surg 1999;36:57–152, Figure 28).

mately 95% of patients diagnosed with biliary obstruction. Complications of percutaneous transhepatic catheter drainage include stent occlusion, hemobilia related to the transhepatic route, bile peritonitis, bile pleural effusion, cholangitis, pancreatitis and acute cholecystitis.[167]

The available data support the use of an endoscopic method as the primary method for nonoperative palliation of jaundice in patients with pancreatic cancer (Table 11). A prospective randomized trial,[168] and numerous other comparisons reviewed by Watanapa and Williamson[169] have shown a comparable success rate for the endoscopic approach, generally associated with a lower degree of procedure-related death and morbidity.[170]

Pain

Tumor-associated pain can be an incapacitating symptom of pancreatic cancer. Unfortunately for many patients such pain is poorly managed, and it can remain a significant problem up until their demise. There are many

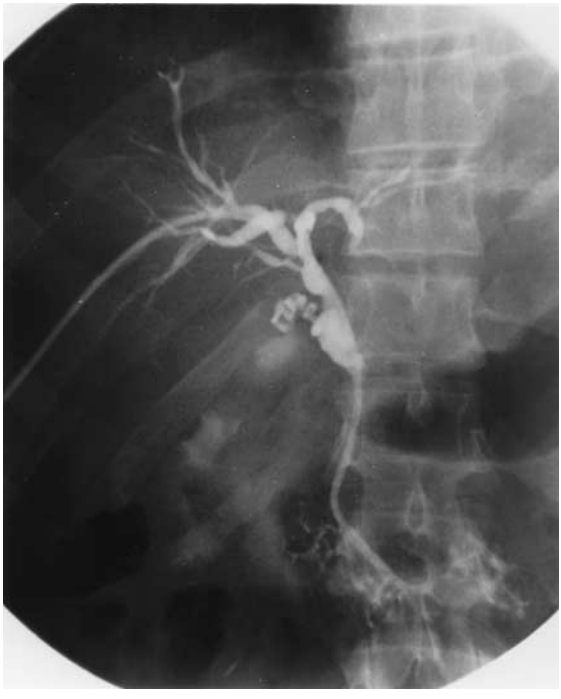

FIG 16. Cholangiogram obtained after placement of internal-external percutaneous transhepatic biliary drainage catheter. The catheter transverses the obstruction in the head of the pancreas. The tip of the catheter resides in the duodenum, distal to the ampulla. (From Yeo CJ, Cameron JL. Pancreatic Cancer. Curr Prob Surg 1999;36:57–152, Figure 29).

TABLE 11. Results of percutaneous and endoscopic stent placement in patients with malignant bile duct obstruction

	Percutaneous stent (n = 490)		Endoscopic stent (n = 689)	
	Range	**Mean**	**Range**	**Mean**
30-day mortality rate (%)	6–33	9	37518	14
Hospital stay (days)	37638	14	37340	7
Success rate (%)	76–100	92	82–100	90
Early complication (%)	4–67	16	8–34	21
Late complication (%)	7–38	28	13–45	28

From Watanapa P, Williamson RCN. Br J Surg 1992;79:8–20, p. 14, Table 5.

postulated causes of tumor-associated pain (tumor infiltration into the retroperitoneal celiac plexus, pain associated with early satiety, gastroduodenal obstruction, gallbladder distention secondary to biliary obstruc-

tion, increased parenchymal pressure secondary to pancreatic ductal obstruction and superimposed pancreatic inflammation). In general, pain is not relieved by endoscopic or percutaneous biliary decompression. Analgesic therapy is guided by the Three Step Analgesic Ladder of the World Health Association. Tumor-associated pain is best treated with long-acting oral analgesics in appropriate doses, with the most common drug used being long-acting morphine sulfate.[171] In patients who cannot take oral medications, topical analgesics (fentanyl patches) worn as continuous-release cutaneous patches can be highly effective. Poorly controlled pain is often the result of inadequate analgesic dosing and may require the expertise of pain management specialists. Several nonoperative treatment modalities may be considered to manage intractable pain that does not respond to oral or topical pain medication. The first modality involves percutaneous or endoscopic celiac nerve block. The second modality uses external beam radiation therapy directed to the primary tumor and celiac plexus. In our experience, most patients can be managed without resorting to nerve blocks or radiation therapy for pain.

Duodenal Obstruction

Until recently there was little option besides surgical bypass (gastrojejunostomy) or feeding tubes for patients with malignant obstruction of the duodenum caused by pancreatic cancer. The recent modification and application of available biliary-type metallic stents with their refined delivery systems have allowed alternative endoluminal approaches. Although thus far these endoluminal approaches have been reported in small numbers, the basic premise is to place metallic endoprostheses within the native duodenum at the site of tumor infiltration or at the site of an obstructed gastrojejunostomy.[165] Careful assessment of this promising approach is required to establish its proper place in the management of malignant duodenal obstruction.

Operative Palliation

Palliative surgery for pancreatic cancer is appropriate in patients with unresectable disease discovered at the time of potentially curative laparotomy, or in patients at good risk whose tumor-related symptoms are poorly alleviated via nonoperative means. Palliative surgery is designed to relieve biliary obstruction, avoid or treat duodenal obstruction, palliate tumor-associated pain, and improve quality of life.

The surgical options for palliation of obstructive jaundice all include some form of an internal biliary bypass. The 3 most common techniques currently used include choledochoduodenostomy, cholecystojejunos-

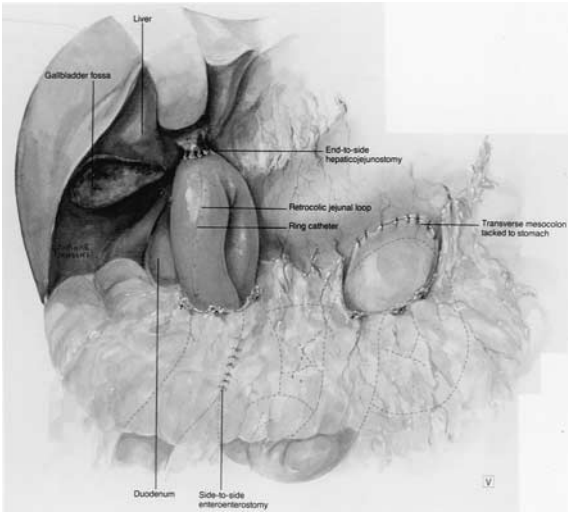

FIG 17. Illustration depicts anatomy after one method of palliative intervention. The biliary-enteric anastomosis is shown as a retrocolic end-to-side hepaticojejunostomy with a jejunal loop. A jejunojejunostomy is performed below the transverse mesocolon, to divert the enteric stream away from the biliary-enteric anastomosis. Also shown is a retrocolic gastrojejunostomy. (From Cameron JL, Atlas of Surgery, Volume 1, B.C. Decker, Toronto, 1990, p 427, Image V).

tomy, or hepatico- (choledocho) jejunostomy. Although choledochoduodenostomy provides effective relief of obstructive jaundice in a number of benign conditions, it has generally been avoided in patients with pancreatic cancer because of concerns regarding the proximity of the anastomosis to the tumor, with the possibility of recurrent jaundice. Although cholecystojejunostomy has been advocated by some surgeons because it can be performed quickly (and can be done laparoscopically) and does not require dissection of the extrahepatic tree, the data do not support its use. Rather, the preferred technique is choledocho- (or hepatico-) jejunostomy, with the gallbladder being removed before mobilization of the biliary tree (Fig 17). A number of retrospective reviews have compared both the short-term and long-term results after cholecystojejunostomy or hepaticojejunostomy for palliation of obstructive jaundice. In a classic review by Sarr and Cameron,[172] although operative mortality and long-term survival rates were similar, the incidence of recurrent jaundice was 0 after hepaticojejunostomy, compared with 8% in patients undergoing cholecystojejunostomy. A metaanalysis[169] found that cholecystojejunostomy carried an 89% success rate for alleviating jaundice, compared with a 97% success rate with choledochojejunostomy.

At the time of diagnosis of pancreatic cancer, approximately one third of patients will have symptoms of nausea and vomiting. Although true mechanical obstruction of the duodenum occurs much less frequently, as the malignant disease progresses, duodenal obstruction may occur in a number of patients. Obstruction can occur at either the duodenal C loop by cancer in the head of the pancreas, or at the ligament of Treitz by a cancer of the body of the pancreas. Over the years, information has accrued regarding the natural history of duodenal obstruction associated with pancreatic cancer. Sarr and Cameron[172] reviewed more than 8000 surgically managed patients and found that 13% of patients who did not undergo gastrojejunostomy at their initial operation required a gastrojejunostomy before their death, and an additional 20% of patients died with symptoms of duodenal obstruction. A review by Singh and Reber[173] found that 21% of patients required a gastrojejunostomy late in the course of their disease. Additionally, an analysis of more than 1600 cases found that 17% of patients who underwent biliary bypass alone had development of duodenal obstruction at a mean of 8.6 months after operation and required subsequent gastric bypass.[169]

To date, only one prospective, randomized trial by Lillemoe et al[174] has evaluated the role of prophylactic gastrojejunostomy in patients found at laparotomy to have unresectable disease. Eighty-seven patients without evidence of preoperative duodenal obstruction or intraoperative tumor encroachment around the duodenum were randomized to receive either a prophylactic retrocolic gastrojejunostomy (n = 44) or no gastrojejunostomy (n = 43). The postoperative mortality rate (0%), morbidity rate (32% to 33%) and length of hospital stay (8 days) were similar, as were the mean survival (8 months). However, 8 of 43 patients (19%) without gastrojejunostomy developed late gastric outlet obstruction requiring intervention (gastrojejunostomy in 7, duodenal stent in 1), whereas no patient in the prophylactic gastrojejunostomy group required such intervention ($P < .01$). On the basis of these data and the results of previous retrospective analyses, we recommend the performance of a retrocolic gastrojejunostomy in patients who are found at laparotomy to have unresectable right-sided pancreatic cancer.

