PancreasFest 2010
Sixth International Symposium on Inherited Diseases of the Pancreas
Recurrent Acute & Chronic Pancreatitis: Defining Disease & Targeting Treatment
Advancing the War on Pancreatic Cancer
CAPER Annual Meeting & Young Investigators’ Session

POSTER ABSTRACTS
as of 7.28.10

July 29, 30 & 31, 2010
#1  Quality of Life After Total Pancreatectomy and Islet Autotransplant for Management of Chronic Pancreatitis in Children

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**Background:** Children with painful chronic pancreatitis (CP) that is refractory to medical and endoscopic therapies are candidates for total pancreatectomy and islet autotransplant (TP/IAT). Initial retrospective studies in pediatric patients undergoing this procedure suggest that the majority experience significant pain relief, and half are insulin independent at 1 year. Objective measures of quality of life are lacking.

**Objectives:** The primary aim was to determine if quality of life is improved in pediatric patients undergoing TP/IAT, using a standardized measure for health-related quality of life (SF-36).

**Methods:** Nineteen consecutive children with CP scheduled for TP/IAT from December 2006 to December 2009 at the University of Minnesota were enrolled in this prospective analysis. Health questionnaires including the Medical Outcomes Study 36-item short form (SF-36) were administered at baseline and 3 months, 6 months, and annually after surgery. Hemoglobin A1c, glucose, and C-peptide levels were measured. Insulin use was recorded.

**Results:** Patients were 14.5 ± 3.6 years (range 5-18 years) at the time of surgery. One patient had pre-existing C-peptide positive diabetes. Six patients had a prior Puestow procedure with or without distal pancreatectomy. Average islet yield was 3,513 ± 2,480 islet equivalents per kilogram body weight (IE/kg) but was substantially lower in those patients with a prior history of Puestow procedure (1,218 ± 1,189 IE/kg compared to 4,457 ± 2,145 IE/kg, p=0.01).

Prior to surgery, average health-related quality of life scores on the SF-36 were nearly 2 standard deviations below the population normal. At baseline, mean Physical Component Score (PCS) was 30.2 and a mean Mental Component Score (MCS) was 34.1 (standardized normal =50, standard deviation =10). Both PCS and MCS significantly improved significantly after surgery (figure 1). By 1 year, mean PCS was 50 and mean MCS was 45.7.

Seven patients achieved and maintained insulin independence and another 4 have minimal insulin requirements (basal insulin or correction scale only, <0.25 u/kg/day), all with HbA1c levels ≤6.5%. A prior Puestow was associated with a higher likelihood of insulin dependence (p=0.04)—5 of 6 patients with a prior Puestow required full insulin supplementation (basal-bolus regimen, >0.25 u/kg/d).

**Conclusions:** This study provides the first objective evidence that quality of life is improved after TP/IAT in selected pediatric patients with severe chronic pancreatitis. Notably, both physical and emotional summary component scores on the SF-36, which were nearly 2 standard deviations below the population normal score before surgery, normalized after TP/IAT in these patients. Nearly two-thirds of patients without pre-existing diabetes mellitus require low dose or no insulin therapy.

**Figure 1:** Change in physical component summary scores (triangles, dashed line) and mental component summary scores (squares, solid line) after total pancreatectomy and islet autotransplant. Asterix indicates statistically significant change from baseline (p<0.05).
Islet Transplant Outcomes in Thirty-Six Children Undergoing Pancreatectomy and Islet Autotransplant

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Background: In children with severe chronic pancreatitis, total pancreatectomy may be considered to relieve pain. However, total pancreatectomy alone would invariably result in diabetes mellitus. Thus, the goal of a simultaneous islet autotransplant (IAT) is to prevent or minimize postsurgical diabetes. Overall, one third of patients are insulin independent following this procedure. A greater number of islets transplanted (islet yield), younger age, and lack of prior pancreatic surgeries have been associated with better transplant outcomes. We reviewed diabetes outcomes in 36 children undergoing this procedure at the University of Minnesota.

Methods: Forty-two pediatric patients (age 5-18 years) underwent pancreatectomy with IAT between 1989 and December 2006. Follow up data was available in 36 cases. Data on insulin use and hemoglobin A1c levels were obtained by review of medical records and/or through health questionnaires administered to patients or their parents. Patients were classified as insulin independent (fasting blood sugar <126 mg/dL and HbA1c <6.5% without exogenous insulin), minimally insulin dependent (required basal insulin alone or intermittent correction scale alone to maintain glycemic goals), or fully insulin dependent (required a basal-bolus insulin regimen).