We typically perform a retrocolic, isoperistaltic loop gastrojejunostomy, using the jejunum 20 to 30 cm beyond the ligament of Treitz, and placing the horizontal gastrotomy somewhat posterior, in the most dependent portion of the gastric greater curvature (Fig 17). Using this technique, the incidence of early delayed emptying appears to be low and hospital discharge is not delayed.[174] Importantly, vagotomy is not performed for the palliation of pancreatic cancer, because it may further contribute to

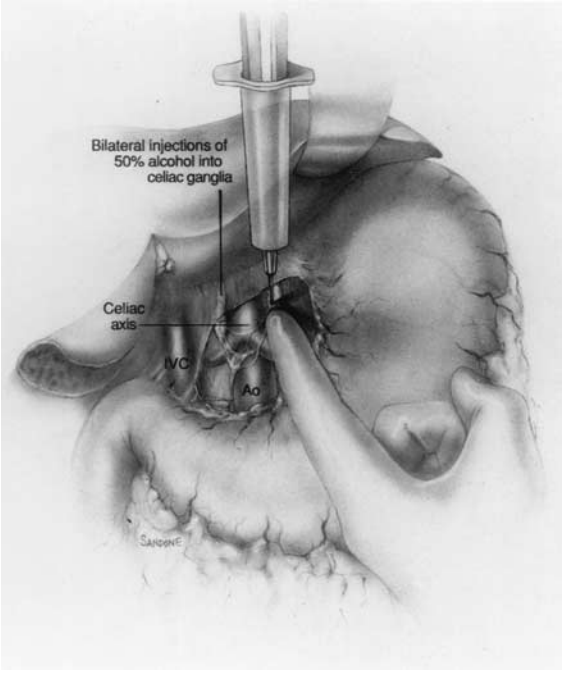

FIG 18. Technique of alcohol celiac nerve block. Twenty milliliters of 50% alcohol are injected on each side of the aorta *(Ao)* at the level of the celiac axis. *IVC,* Inferior vena cava. (From Lillemoe KD et al. Ann Surg 1993;217:447–57).

delayed gastric emptying. Instead, routine gastric acid secretory inhibition agents are used to prevent marginal ulceration, such as histamine H_2-receptor antagonists or proton pump inhibitors.

The abdominal and back pain associated with an unresectable pancreatic cancer can be the major debilitating symptom for the patient. At the time of palliative surgery, this symptom can be addressed by intraoperative chemical splanchnicectomy. The use of intraoperative chemical splanchnicectomy for this indication was first introduced by Copping et al[175] in 1969 and was reported in 41 patients in 1978. In this uncontrolled study, 88% of patients with pain caused by pancreatic cancer were reported to experience relief of pain after chemical splanchnicectomy. Only one prospective, randomized placebo-controlled study of intraoperative chemical splanchnicectomy has been reported. In this study at Johns Hopkins, chemical splanchnicectomy was performed by injection of either 20 mL of 50% alcohol or a saline solution placebo on either side of the aorta at the level of the celiac axis (Fig 18).[176] The data clearly

TABLE 12. The Johns Hopkins experience with surgical palliation (December 1991–December 1997; n = 256 patients)

Age	64 years
Sex	57% male
Presenting symptoms	
Abdominal pain	64%
Jaundice	57%
Procedures	
Chemical splanchnicectomy	75%
Biliary and gastric bypass	51%
Gastric bypass	19%
Operative time	3.9 hours
Transfusions (mean)	0
Operative mortality rate	3.1%
Overall morbidity rate	22%
Postoperative length of stay	10 days
Median survival	6.5 months
1-year survival rate	25%
2-year survival rate	9%

From Sohn TA, et al. J Am Coll Surg 1999;188:658–69.

indicated that mean pain scores (as defined by a visual analog scale) were significantly lower in the patients who received alcohol block at all postoperative time points, as compared with the placebo group. These data support the routine performance of intraoperative chemical splanchnicectomy with alcohol in all patients undergoing operative palliation for unresectable pancreatic carcinoma.

A recent review describes the Johns Hopkins experience with surgical palliation of unresectable pancreatic and periampullary adenocarcinoma.[177] Over a 6-year period, 256 patients underwent operative palliation (Table 12). Sixty-eight percent of patients were unresectable because of liver or peritoneal metastases, and 32% were unresectable because of local vascular invasion. The most common operative procedures were chemical splanchnicectomy (75%), biliary and gastric bypass (51%), and gastric bypass alone (19%). The postoperative mortality rate was 3.1%, the complication rate was 22%, and the length of hospital stay was 10 days. Median survival was 6.5 months, with 1- and 2-year survival rates of 25% and 9%, respectively.

Resectional Therapy

Pancreaticoduodenectomy for Tumors of the Head, Neck, or Uncinate Process

In 1912 Kausch, a German surgeon from Berlin, reported the first successful resection of the duodenum and a portion of the pancreas for an ampullary cancer.[178] More than 2 decades later, Whipple et al[179] in New York City reported 3 cases of pancreaticoduodenal resection. Although the early reports of Kausch and Whipple describe pancreaticoduodenal resections that spared the pylorus and retained the entire stomach, by the mid 1970s pancreaticoduodenectomy was most commonly performed in combination with a distal gastrectomy. In 1978 Traverso and Longmire[180] repopularized the concept of pylorus preservation during pancreaticoduodenectomy. Several reports have reviewed large experiences with pylorus preservation, and this operation is the most typical variant of pancreaticoduodenectomy performed currently (Fig 19). Pylorus preservation is favored because it preserves the entire gastric reservoir, maintains the pyloric sphincter mechanism, somewhat shortens the operative time, appears to be associated with no consistent adverse sequelae, and no long-term decrement in quality of life. Although there have been some who have cautioned that pylorus preservation may compromise anticancer therapy, this has not been supported by our data.[181–184] In approximately 85% of our patients, the pylorus can be successfully preserved, with the 2 most common reasons for sacrificing the pylorus and performing a distal gastrectomy being (1) ischemia of the duodenal cuff after resection (related to devascularization of the duodenal cuff by sacrifice of branches of the right gastric artery or an incomplete right gastroepiploic arcade) or (2) intraoperative findings of tumor involvement of the first portion of the duodenum, pylorus, or distal stomach.

Operative Technique

In patients undergoing exploration for potential pancreaticoduodenectomy, the initial portion of the operative procedure is dedicated to the assessment of resectability. This involves assessing the liver for metastases not seen by preoperative imaging studies, evaluating the parietal and visceral peritoneal surfaces thoroughly, assessing at the level of the celiac axis for enlarged lymph nodes, and carefully examining the omentum, the ligament of Treitz and the entire jejunoileum for tumor involvement. An

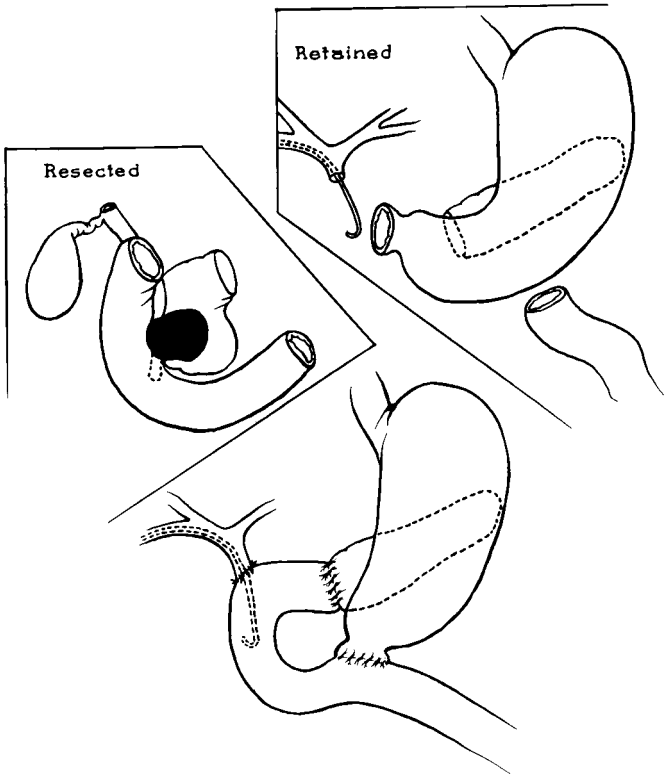

FIG 19. Pylorus-preserving pancreaticoduodenectomy. **Top left:** The structures resected include the duodenum (except for the initial 1 to 2 cm beyond the pylorus); head, neck and uncinate process of the pancreas, with tumor *(black)*; gallbladder; and distal extrahepatic biliary tree. **Top right:** The structures retained include the entire stomach, pylorus, proximal 1 to 2 cm of duodenum, body and tail of the pancreas, proximal biliary tree, and jejunum distal to the ligament of Treitz. **Bottom:** The reconstruction is shown as a proximal end-to-end pancreaticojejunostomy, hepaticojejunostomy decompressed via a percutaneous transhepatic catheter and a distal duodenojejunostomy. (From Yeo CJ, Cameron JL. The pancreas. In: Hardy JD, editor. Hardy's textbook of surgery. 2nd ed. Philadelphia: JB Lippincott; 1988. p 718, Fig 28-9).

extensive Kocher maneuver is performed, elevating the duodenum out of the retroperitoneum, assessing the superior mesenteric vein and its branches, and palpating the superior mesenteric artery pulse in its retropancreatic position. The porta hepatis is carefully assessed by mobilizing the gallbladder out of the gallbladder fossa, and following the cystic duct down to its junction with the common hepatic duct. In favorable cases, the intraoperative assessment will determine that the tumor is localized only to the area of the head, neck or uncinate process

of the pancreas, with no evidence of tumor involvement outside of the resection zone.

Several maneuvers can speed the performance of a pancreaticoduodenectomy. Early division of the extrahepatic biliary tree allows caudal retraction of the distal common bile duct and opens the plane to visualize the anterior portion of the portal vein. The superior mesenteric vein is most easily identified during the performance of an extensive Kocher maneuver, where it is identified running anterior to the third portion of the duodenum, frequently surrounded by adipose tissue, and receiving tributaries from both the uncinate process and from the transverse mesocolon.[185] The division of the proximal gastrointestinal tract is typically performed approximately 2 cm distal to the pylorus with a linear stapling device. In similar fashion, the jejunum 10 to 15 cm beyond the ligament of Treitz is cleared circumferentially and divided with a linear stapling device. Subsequently the proximal jejunum and distal duodenum can be delivered dorsal to the superior mesenteric vessels from the left to the right side, allowing easier dissection of the uncinate process off of the right lateral aspect of the superior mesenteric vein. Further steps in pancreaticoduodenal resection involve the division of the pancreatic neck and the final cautious dissection of the head and uncinate process from the superior mesenteric vein, portal vein and superior mesenteric artery. A more complete description of the details of pancreaticoduodenal resection is available from numerous sources.[181,186,187]

Multiple options exist for the reconstruction of the pancreas, bile duct and gastrointestinal tract. Most commonly, the reconstructive technique anastomoses the pancreas first, followed by the bile duct and the duodenum (Fig 19). The pancreatic-enteric anastomosis is typically performed as a pancreaticojejunostomy, either in an end-to-end or end-to-side fashion. Controversy continues regarding the optimal configuration of the pancreaticojejunostomy, the importance of duct-to-mucosal sutures, and the use of pancreatic duct stents. An alternative for pancreatic-enteric reconstruction involves the use of a pancreaticogastrostomy.[188–190] The biliary-enteric anastomosis is typically performed in end-to-side fashion approximately 10 cm down the jejunal limb from the pancreatic-enteric anastomosis. The third anastomosis is the duodenojejunostomy, typically performed 10 to 15 cm downstream from the biliary-enteric anastomosis.

Complications

The operative mortality rate for pancreaticoduodenectomy is currently less than 3% in major surgical centers with significant experience with the procedure.[181,182,191,192] Leading causes of postoperative in-hospital death

TABLE 13. Complications after pancreaticoduodenectomy

Common
Delayed gastric emptying
Pancreatic fistula
Intraabdominal abscess
Hemorrhage
Wound infection
Metabolic
Diabetes
Pancreatic exocrine insufficiency
Uncommon
Fistula
Biliary
Duodenal
Gastric
Organ failure
Heart
Liver
Lung
Kidney
Pancreatitis
Marginal ulceration

From Yeo CJ. Surg Clin North Am 1995;75:913–24.

include postoperative sepsis, hemorrhage, and cardiovascular events. In contrast to the low mortality rate, the incidence of postoperative complications can approach 40% to 50%.[192–194] The leading causes of morbidity include early delayed gastric emptying, disruption or failure of healing of the pancreatic anastomosis (pancreatic fistula), intraabdominal abscess, hemorrhage and others (Table 13). These complications may have minimal impact on postoperative length of hospital stay or they may prolong hospitalization and require either interventional radiologic techniques[194] or reoperation.

Controversies

Several controversial areas pertaining to the technique and performance of pancreaticoduodenectomy exist.[195] These include (1) classic pancreaticoduodenectomy versus pylorus-preserving pancreaticoduodenectomy, (2) extent of pancreatic resection: partial pancreatectomy versus total pancreatectomy; (3) extent of peripancreatic and nodal resection: standard pancreaticoduodenectomy versus extended (or radical) pancreaticoduodenectomy. As stated above, pylorus-preserving pancreaticoduodenectomy does not appear to be associated with a consistent increased rate of adverse sequelae, and has equivalent survival and quality of life as compared to classic pancreaticoduodenectomy. Additionally, gastric acid

secretion and hormone release are more normal in patients who undergo pylorus preservation. On the basis of these data, pylorus preservation is favored in most patients who undergo pancreaticoduodenectomy.

The controversy regarding the use of total pancreatectomy as a treatment for patients with cancer of the head of the pancreas has diminished in recent years. Current practice avoids total pancreatectomy and favors the performance of a partial resection. By avoiding total pancreatectomy, one avoids the obligate requirements for exogenous pancreatic enzyme supplements, avoids the inevitable generation of insulin-dependent diabetes mellitus, reduces the potential for increased intraoperative blood loss, and avoids splenectomy and the loss of splenic function. Total pancreatectomy is reserved for cases where the pancreatic cancer extends across the neck and body of the gland, or when the pancreatic remnant is too soft and friable to allow a safe pancreatic-enteric anastomosis.

Numerous retrospective reports and a few prospective randomized trials have suggested that extended (radical) pancreaticoduodenectomy may improve survival rates in patients with pancreatic cancer.[196–198] However, a recently completed study at Johns Hopkins failed to reveal a survival advantage for one type of extended resection.[181,183] In this prospective, randomized single-institution trial, 294 patients with periampullary adenocarcinoma were analyzed, after having been allocated to standard pylorus-preserving pancreaticoduodenectomy or extended pancreaticoduodenectomy (to include distal gastrectomy and retroperitoneal lymphadenectomy). Although the mortality rates between the two groups were similar (4% standard vs 2% extended; P = NS), there were significantly more complications in the radical group (29% standard vs 43% radical; $P < .01$). For the patients with pancreatic adenocarcinoma (n = 163), there were no differences in either median, 1-year, 3-year, or 5-year survival rates when comparing between the standard and radical groups (median survival 20 to 21 months; 1-year survival rate, 75%; 3-year survival rate, 37%; 5-year survival rate, 17%). From this, the largest prospective randomized clinical trial of standard versus extended resection, there appears to be no survival benefit derived from the addition of distal gastrectomy and retroperitoneal lymphadenectomy to a pylorus-preserving pancreaticoduodenectomy.[183]

Distal Pancreatectomy for Tumors of the Body and Tail

A minority of patients with pancreatic cancer have tumors arising in the body and tail of the gland. Such primary tumors in the left side of the

pancreas do not obstruct the bile duct and thus do not present with early jaundice. Typically, diagnosis of these tumors is delayed. Tumors of the body and tail are often larger than tumors of the head and are associated with a much higher incidence of metastatic disease. As a result, the likelihood that curative resection will be possible is lower for left-sided, as compared with right-sided primaries. However, if the diagnosis is made when the tumor is localized and not encasing the celiac axis, the superior mesenteric vessels, or the portal vein, then resection remains a surgical option. Importantly, involvement of either the splenic artery or vein does not alone render the patient unresectable, because the entirety of these vessels can be resected en bloc with the tumor. As has been mentioned previously, in addition to routine staging studies to include abdominal CT or MRI, there appears to be an important role for staging laparoscopy in patients with left-sided tumors. Should staging studies fail to reveal evidence of disseminated tumor or unresectable local disease, then exploration is appropriate.

The entire abdomen is explored to search for metastatic disease. A careful search of the liver for metastatic deposits is undertaken, as well as a thorough evaluation of all the peritoneal surfaces. The lesser omentum is opened to allow assessment of the celiac axis and periaortic region. The ligament of Treitz is carefully evaluated, because tumors in the body of the pancreas may invade the fourth portion of the duodenum at the ligament. Additionally, the gastrocolic ligament should be opened to allow assessment of the body and tail of the pancreas and better assessment of the tumor's proximity to the ligament of Treitz and to the superior mesenteric vessels.

Localized tumors without extensive vascular or retroperitoneal involvement are appropriate for resection. Involvement of the splenic artery and vein does not indicate unresectability. Splenic preservation is not indicated when the resection is being performed for pancreatic adenocarcinoma, therefore the spleen is mobilized out of the retroperitoneum, often with early ligation of the splenic artery. The short gastric vessels along the gastric greater curvature require division, as do the vessels within the splenocolic ligament. Mobilization of the spleen from the retroperitoneum facilitates dissection of the tail of the pancreas from the retroperitoneum (Fig 20).