Results: The 36 patients underwent pancreatectomy and IAT at an average age of 13.6 ± 4.0 years. In 1 case, the extent of pancreatectomy was partial; in the remaining 35 cases, total pancreatectomy was performed. Patients received 4,444 ± 3,461 IE/kg. The most common underlying causes of pancreatitis were predisposing genetic mutations or idiopathic disease. Eleven patients had a prior history of distal pancreatectomy (n=3), lateral pancreaticojejunostomy (n=5), or both (n=3). At most recent follow up, patients were 3.0 ± 3.2 years posttransplant (range 0.4-17 years). After transplant, 16 patients achieved and maintained insulin independence, 7 patients had only minimal insulin requirements, and 13 patients were fully insulin dependent. Hemoglobin A1c was documented for the majority of patients, and was consistently ≤6.5% for those patients requiring no or minimal insulin. Factors associated with better islet transplant outcomes included younger age at transplant (p=0.04), greater islet yield (islet equivalents/kg body weight) transplanted (p=0.002), and lack of prior distal
pancreatectomy or lateral pancreaticojejunostomy (p=0.0003). Notably, none of the 11 patients with a prior distal pancreatectomy or pancreaticojejunostomy achieved insulin independence, and only 2 had minimal insulin needs (figure 1). There was a trend towards better outcomes in those with a shorter duration of disease (p=0.08).

Conclusions: Forty-five percent of the children in this series achieved and maintained insulin independence, while another ~20% required minimal insulin to maintain euglycemia. Prior partial pancreatic resections (without IAT) and surgical drainage procedures conferred a particularly poor prognosis and should be avoided in patients with diffuse pancreatic disease who are likely to require a total pancreatectomy.

Figure 1: Insulin requirements for patients with prior distal pancreatectomy and/or surgical drainage procedures (n=11, grey pattern) compared to those without prior surgery (n=24, solid grey) or whipple alone (n=1, spotted pattern).

#3 Chronic and Recurrent Acute Pancreatitis in Children

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Introduction: Etiologies of chronic (CP) and recurrent acute pancreatitis (RAP) (defined as 2 or more episodes of acute pancreatitis) in children include anatomic abnormalities, hereditary, metabolic and autoimmune disorders with the majority of cases being labeled as idiopathic. With the increasing use of modern imaging studies and genetic testing, CP and RAP are increasingly recognized in children. Hereditary pancreatitis is caused by mutations of the cationic trypsinogen (PRSS1) gene. Other genes that are associated with RAP and CP are serine protease inhibitor kazal type 1 (SPINK1), cystic fibrosis
transmembrane conductor regulator gene (CFTR) and chymotrypsinogen C gene (CTRC). Although the diagnosis of CP and RAP are increasing, there is a paucity of literature regarding the clinical profile of genetic pancreatitis in children.

**Aims:** 1) To estimate the prevalence of genetic etiology in children with RAP and CP and 2) to describe the clinical characteristics and outcome of patients with genetic pancreatitis.

**Methods:** We reviewed the charts of children younger than 18 years of age with RAP or CP diagnosed between 2000 and 2009 at the Children’s Hospital of Wisconsin. Data collected included demographics, clinical presentation, imaging studies and genetic screening (PRSS1, SPINK1, and CFTR genes). Patients who tested positive for any of these 3 genes were included in the study.

**Results:** 23 RAP or CP patients were identified. The mean age of onset of symptoms was 6.7 years (range 9 months to 15 years) and the mean age of diagnosis was 7.4 years (range 1 to 16 years). 21 were Caucasian and 14 were females. The most common presenting symptoms were abdominal pain (100%), vomiting (74%) and nausea (40%). The patients with RAP had 2 to 8 episodes within 3.6 years average follow-up. A family history was present in 5 (21%) patients (2 with PRSS1 and 3 with CFTR mutations). 8/23 (34%) tested had PRSS1 mutations, 7/23 (30%) had SPINK1 mutations and 14/23 (60%) had CFTR mutations (8 homozygote and 6 heterozygote). Six patients with CP had a combination of CFTR and SPINK1 or PRSS1 mutations. 10/23 (43%) patients met the criteria for chronic pancreatitis as documented by radiological studies. All patients heterozygote for both CFTR and SPINK1 mutations had chronic pancreatitis. Eight patients developed chronic pain syndrome and 2 developed exocrine pancreatic insufficiency.