The resectability rates for adenocarcinoma of the body and tail of the pancreas in the era before routine staging laparoscopy were approximately 10%. The routine use of staging laparoscopy to identify metastases not visualized by CT or MRI have improved the resectability rates. Overall patients undergoing left-sided pancreatic resection have median

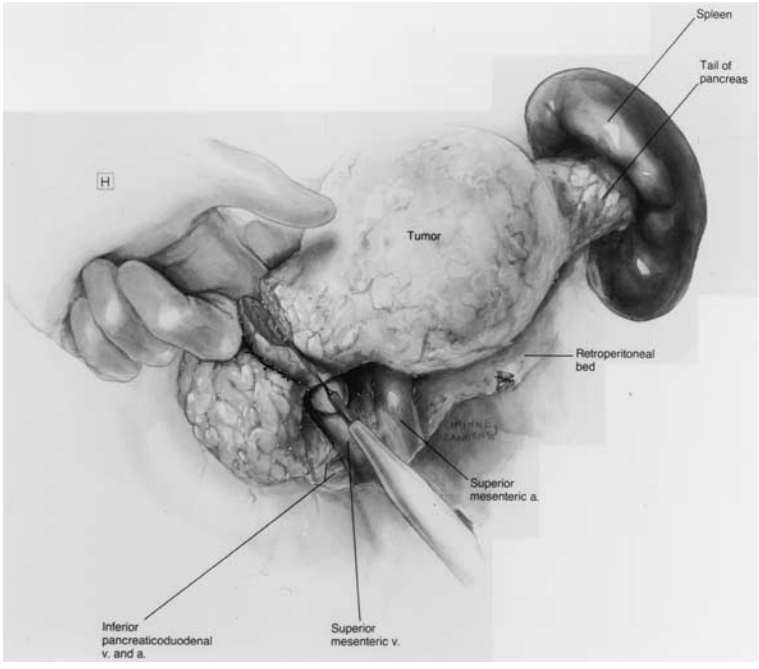

FIG 20. Illustration near completion of distal pancreatectomy and splenectomy for large tumor in body of pancreas. The spleen and tail of the pancreas have been mobilized out of the retroperitoneum. The pancreatic parenchyma is being divided by use of electrocautery. (From Cameron JL. Atlas of surgery. Vol 1. Toronto: BC Decker; 1990. p. 435, Image H).

TABLE 14. Right-sided versus left-sided pancreatic resection: recent Johns Hopkins Experience (1984–1999)

	Right-sided (Pancreatico-duodenectomy) (n = 564)	**Left-sided (Distal pancreatectomy) (n = 52)**	***P* value**
Tumor diameter	3.1 cm	4.7 cm	<.001
Positive resection margins	30%	20%	NS
Positive lymph node status (N1)	73%	59%	0.03
Postoperative mortality rate	2.3%	1.9%	NS
Overall complications	31%	25%	NS
Median postoperative length of hospital stay	11 days	7 days	NS
Survival			
1 year	64%	50%	NS
5 years	17%	15%	
Median	18 mo	12 mo	

From Sohn TA et al. J Gastrointestinal Surg 2000;4:567–79.

survival rates ranging from 7 to 14 months, with 5-year survival rates of approximately 15% or less.[182,199,200] A comparison between results for right-sided pancreatic resection (pancreaticoduodenectomy) and left-sided pancreatic resection (distal pancreatectomy) is shown in Table 14. In general, at the time of resection, left-sided tumors are larger, have a lesser degree of lymph node involvement, and are associated with a somewhat poorer outcome.

Chemotherapy and Radiation Therapy

For patients with right-sided pancreatic cancer presenting without contraindication on the basis of clinical staging or associated comorbidity, pancreaticoduodenectomy is undertaken with curative intent and is understood to be sine qua non for affecting cure in this clinical context. Nevertheless, for most patients undergoing this operation, cure does not result. Similarly, for patients with locally-regionally unresectable disease, therapeutic interventions aimed at palliating symptoms and temporarily prolonging life are needed. In both contexts, chemoradiotherapy is used on the basis of well-established principles of chemosensitization and results of limited trials. In this article we review recent trials, results, and evolving areas of study.

Combined Modality Therapy in the Adjuvant Setting

The current standard of 5-fluorouracil (5-FU)–based combined modality chemoradiotherapy has evolved from in vitro data, animal studies, and a series of human studies, most notable being those from the Gastrointestinal Tumor Study Group (GITSG). This study, with split-course irradiation used in modest doses with concurrent bolus 5-FU followed by maintenance 5-FU, demonstrated a survival advantage for the therapy in comparison to surgery alone.[201] Although criticized for slow and limited accrual, this study was the first to document that adjuvant therapy after surgical resection for pancreatic surgery prolonged survival. Additional studies by the GITSG demonstrated the benefit of combined chemoradiotherapy versus chemotherapy alone or radiation therapy alone for patients with local regionally-advanced unresectable disease.[202–204]

A number of groups have further developed this approach (Table 15). The Johns Hopkins group published results of 2 single-institution prospective but nonrandomized trials that were designed to evaluate survival benefit in patients with pancreatic cancer after surgical resection.[205,206] The first report, involving 174 patients, demonstrated that patients receiving GITSG-style chemoradiotherapy with maintenance 5-FU truncated at 6 months (rather than 2 years), or a more intensive regimen involving higher doses of irradiation, as well as hepatic irradiation administered without a split (planned interruption) and with 5-FU

TABLE 15. Recent adjuvant studies in pancreatic cancer

Study	Regimen	No. of patients	Median survival (mos)
UCLA	5FU, MMC, LV, dipyridamole	38	15.5
Johns Hopkins	Standard regimen	99	21
	Intensive regimen	21	17.5
	Observation	53	13.5
Johns Hopkins	50 Gy, 5FU, MMC, LV, dipyridamole	39	16
Stanford	54 Gy and 5FU CI	52	32
Virginia Mason	54 Gy and 5FU CI, cisplatin, IFN	33	45
EORTC	40 Gy in 2 modules and 5FU vs observation	218	24.5 vs 19
ESPAC-1	Chemotherapy vs observation	541	19.7 vs 14
	Chemoradiation vs observation		15.5 vs 16.1
RTOG-9704	5FU CI, 5FU/XRT (50 Gy), 5FU × 2 vs Gem, 5FU/XRT, Gem × 3	518	Study to close July 2002

5FU, 5-Fluorouracil; *LV*, leucovorin; *CI*, continuous infusion; *MMC*, mitomycin C; *IFN*, interferon-α; *Gy*, Gray; *EORTC*, European Organization for Research and Treatment of Cancer.

chemotherapy given as continuous infusion and augmented with leucovorin, did better than patients receiving no postsurgical therapy.[205] The median survival for the more standard regimen was 21 months, with 1- and 2-year survival rates of 80% and 44%. For the intensive regimen, the median survival was 17.5 months, with 1- and 2-year survival rates of 70% and 22%. For the control arm the median survival was 13.5 months, with survival rates at 1 and 2 years of 54% and 30%. The intensive therapy had no survival advantage when compared with the standard therapy group, but there was a statistically significant difference between the standard arm versus control ($P < .002$). Multivariate analysis confirmed that prognostic factors for disease recurrence included margin and lymph node status, tumor size, and degree of differentiation. This approach, showing the importance of multiple prognostic factors—in addition to adjuvant therapy—on postsurgical outcomes, has been further refined by Abrams et al.[206] The critical factors appear to be the histologic status of resection margins, lymph node involvement, especially with more than 3 lymph nodes involved, tumor size greater than 3 cm, and the presence of a poorly differentiated component within the tumor. With these factors, patients can be segregated into high-risk and low-risk groups, with median survival after standard adjuvant therapy being 30.5 months for patients at low risk and 14.0 months for patients at high risk.

Attempting to enhance the activity of chemotherapy in pancreatic cancer, other agents have been examined in combination with 5-FU. Mitomycin-C (MMC) is an antitumor antibiotic with activity in several

gastrointestinal cancers, including pancreatic cancer. The UCLA group has published their experience with MMC (10 mg/m^2 administered intravenously every 6 weeks) and 5-FU (200 mg/m^2/d administered via continuous infusion), in combination with leukovorin (30 mg/m^2 weekly)and dipyridamole (75 mg orally daily) in 38 patients with locally advanced pancreatic carcinoma.[207] There were noted to be 14 partial responders with 1 complete response. The median survival for all patients was 15.5 months, which is an improvement over historical data for local-regionally advanced disease. This regimen has subsequently been applied to pancreatic cancer in combination with radiotherapy.[208] The Johns Hopkins group has recently presented data on 39 patients with resected pancreatic cancer treated with combined radiotherapy (50 Gy in 25 fractions with a planned 2-week break after 25 Gy) and chemotherapy consisting of 5-FU 400 mg/m^2 D1-3, MMC 10 mg/m^2 D1, leukovorin 20 mg/m^2 D1-3 and dipyridamole 75 mg administered orally 4 times daily D0-4, administered on weeks 1 and 4. One month after combined chemoradiotherapy, patients received 4 additional cycles (4 months) of the same chemotherapy alone. At 12.6 months median follow-up, median survival was 16 months.[208]

The Stanford group has recently published their experience in 52 patients with resected pancreatic cancer, using combined radiotherapy (45 Gy to tumor bed and nodes in 1.8 Gy fractions with boost to total of 54 Gy if surgical margins were positive) and chemotherapy (5-FU 200-250 mg/m^2/d administered without break throughout radiation therapy). All patients were able to complete therapy without grade IV toxicities. With median follow-up of 24 months, the median survival was reported to be 32 months.[209]

Recently the Virginia Mason Clinic published their experience in 33 patients with resected pancreatic adenocarcinoma who received combined radiotherapy (external beam at a dose of 4500 to 5400 cGy in standard fractions d1-35) and chemotherapy (5-FU 200 mg/m^2/d as continuous infusion, weekly cisplatin 30 mg/m^2 intravenous bolus, and interferon-α 3 million units administered subcutaneously every other day) during radiation.[210] After combined modality chemoradiotherapy, chemotherapy alone was administered (5-FU 200 mg/m^2/d as continuous infusion) in two 6-week courses during weeks 9 to 14 and 17 to 22. There were significant grade III/IV gastrointestinal toxicities, including vomiting, mucositis, diarrhea, and gastrointestinal bleeding. With a median follow-up of 26 months, the 2-year survival rate was 84%, with a median survival of 45 months.[210] These encouraging data await further confirmation.

In July 2002 the Radiation Therapy Oncology Group (RTOG) closed study R97-04. This phase III study of 518 patients with pancreatic cancer randomized between 5-FU continuous infusion (250 mg/m^2/d for 3 weeks) followed by 5-FU continuous infusion (250 mg/m^2/d) during radiation therapy (50.4 Gy in 1.8 Gy/fractions) followed by 2 cycles of 5-FU continuous infusion, versus gemcitabine 1000 mg/m^2 weekly $\times$ 3 followed by 5-FU continuous infusion during radiation therapy followed by 3 cycles gemcitabine alone. The experimental question being asked was whether gemcitabine before and after 5-FU–based chemoradiotherapy would be more efficacious than continuous infusion of 5-FU before and after the same 5-FU–based chemoradiotherapy. In 1997, when this study was designed, there was inadequate knowledge regarding how to safely administer gemcitabine concurrently with irradiation to allow for concurrent gemcitabine and radiotherapy. This study was the first North American cooperative group trial since the GITSG trial. Although the survival results for this trial will not be known until sometime in 2003, a number of important observations have already resulted. These include that neither arm was observed to have unacceptable acute toxicity during the trial, that accrual was quite rapid (12 to 14 patients per month), reflecting both the support of the Eastern Cooperative Oncology Group (ECOG) and the Southwest Oncology Group, and the willingness of patients and their physicians to participate in adjuvant trials for pancreatic cancer.

In spite of a growing body of literature supporting the benefit of adjuvant combined modality therapy after resection in patients with pancreatic cancer, adjuvant chemoradiation has not been universally accepted as standard of care. One of the criticisms has been that none of these studies included an observation-only arm. Two European studies have demonstrated contrasting conclusions.

A European Organization for Research and Treatment of Cancer trial randomized 218 patients with pancreatic and nonpancreatic periampullary adenocarcinoma 2 to 8 weeks after potentially curative resection to either observation or to combined radiotherapy (40 Gy with a 3 or 4 field technique in 2 Gy fractions with a 2-week break at mid-treatment) and chemotherapy (5-FU administered as a continuous infusion 25 mg/kg/d during the first week of each 2 week radiation therapy module only).[211] No postradiation chemotherapy was administered. Median survival was 19 months in the observation group versus 24.5 months in the treatment group, but this difference was not statistically significant ($P = .737$). For the subgroup of patients with pancreatic adenocarcinoma (n = 114), the median survival was 12.6 months in the observation group versus 17.1

months in the treatment arm, again not statistically significant ($P = .099$). Of note, 21 of 104 patients randomized to the treatment arm were not treated. In addition, although the original dose of 5-FU was already modest, 35 patients in the treatment arm received only 3 days of 5-FU during the second module of radiotherapy, secondary to grade I/II toxicities. Therefore, although controversial, this study may be better described as an underpowered but trending toward positive study.[211]

Recently, the European Study Group for Pancreatic Cancer (ESPAC) randomized 541 patients with pancreatic adenocarcinoma in a 4-arm design, on the basis of a 2×2 factorial design: (1) observation, (2) concomitant chemoradiotherapy alone (20 Gy in 10 fractions over 2 weeks with 500 mg/m^2 5-FU intravenous bolus during the first 3 days of radiation therapy; the module is repeated after a planned 2-week break) followed by no additional chemotherapy, (3) chemotherapy alone (leukovorin 20 mg/m^2 bolus followed by 5-FU 425 mg/m^2 administered for 5 consecutive days repeated every 28 days for 6 cycles), or (4) chemoradiotherapy followed by chemotherapy.[212] There was no significant difference in survival between patients assigned to chemoradiotherapy (median survival 15.5 months) versus observation (median survival 16.1 months; $P = .24$). The survival data were similar in the subset (n = 285 patients) randomized through the 2×2 design. In contrast, there was a survival advantage for those patients treated with chemotherapy alone (median survival 19.7 months) versus observation (median survival 14 months, $P = .0005$). For the same subset randomized through the original 2×2 design, survival demonstrated a trend toward improved survival for chemotherapy alone (median survival 17.4 months) versus observation alone (15.9 months), but it was not statistically significant ($P = .19$). Multivariate analysis for known prognostic factors including margin status, lymph node involvement, and tumor grade and size did not alter the effect for chemoradiotherapy treatment. The ESPAC-1 authors concluded that there was no survival benefit for adjuvant chemoradiotherapy. In addition, the authors concluded that a potential benefit existed for adjuvant chemotherapy alone after surgical resection.

Although this ESPAC-1 trial was a randomized study consisting of more than 500 patients, the conclusions of the study should be carefully measured. To encourage maximal patient recruitment, the study was modified in that 68 patients were assigned separately and randomized to either chemoradiotherapy or observation alone. In addition, 188 patients were assigned separately and randomized to either chemotherapy alone or observation. In a sense, 3 randomizations were possible for inclusion into the same study. Also, patients in the additional 2 randomizations could

TABLE 16. Selected active or planned adjuvant studies

Study	Regimen
RTOG1091	Gem 1000 mg/m^2 × 3 w, XRT 50 Gy 1.8 Gy fx vs Gem 600 mg/m^2 weekly, followed by gem × 3 cycles
ACOSOG Z5006	Arm 1: 5FU CI/LV/DPM/MMC, 5FU/XRT (50 Gy), 5FU CI × 2 cycles Arm 2: 5FU/LV/DPM/MMC × 6 cycles Arm 3: XRT (50 GY)/ 5FU CI/cisplatin/IFN, 5FU CI × 2 cycles
Johns Hopkins	GM-CSF allo vaccine, 5-FU CI/LV/DPM/MMC, 5FU/XRT, 5-FU CI × 2 cycles followed by GM-CSF allogeneic vaccine × 3

Gem, Gemcitabine; *LV*, leucovorin; *IFN*, interferon-α; *CI*, continuous infusion; *MMC*, mitomycin; *DPM*, dipyridamole; *GM-CSF*, granulocyte-macrophage colony-stimulating factor.
All are planned as phase II studies.

have potentially received "background chemotherapy or chemotherapy," which was not specifically defined. The background treatment was not known in 82 eligible patients. Of note, these patients were still assigned into an arm of the study in spite of the lack of definitive knowledge of prior therapy. Finally, 25 of the eligible 541 patients refused to accept their randomization, and an additional 25 patients withdrew as a result of treatment toxicities.

As the debate regarding the optimal adjuvant therapy for pancreatic cancer continues, several studies have recently opened or have been proposed by either the cooperative groups or through single institutions. Table 16 summarizes some open or planned studies in the adjuvant setting. These future studies will be notable for the addition of multiagent chemotherapy to irradiation at the cooperative group level, or by the addition of gemcitabine to the period of chemoirradiation, and by the use of conformal, 3-dimensional planned irradiation, planned to patient-specific anatomic and surgical pathologic data.

Combined Modality Therapy for Locally Advanced Disease

In patients with metastatic pancreatic cancer, the current standard of care is single-agent gemcitabine. There has therefore been significant interest in using gemcitabine either earlier in treatment or in combination with radiation therapy; however, gemcitabine is also a potent radiosensitizer.[213] Because of this, studies combining radiotherapy with gemcitabine have found it necessary to proceed cautiously. Blackstock et al[214] examined in a phase I study, gemcitabine (starting at 20 mg/m^2) administered twice weekly in combination with radiation therapy (total dose 50.4 Gy in 1.8 Gy fractions) in 19 patients with locally advanced

pancreatic adenocarcinoma. Thrombocytopenia, neutropenia and nausea/vomiting were dose-limiting toxicities. Of the 15 patients assessable for response, 3 partial responses were identified. A dose of 40 mg/m^2 administered twice weekly in combination with radiotherapy (to a total dose of 50.4 Gy) was subsequently examined by the Cancer and Leukemia Group B in a phase II study of 38 patients with locally advanced pancreatic cancer.[215] After chemoradiotherapy, patients without disease progression received gemcitabine alone 1000 mg/m^2 weekly $\times$ 3 every 4 weeks for 5 additional cycles. Grade III/IV hematologic toxicity was significant and was identified in 60% of patients. In addition, grade III/IV gastrointestinal toxicity was identified in 42% of patients. With median follow-up of 10 months, median survival was 7.9 months.