**Conclusion:** RAP and CP due to mutations in PRSS1, SPINK1 and CFTR genes may be more common than previously thought. Genetic pancreatitis is associated with early and severe pancreatitis, high risk of chronic pancreatitis, and chronic pain syndrome. Patients with combined CFTR and SPINK1 mutation might be at higher risk for chronic pancreatitis. A prospective study with larger number of patients is needed to better clarify the clinical presentation and outcome of patients with genetic pancreatitis.

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**#4 Inflammation Contributes to the Pathogenesis of Pancreatic Lesions in Cystic Fibrosis**

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**Background:** Pancreatic disease begins *in utero* in the majority of patients with cystic fibrosis (CF) and progresses over time to complete destruction of the organ. Although inflammatory cells have been well-described in the pancreas of humans with CF and the CFTR–/– pig model, it is not known whether inflammation plays a role in the destruction of pancreas in CF.

**Hypothesis:** Inflammation plays a role in the progression of pancreatic disease in CF.

**Methods:** Pancreatic histopathology was studied with H&E and PAS staining and slides were reviewed by a veterinary pathologist (D.K.M). Microarray expression profiling was done to explore the differences in gene transcription between CF (4 CFTR–/–), WT (4 CFTR+/+) and heterozygous (4 CFTR+/–) pig pancreata using an Affymetrix Porcine GeneChip. Using four-color flow cytometry, the surface phenotype of leukocytes in the pancreas, blood and mesenteric lymph nodes (MLNs) of CF pigs (4 CFTR–/–, 1 CFTRΔF508/ΔF508) and WT and heterozygous pigs (3 CFTR+/–, 3 CFTR+/+, 1 CFTRΔF508/) were analyzed immediately after birth. Cell suspensions were incubated with primary monoclonal antibodies against CD2, CD3, CD4, CD8, CD14, CD21, CD25, IgM, TCR γδ SWC1, SWC7, SWC8 and MHC-II antigens. Following incubation with secondary antibodies, measurements were made using a FACS Calibur flow cytometer.

**Results:** 1.) WT and heterozygous pigs had identical pancreatic histology, microarray gene expression and leukocyte cell populations. 2.) Histopathological tissue examination showed clear differences in CF pig pancreas compared with non-CF pancreas. CF pancreas had abundant inflammatory cell infiltrate in the interstitium, remnants of atrophic acini, and severe pancreatic architectural destruction that included...
ductal dilations with mucinous plugs, along with mucinous metaplasia. Acini and ducts had zymogen material in their lumens with scattered neutrophilic infiltrates. 3.) Microarray gene expression profiling of newborn pigs showed significant upregulation of proinflammatory and complement pathway genes compared to non-CF pigs. 4.) CF pig pancreas had an increased proportion of B cells (IgM+); effector (MHC-II+) and cytotoxic (CD8+CD8+) γδ T cells; activated (MHC-II+ and/or CD25+) and effector (CD4+CD8+) αβ T helper cells; effector natural killer cells (MHC-II+CD3-CD8+); monocytes/macrophages (SWC8-CD14+) and neutrophils (SWC8-CD14+) compared to pigs without CF. 5.) Flow cytometry from blood and MLNs did not indicate that leukocyte populations differ between CF and non-CF pigs. Conclusion: Both the innate and adaptive immune systems were activated and proinflammatory and complement genes were upregulated in the newborn CF pig pancreas. Blood and MLN inflammatory cell populations were not different between CF and non-CF pigs, suggesting that the pancreatic immune response represents a localized rather than systemic inflammation. An activated immune response contributes to the pathogenesis of pancreatic lesions in CF.