Two groups have published phase I experiences with gemcitabine dose escalation, starting at 300 mg/m^2 with radiation therapy (5040 cGy in standard fractions) in patients with resectable or locally advanced disease. Dose-limiting toxicities were identified at a gemcitabine dose of 700 mg/m^2. In addition, late toxicities were identified in 2 of 6 patients at 600 mg/m^2. Of note, partial responses were seen in 3 of 6 patients with locally advanced disease at a dose of 600 mg/m^2.[216–218]

The MD Anderson Cancer Center (MDACC) has since published a corollary phase I study of 18 patients with locally advanced disease by use of rapid fractionation external beam radiation.[219] Patients received dose escalation gemcitabine from 350 mg/m^2 to 500 mg/m^{2}weekly $\times$ 7, with concurrent rapid fractionation 3000 cGy external beam radiation therapy during the first 2 weeks of therapy. Hematologic and nonhematologic toxicities were significant in all 3 patient cohorts. There were 8 responses (4 minor and 4 partial). One of 2 patients who were subsequently explored had a curative resection. The recommended phase II testing dose of gemcitabine was 350 mg/m^2.[219]

Recently, the University of Michigan has also described an alternative approach, using standard doses of gemcitabine at 1000 mg/m^2 weekly $\times$ 3 every 4 weeks and administering radiation therapy as dose escalation beginning at 24 Gy (1.6 Gy fractions in 15 fractions) in 37 patients with locally advanced disease.[220] Most patients received postchemoradiotherapy chemotherapy at the discretion of the treating physician. Seventy-five percent of the patients received at least 85% of the planned gemcitabine. Two of 6 assessable patients experience dose-limiting toxicity at the final planned radiation dose of 42 Gy in 2.8 Gy fractions. An additional 2 patients had development of late gastrointestinal toxicities at this dose level. Six patients were documented to have a partial response, with a complete radiographic response in 2 patients. In addition,

4 patients with documented stable disease at the time of study entry experienced objective responses (2 partial and 2 complete reponses). Definitive resection was achieved in 1 of 3 patients. With median follow-up of 22 months, median survival for the entire group was 11.6 months. The recommended phase II radiation dose was 36 Gy in 2.4 Gy fractions.

There have also been attempts to optimize chemotherapy when combined with radiation therapy. The ECOG published a phase I study of 7 patients with locally advanced disease by use of combination chemotherapy consisting of radiation therapy to a maximum 59.4 Gy in 1.8 Gy fractions.[221] The 5-FU (200 mg/m^2/d as continuous infusion throughout radiation therapy) was administered with weekly gemcitabine dose escalation beginning at 100 mg/m^2. Because of dose-limiting toxicities seen in 2 of the first 3 patients, the study was amended to lower the initial dose of gemcitabine to 50 mg/m^2. However, dose-limiting toxicities were subsequently seen in 3 of 4 patients at the 50 mg/m^2 dose. Three of the 5 dose-limiting toxicities occurred at radiation doses less than 36 Gy. The study was subsequently closed.

A more promising combination may be gemcitabine and cisplatin. This combination has demonstrated synergy in a variety of human tumor cell lines and has been demonstrated to have clinical benefit when used in the metastatic setting.[222–224] This combination is currently being further evaluated in clinical studies.

Given the current published data, would 5-FU or gemcitabine be better suited to be used concurrently with radiation therapy for either resected or locally advanced disease? The MDACC retrospectively examined their database of 114 patients with locally advanced disease treated with combination radiation therapy (rapid fractionation 30 Gy in 10 fractions) and either 5-FU continuous infusion 200 to 300 mg/m^2 (61 patients) or gemcitabine 250 to 500 mg/m^2 weekly $\times$ 7 (53 patients).[225] Patients receiving gemcitabine developed a significantly higher incidence of severe acute toxicity: defined as toxicity requiring a hospital stay of more than 5 days, mucosal ulceration with bleeding, more than 3 dose deletions of gemcitabine or discontinuation of 5-FU, or toxicity resulting in surgical intervention or death, compared with those patients receiving 5-FU (23% vs 2%, $P < .0001$). Five of 53 patients treated with gemcitabine/radiation therapy subsequently underwent surgical resection compared with 1 of 61 patients treated with 5-FU/radiation therapy. However, with a short median follow-up, median survival was similar (11 months vs 9 months, $P = .19$).[225]

Neoadjuvant Therapy

Neoadjuvant therapy is a potentially attractive alternative to current adjuvant therapies for several reasons: (1) radiation is more effective on well-oxygenated cells that have not been devascularized by surgery, (2) contamination and subsequent seeding of the peritoneum with tumor cells resulting from surgery could theoretically be reduced, (3) patients with metastatic disease on restaging after neoadjuvant therapy would not need to undergo exploration, and (4) the risk of delaying adjuvant therapy after resection would be eliminated, because it would be delivered in the neoadjuvant setting.

The MDACC published their experience of 142 patients with localized resectable pancreatic adenocarcinoma who were either treated (1) before operation with radiation therapy (50.4 Gy in either standard 1.8 Gy fractions or consisting of 30 Gy rapid fractionation in 3 Gy/fraction) combined with 5-FU continuous infusion 300 mg/m^2/d followed by surgical resection or (2) by resection followed by postoperative chemotherapy (5-FU continuous infusion 300 mg/m^2/d) and radiation therapy (50.4 Gy in standard fraction). There were no delays to surgery in the neoadjuvant group, but there were noted to be delays to initiate postoperative therapy in 6 of 25 patients who underwent surgical resection first. At a median follow-up of 19 months, no significant differences in survival were noted.[226]

The Fox Chase Cancer Center published their experience of 53 patients with localized resectable pancreatic cancer who were treated before operation with radiation therapy (5040 cGy in 180 cGy fractions) and chemotherapy (MMC 10 mg/m^2 on day 2 with 5-FU 1000 mg/m^2/d continuous infusion on days 2 to 5 and 29 to 32). Forty-one patients subsequently underwent exploratory laparotomy at the conclusion of preoperative chemoradiation. From this group of patients, 17 were not resectable (11 patients with hepatic or peritoneal metastases and 6 patients with local extension that precluded resection). Twenty-four patients underwent resection. There were significant treatment-related hematologic and nonhematologic toxicities identified, including one patient with treatment-related toxicities that precluded reexploration. Median survival for the entire group was 9.7 months, and 15.7 months for the group that underwent surgical resection.[227]

The Fox Chase group has since published a follow-up study of 30 patients with localized left-sided resectable pancreatic cancer, of which 26 received preoperative radiation therapy (50.4 Gy) with 5-FU continuous infusion. Ten patients who received such preoperative therapy

subsequently underwent resection. Median survival was 34 months for the resected group.[228]

Other potential radiation sensitizers have also been examined in the preoperative setting. The MDACC have used paclitaxel 60 mg/m^2 over 3 hours weekly with 30 Gy radiation therapy rapid fractionation.[229] Of note, if patients could undergo surgical resection, they could also receive intraoperative radiation therapy. Grade III hematologic and nonhematologic toxicities were identified in 16 patients. No delays in surgery were attributable to preoperative therapy. Twenty of 25 patients who underwent exploratory laparotomy underwent resection. There were no histologic complete responders. With a median follow-up of 45 months, the 3-year survival rate for those patients after potentially curative resection was 28%, with an overall median survival of 19 months.

Currently, ECOG is planning to open a prospective neoadjuvant trial, randomizing patients to intensified gemcitabine-based or 5-FU/platinum–based chemoradiotherapy. This trial makes an important distinction between clearly unresectable disease, focusing on the issues of partial versus complete encasement of the superior mesenteric artery and length of superior mesenteric vein involved by tumor at initial presentation.

To date, the current data demonstrate that although neoadjuvant chemoradiotherapy can be administered safely, there is no clear advantage to this strategy compared with postoperative therapy. In the cohort of questionably resectable patients, it remains to be seen whether there are patients for whom this approach may represent an important therapeutic advantage.

Immunotherapy

Immunotherapy offers the potential of a non-cross-resistant mechanism of antitumor activity that can be integrated with surgery, radiation, and chemotherapy. A major advantage of immune-based therapies is their ability to specifically target a tumor cell relative to the normal cell of origin, thereby minimizing nonspecific toxicities. Both B and T cells have an unlimited capacity to recognize specific motifs expressed by tumor cells relative to their normal cellular counterparts. For pancreatic cancer, these specific targets have not yet been defined. Currently, immune-based approaches either target a small group of candidate antigens expressed by the tumor, or rely on whole tumor cells as the immunogen. However, with the recent sequencing of the human genome and the development of rapid methods for identifying genes that are differentially expressed by tumor cells, many more candidate immune targets are expected to be identified that may serve as immunogens for treatment, as well as prevention. This section will highlight the important features of an effective antitumor immune response, summarize the results of some of the more promising strategies that are currently under clinical development, and predict what can be expected in the near future.