#5 A New Paradigm for CF Drug Development

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Cystic fibrosis (CF) is the most common lethal monogenic disease in the U.S. The CF gene was identified by positional cloning in 1989 and this discovery led to rapid advances from the academic sector in our understanding of the genetics, pathogenesis and clinical management of CF. One reason to push for better basic and clinical knowledge of CF in the early 1990s was the urgent need for better treatments for CF. However, it was difficult to translate new information about CF into new CF treatments because private companies did not view CF as a favorable business opportunity. This led the CF Foundation (CFF) to develop a novel strategy for supporting the development of new CF drugs. The general goal of the CFF’s drug development effort has been to encourage companies to develop CF drugs by reducing their financial, scientific & clinical risks. Several CFF-supported activities aim to reduce drug development risks in different ways: (1) CFF-supported basic research on CF pathogenesis identified potential drug targets, provided assays for high throughput screening (HTS) and generated a community of experts in the academic sector who have served as advisors, consultants or founders for companies developing new CF drugs. (2) Most American CF patients are seen at CFF-accredited care centers supported by dedicated clinicians, site visits and benchmarking. These care centers also are noted for high participation in clinical trials and for rapid uptake of new therapies once shown to be effective. (3) CF care centers have been organized into a Therapeutics Development Network (TDN) and this provides centralized support for statistics, standard operating procedure development, centralized testing for specialized assays, training/support for study coordinators, and a dedicated Data Safety Monitoring Board. (4) The clinical data for most U.S. CF patients is collected in a Patient Data Registry, a dataset with more than four decades of prospectively collected longitudinal data for all patients followed at CFF-accredited care centers. Thus, the CFF has many programs designed to reduce costs and risks for companies capable of developing new drugs. During the last decade, the CFF expanded and reinforced these programs as part of an effort to encourage private companies to work on drugs for CF. Beyond this, the CFF extended the translational impact of these activities by providing financial support to dozens of companies and academic labs for a wide range of milestone-driven drug discovery and drug development programs for CF. One of the largest and most advanced of these programs is the CFF’s partnership with Vertex. The partnership began when a small company (now part of Vertex) proposed to perform HTS for CFTR correctors and potentiators in response to a request
for applications issued by the CFF. The initial HTS studies were productive and this led to a full-scale drug development effort which is now at the stage of phase 3 (pivotal) clinical trials. The CFF supported this drug development effort with significant funding (more than $80 million, to date); the CFF also helped the company identify advisors from the academic sector to guide or help perform several stages of drug discovery, including the HTS screens and secondary target validation assays, the preclinical testing, and the clinical trials. If the CFF had not provided this support, this company would not be developing drugs for CF. This approach has been termed “venture philanthropy” and it is a new paradigm for developing drugs to treat orphan diseases.

#6 Hereditary Pancreatitis Caused by an Intragenic Duplication Affecting the Activation Peptide of Human Cationic Trypsinogen (PRSS1)

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Background and Aims: Hereditary pancreatitis is caused by missense mutations in human cationic trypsinogen. A subset of mutations alters the activation peptide and increases autoactivation of trypsinogen to trypsin. At the cellular level, autoactivation results in decreased trypsinogen secretion and acinar cell death. In a hereditary pancreatitis family from Denmark we identified a novel intragenic duplication of 9 nucleotides in exon-2 of the PRSS1 gene (c.63_71dup) which at the amino-acid level altered the activation peptide. The aim of the present study was to characterize the effect of this unique genetic defect on the function of human cationic trypsinogen.

Methods. Wild-type and mutant cationic trypsinogens were produced recombinantly and purified to homogeneity. Trypsinogen activation was followed by enzymatic assays and SDS-PAGE. Trypsinogen secretion was measured from transfected HEK 293T cells.

Results. The intragenic duplication within exon-2 results in the insertion of three amino acids (p.K23_I24insIDK) within the activation peptide of cationic trypsinogen changing the sequence to APFDDDDKIDK. The mutated activation peptide thus contains two potential tryptic cleavage sites, however, activation would only occur upon cleavage of the second site. Recombinant cationic trypsinogen carrying the p.K23_I24insDK mutation exhibited >10-fold increased autoactivation. Activation by human cathepsin B was also accelerated by 10-fold. Activation by human enteropeptidase was unaffected. Secretion of the p.K23_I24insDK mutant from transfected cells was diminished, consistent with intracellular autoactivation.

Conclusions. This is the first report of an intragenic duplication within the PRSS1 gene causing hereditary pancreatitis. The robust autoactivation of the novel mutant is consistent with the similar phenotypic behavior of previously described activation peptide mutants such as p.D19A, p.D22G, and p.K23R. The accelerated activation by cathepsin B is a unique biochemical property not found in any other pancreatitis-associated trypsinogen mutant and, therefore, it is unlikely to be of pathogenic significance. Finally, the observations confirm and extend the notion that increased autoactivation is a disease-relevant biochemical alteration of cationic trypsinogen mutants.