Components of the Immune System Required for Cancer Immunotherapy

There are a number of cell types that, when activated, are extremely efficient at recognizing and killing their target. B and T cells each have combinatorial clonally distributed antigen receptors that provide the specificity to recognize foreign antigens and to discriminate self from nonself. Through the recombination of genes encoding subunits of their receptors, both B and T cells can recognize more than a million different antigens whether they are in the form of the product of a new genetic alteration, a reactivated embryonic gene, or an overexpressed gene. The B cell recognizes free antigenic determinants or whole-surface molecules, eliminating the need for special antigen processing. In contrast, the T-cell receptor recognizes fragments of the antigenic protein bound to human leulocyte antigen (HLA) class I and II molecules on tumor cells or specialized cell types known as *antigen-presenting cells* (APCs), respectively. These peptide-HLA complexes are formed as a result of fragmentation of proteins within specialized cellular compartments and subse-

TABLE 17. Immunotherapy strategies

Passive immunotherapy
Unlabeled monoclonal antibodies
Radioimmunotherapy
Antibody-directed immunotoxins
T cell adoptive transfer
Active nonspecific immunotherapy
Whole tumor cells/ tumor lysate mixed with bacterial adjuvant
Systemic cytokines
Active specific immunotherapy (vaccines)
Genetically modified whole tumor cells
Protein/peptide/carbohydrate–*based antigen vaccines
Dendritic cell-based antigen vaccines
DNA-based vaccines
Recombinant viral-based antigen vaccines

*Glycoprotein antigens are recognized by T and B cells in a similar way as protein/peptide antigens.

quent association with a binding site on the HLA molecule. APCs (macrophages, B cells, and dendritic cells) have the ability to capture extracellular proteins that are released by the tumor through secretion, shedding, or tumor lysis. These proteins are subsequently internalized via endocytosis and processed through the exogenous pathway. Peptide fragments (10 to 25 amino acids in length) then bind to the HLA class II protein, before expression of the complex on the cell surface. This complex is recognized exclusively by $CD4^+$ helper T cells in the context of a second costimulatory molecule such as B7. In contrast, most tumor cells cannot process and present antigen through the exogenous pathway, because they usually do not derive from professional APCs. However, all cells including tumor cells have the ability to process and present antigens that derive from cellular proteins through the endogenous pathway. Any protein within a tumor cell can gain access to the cytosol and undergo enzymatic degradation into 8 to 10 amino acid fragments by specialized machinery (the proteasome) via the endogenous pathway. The peptide fragments are subsequently transported into the endoplasmic reticulum, where they bind to HLA class I molecules and are transported to the cell surface for recognition by $CD8^+$ T cells. In general, $CD4^+$ T cells provide helper or regulatory function whereas $CD8^+$ T cells carry out direct tumor lysis.

Immunotherapy in Clinical Practice

Immunotherapy can be broadly divided into passive and active therapeutic approaches (Table 17). Passive immunotherapy mainly involves

the use of unlabeled or labeled monoclonal antibodies that are specifically raised against tumor antigens. To date, antibodies have been the most successful form of immunotherapy clinically. They are being used as diagnostic tools, prognostic indicators, and as primary therapy. Advantages include specific targeting of tumor cells while sparing normal tissue, relative ease of administration, and low toxicity profile. Their major disadvantages include the absence of T-cell activation and the lack of induction of memory immune responses. In addition, all tumor cells within a proliferating mass may not express the antigen being targeted by the antibody because of tumor heterogeneity. In spite of some disadvantages, it is encouraging to note that passively administered antibodies have already been shown to induce significant clinical responses in several diseases, including lymphoma and breast cancer. A number of monoclonal antibodies have also undergone testing to assess their ability to treat pancreatic cancer. In one study of 41 patients with advanced pancreatic cancer, an antibody to epidermal growth factor receptor (Erbitux; Imclone Systems, New York, NY) was given in combination with gemcitabine at standard infusion and schedule.[230] The most commonly reported toxicities included grade I/II acne-form rash, folliculitis, and fatigue. After 2 cycles of therapy, 5 patients (12%) achieved a partial response, and 16 patients (39%) had stable disease. The median time to progression was 16 weeks, with median survival not reached at time of abstract submission. These results are encouraging and provide the rationale for developing vaccine approaches that can induce natural tumor-specific antibody responses in pancreatic cancer.

In contrast to passive immunotherapy, active specific immunotherapy (vaccine therapy) targets specific tumor antigens as a result of the induction of antigen-specific B-cell– or T-cell–mediated immune responses. Active specific therapy can also generate antigen-specific memory T-cell responses that are capable of being reactivated if tumor cells expressing the same antigen profile recur. Furthermore, the induction of cellular immune responses has the added benefit of allowing natural access to the microenvironment of the tumor. Preclinical studies have already shown that T-cell–mediated vaccine therapy can induce antitumor immune responses that are potent enough to eradicate many murine tumors. Translation of these vaccine approaches into therapies for patients with pancreatic cancer are in the early phases of clinical development. Examples of the different vaccine approaches that are currently undergoing clinical testing include peptide- and protein-based vaccines or whole tumor cell vaccines.

TABLE 18. Some current clinical trials testing vaccines in pancreatic adenocarcinoma

Approach	Sponsor	Stage	Trial phase
rFowlpox CEA/TRICOM ± GM-CSF	NCI/Fox Chase NCI	Metastatic	Phase I
CEA peptide/adjuvant + GM-CSF	NCI	Metastatic	Phase II
Virulizin/Gem vs Gemcitabine	Lorus Therapeutic	Metastatic	Phase III
G17DT/Gem vs Gemcitabine	Aphton	Metastatic	Phase III
K-*ras*/adjuvant ± IL-2 or GM-CSF	NCI	Metastatic	Phase I
Gem/XRT vs K-*ras* + Gem/XRT	RTOG	Resected	Phase II
GM-CSF allogeneic whole tumor cell	Johns Hopkins	Resected	Phase II
GM-CSF allogeneic whole tumor cell	Johns Hopkins	Metastatic	Phase I

Gem, Gemcitabine; *GM-CSF*, granulocyte-macrophage colony-stimulating factor.

Peptide- and Protein-based Vaccines

There are at least 2 major advantages to peptide- and protein-based vaccines: they are inexpensive and simple to produce, and they can be given in large quantities, thereby allowing for maximal immunization with relevant antigens. Point mutations in a variety of oncogenes (K-*ras*) or tumor suppressor genes (p53, p16, DPC4, BRCA2, Her-2/*neu*) have been associated with different histologically defined precursor lesions, and some are being studied as candidate immune targets. Mutated K-*ras* is a particularly attractive immune target because it is mutated in >90% of pancreatic adenocarcinomas.[231,232] This antigen has been tested for the induction of antitumor immunity in several trials. However, post vaccination responses have been observed in very few patients and have not correlated with clinical responses.[233–235] Peptide- and protein-based vaccines that are currently under development are listed in Table 18.

Heat shock protein (HSP)–based vaccines are a newer approach that have demonstrated promise. HSPs are ubiquitous and highly conserved cellular proteins that have multiple functions, including helping newly synthesized polypeptides fold, assisting in protein transport, and associating with peptides generated during protein degradation. They are also believed to stimulate macrophage and dendritic cell activation and assist in representation of peptides. Preclinical studies have shown that HSPs isolated from tumor cells can serve as potent vaccines by taking advantage of their role as a peptide transporter and as a stimulator of APCs. This approach has been tested in patients with resected pancreatic adenocarcinoma from whom HSP could be obtained and purified. Eligible patients were administered 5 mg of protein (HSP-96) subcutaneously weekly for 4 weeks.[236] The vaccine was well tolerated. In addition, an increase in postvaccination $CD8^+$ T cells specific for autologous tumor was observed in one patient.

Whole Tumor Cell Vaccines

Currently, the major limitation of antigen-based vaccines is the lack of identified pancreatic tumor antigens that are the known targets of the immune response. Until a panel of pancreatic tumor–specific antigens is discovered, the whole tumor cell represents the best source of immunogens. A whole tumor cell vaccine approach involves the use of autologous or allogeneic tumor cells to stimulate an immune response. However, studies aimed at dissecting antitumor immune responses have confirmed that most tumors are not naturally immunogenic. Evidence from preclinical models suggests that the failure of the immune system to reject spontaneously arising tumors is unrelated to the absence of sufficiently immunogenic tumor antigens. Instead, the problem is derived from the immune system's inability to appropriately respond to these antigens. The importance of the local release of stimulatory cytokines to provide an immunologic boost and attract other immune cells has been extensively examined. These findings have led to the concept that a tumor cell can become more immunogenic if engineered to secrete immune activating cytokines.

Tumor cells genetically modified to secrete immune-activating cytokines have been extensively studied for their ability to induce systemic antitumor immune responses.[237] Preclinical studies have shown that these vaccines can induce immune responses potent enough to cure mice of preestablished tumor. In one comparison study of 10 cytokines, granulocyte and macrophage-colony stimulating factor (GM-CSF) was the most potent cytokine, generating systemic immunity dependent on both $CD4^+$ and $CD8^+$ T cells.[238] GM-CSF is known to be involved in the recruitment and differentiation of bone marrow–derived dendritic cells, and dendritic cells are known to be the most efficient APCs at activating T cells. In addition, GM-CSF is produced by activated $CD4^+$ T helper cells, further supporting the concept that this cytokine may function by priming immune effector cells.[239,240] Studies aimed at optimizing this cytokine-secreting tumor vaccine approach confirmed that GM-CSF secretion must be at the site of relevant tumor antigen. Simple injection of soluble GM-CSF along with the appropriate tumor cells does not provide the sustained local levels required to provide a sufficient immunologic boost. Furthermore, high levels must be sustained for several days. In the preclinical data, it appeared that a minimum of 35 ng/10^{235} cells/24 hours is necessary to generate effective antitumor immunity.[241] Autologous tumor cells in theory are the ideal source of tumor antigens, because they would preserve unique antigens expressed by each patient's cancer.

Unfortunately, the development of an autologous vaccine requires that extensive processing, in vitro expansion, and regulatory testing be performed for each individual patient's vaccine. In the case of metastatic disease, the development of autologous tumor vaccine would also require the ability to obtain an adequate volume of tissue. These limitations preclude the use of autologous cellular vaccines for most cancers, particularly pancreatic adenocarcinoma.

A growing number of preclinical studies support the use of allogeneic pancreatic tumor cells as an alternative antigen source. Recently, the results of a phase I study with irradiated allogeneic pancreatic tumor cell lines transfected with GM-CSF administered in sequence with adjuvant chemoradiation was conducted in patients with resected adenocarcinoma of the pancreas.[242] Fourteen patients with stage 2 or 3 disease received an initial vaccination 8 weeks after pancreaticoduodenectomy. This was a dose escalation study in which 3 patients each received 1×10^{236}, 5×10^{236},and 1×10^{237}cells. An additional 5 patients received 5×10^{237} vaccine cells. Study patients were jointly enrolled in an adjuvant chemoradiation protocol for 6 months. After the completion of adjuvant chemoradiation, patients were reassessed, and those who were still in remission were treated with 3 additional vaccinations (booster doses), given 1 month apart at the same original dose that they received for the first vaccination. This was the first GM-CSF–secreting vaccine study to escalate the vaccine dose to 5×10^{237} GM-CSF–secreting cells. However, toxicities remained mostly limited to grade I/II local reactions at the vaccine site. In addition, there were self-limited systemic rashes, including one documented case of Grover's syndrome. Systemic GM-CSF levels were evaluated as an indirect measure of the longevity of vaccine cells at the immunizing site. GM-CSF levels peaked at 48 hours after vaccination, similar to what was observed in preclinical models. In addition, serum GM-CSF levels could be detected for up to 96 hours after vaccination. These data, together with data from preclinical models,[241] suggest that detectable serum GM-CSF levels may serve as a biomarker of immune response. The vaccine sites were also evaluated as a measure of the local immune reaction to the vaccine. Eleven of 14 patients demonstrated a similar local inflammatory response. Postvaccination DTH responses to autologous tumor cells have been used in previously reported vaccine studies as a surrogate, to identify and characterize specific immune responses that are associated with vaccination.[243] In the phase I pancreatic cancer vaccine trial just discussed, postvaccination DTH responses to autologous tumor cells were observed in 1 of 3 patients receiving 1×10^{237} and in 2 of 4 patients receiving 5×10^{237} vaccine

cells.[242] These data demonstrate that this vaccine approach is safe and can induce tumor-directed immune responses. Follow-up studies are ongoing to determine whether these promising effects on immune activation will translate into a true clinical benefit for patients with pancreatic cancer.

A Look to the Future

There are significant challenges that must be overcome if immune-based therapies are to play an important role in the treatment of pancreatic cancer. First, immune-based strategies must be able to circumvent the genetic alterations within a tumor cell, which allow the tumor cell to evade immunologic recognition and eradication. Typically, genetic alterations result in the loss of either antigen expression or the ability to adequately present antigen to T cells. One possible solution to this problem is to design polyvalent vaccines and antibodies that target immunity against several tumor rejection antigens. Second, additional candidate pancreatic tumor antigens are needed to serve as immune-relevant tumor targets. New and more rapid methods need to be developed to identify these targets. Third, it is unlikely that immunotherapy alone will be able to overcome mechanisms that functionally inactivate tumor-specific T cells, a recognized problem that limits its effectiveness in patients with large tumor burdens (like pancreatic cancer). Combinatorial therapies that provide tumor debulking and immune modulation given in sequence with immune-based therapies will be required to overcome this problem. Consequently, it might be possible to enhance the effects of vaccine based approaches by combining the cytoreductive and immune-modulating elements of chemotherapy with the tumor cell cytotoxic specificity of immunotherapy. Both combinations have been shown to overcome peripheral tolerance to tumor antigens in preclinical models.[244–247]

Small Molecule Therapy

The recent advances in the understanding of the process of tumor generation, growth, invasion, and metastasis have led to the identification of a substantial number of new targets for novel therapeutic interventions. These targets include membrane growth factor receptors, signal transduction pathways, and molecules involved in angiogenesis and cell cycle regulation. As discussed in previous sections of this monograph dealing with the biologic and pathologic study of pancreatic cancer, most of these alterations are present in pancreatic cancer and are believed to be implicated in the pathogenesis of this disease. Recently, specific therapeutic agents targeted against some of the most relevant pathways noted above have been tested in clinical trials in patients with pancreatic cancer, as well as other malignancies. Although many of these studies are in their infancy, for some agents definitive or preliminary results have been published and are summarized here. The 3 class of agents for which more advanced clinical data are available are (1) the matrix metalloprotease (MMP) inhibitors, (2) agents that interact with the *ras* oncogene signaling pathway, and (3) inhibitors of the *ERB* family of membrane receptors.

MMP Inhibitors

The MMPs are a group of closely related endopeptidases collectively capable of degrading essentially all components of the extracellular matrix.[248] These proteins are overexpressed in the majority of human neoplasms and are believed to contribute to the process of tumor invasion, angiogenesis and metastasis. A number of agents whose primary mechanism of action is inhibition of the MMP have been developed in the clinic, and at least 2 of the them have been specifically explored in pancreatic cancer: Bay 12-9566 and Marimastat. Bay 12-9566 is a biphenyl matrix metalloprotease inhibitor (MMPI) that selectively inhibits the proteolytic activity of two of the more relevant members of the MMP family.[248,249] This agent was tested in a randomized clinical trial in 277 patients with advanced pancreatic cancer in comparison with gemcitabine. Unfortunately, in spite of promising activity in preclinical studies, the median survival of patients treated with this agent was 3.2 months, significantly worse than the 6.4-month median survival of patients treated with gemcitabine, leading to a halt in the development of this agent in patients with pancreatic cancer.[249] The second MMPI that

has been actively developed in pancreatic cancer is Marimastast, a nonselective hydroxanate MMPI. Phase II studies conducted with Marimastat indicated that treatment with the agent reduced the levels of circulating tumor markers in patients with advanced pancreatic cancer, leading to further study.[250] In a randomized phase II trial, treatment with 25 mg of Marimastat daily was equivalent to gemcitabine in patients with advanced disease, supporting the activity, albeit modest, of this compound in patients with advanced pancreatic cancer.[251] On the basis of these results, the development of Marimastat in patients with pancreatic cancer (either alone or in combination with standard treatments) continues.

Ras Pathway Inhibitors

The second group of molecularly targeted agents being explored in pancreatic cancer are the inhibitors of farnesyl transferase (FT), the enzyme that mediates the posttranslational modification of the *ras* protein required for this oncogene to be activated.[252] More than 90% to 95% of pancreatic cancers harbor mutations in this gene, making it theoretically an attractive target. Several FT inhibitors have been tested in clinical studies to date, and one of these agents, R115777 (Zarnestra) has been tested in both phase II and III clinical trials. Treatment of a total of 76 patients with advanced pancreatic cancer in phase II studies did not show any objective response and resulted in a median survival in treated patients of less than 4.5 months.[253,254] Parallel pharmacodynamic studies indicated, however, that the agent inhibited the FT in peripheral blood mononuclear cells.[253] Phase III evaluation has been equally disappointing, because the addition of R115777 to gemcitabine did not result in better outcome compared with gemcitabine alone.[255] The negative results could, however, be related to several factors. First, it is not clear that the *ras* oncogene itself is the target of these agents, because tumors with wild type *ras* are also inhibited by FT inhibitors.[252] Second, the most prevalent mutation of *ras* in pancreatic cancer involves the K-*ras* oncogene, which is known to be activated by a process different from farnesylation.[252]

Erb Family Inhibitors

The third class of novel targeted compounds that have been studied in pancreatic cancer are the inhibitors of the *ERB* family of receptors.[256] This is a family of transmembrane receptors composed of an extracellular domain, a transmembrane anchoring domain and an intracellular domain with tyrosine kinase activity.[256] Most human epithelial tumors, including cancer of the pancreas, are characterized by dysregulation of this receptor

family. Two members of the family, *ERB*-1 (EGFR) and *ERB*-2 have been targeted therapeutically with either monoclonal antibodies against the extracellular domain of the receptor such as C-225 (*ERB*-1) and Herceptin (*ERB*-2), or small molecules that target the intracellular TK domain of the receptor (OSI-774).[256] The combination of C-225 and Herceptin with gemcitabine has been tested in phase I-II studies, demonstrating a promising response rate in the 15% to 20% range in patients with advanced disease, with a mild to moderate toxicity profile.[257,258] OSI-774 is currently been explored in combination with gemcitabine (compared with gemcitabine alone) in patients with advanced pancreatic cancer.

In conclusion, a variety of novel targeted agents are being explored in patients with pancreatic cancer. Although some trials have been negative, studies with other compounds appear to be more promising. The development of novel targeted agents in pancreatic cancer will continue over the next few years. It should be emphasized, however, that most of these trials have been conducted with classic clinical trials methods used, with no attempt to determine the relationship between molecular target expression and functionality and modulation by treatment. It is likely that the implementation of such biologic end points in these studies will provide critical information to better understand the pharmacology of these novel agents.

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