#7 Use of Mathematical and Statistical Model Predictions to Identify a Novel Class of CFTR Variants Linked to SPINK1 Mutations and Chronic Pancreatitis
Chronic pancreatitis (CP) is a complex disorder with multiple genetic and environmental risks. Our mathematical model of the pancreatic duct physiology predicted that CFTR variants that inhibit bicarbonate conductance will impair pancreatic juice secretion and predispose to recurrent acute pancreatitis through a trypsin-dependent mechanism. Mutations in the trypsin inhibitor SPINK1 are predicted to further increase the risk of CP in these patients.

To determine if common CFTR mutations in CP that do not cause cystic fibrosis (CF) have defective bicarbonate secretion and increase risk of CP with SPINK1 mutations, patients and controls from our NAPS2 study and familial pancreatitis studies were screened for high-risk SPINK1 variants and CFTR variants in all 27 exons.

The final study group included idiopathic CP (n=53) and probands of familial pancreatitis (n=27) and controls (n=150). SPINK1 variants were identified in 3% of controls and 36% of subjects (OR 16.5, CI 6.1-44.9). An atypical CFTR variant was found in 16% of subjects and 7% of controls (OR 2.5, CI 1.0-5.8). Co-inheritance of the novel CFTR variant plus SPINK1 variants were identified in 8.75% of CP patients (OR 40.0, CI 16.6-95.4) and no controls. The atypical CFTR variant was cloned into wild-type (wt) CFTR and expressed in HEK293 cells to measure the relative conductances of CFTR wt and variant proteins to HCO$_3^-$ and Cl$^-$. Patch-clamp recordings of atypical variant CFTR demonstrated normal chloride currents but significantly reduced bicarbonate current (p=0.0001).

This study validates the prediction of our pancreatic duct mathematical model of bicarbonate secretion and development of pancreatitis. While the isolated risk is 2 to 3-fold, the multiplicative risk in patients with SPINK1 mutations confirms a trypsin-dependent mechanism. Future studies are warranted to determine if other CFTR-variants that are not associated with CF will lead to an increase risk for the development of CP through impairment of bicarbonate flow.

#8 Use of Reverse Engineering to Resolve Complex Pancreatic Disorders and Mathematical Modeling to Deliver Personalized Medicine

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Background: Pancreatic disorders tend to be complex syndromes and different people developing inflammation, scarring, pain, dysfunction and/or cancer for different reasons, including complex gene-environment interactions. Currently, susceptibility, onset, severity and progression are unpredictable, and treatments are non-specific and often ineffective.

Aims: the goal of Dr. Whitcomb’s program is to provide effective preventative and curative treatments of people with a variety of pancreatic disorders.

Methods: The approach has been to begin with a holistic approach, with systematic focus on each major anatomical and/or physiologic component to determine their specific mechanisms and effects of a variety of stressors. We focused on the acinar cells, the duct cells, the islet cells, the nervous system, the immune system and regenerative mechanisms. Clinical observations and human syndromes were used to develop animal, statistical and mathematical models studies based on data collected in multi-center studies of genetic and environmental factors, pathologic features and endophenotyping. A
pathologic progression model was used to study acute pancreatitis, chronic pancreatitis and pancreatic cancer.

**Results:** We discovered that hereditary pancreatitis patients with acute pancreatitis, chronic pancreatitis and pancreatic cancer had gain-of-function mutations in the cationic trypsin gene (PRSS1). Multiple genes interacting with trypsin were also discovered. Mathematical modes of the pancreatic duct cell predicted pancreas-specific CFTR functions. Animal models of alcoholic pancreatitis demonstrated the effects on neurohormonal control and altered immune response to injury – leading to alcoholic pancreatitis. Studies on acute pancreatitis have resolved the primary factors causing severity. Risks for pancreatic cancer have also been described.

**Conclusion:** A structured approach to resolve the component parts of a complex mechanistic system followed by modeling of the principals and primary variables, allows for the prediction of the major features of pancreatic diseases.

#9 SPINK1 Mutations in Japanese Chronic Pancreatitis

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**Background / Aim:** Chronic pancreatitis (CP) is an inflammatory disease with multifactorial pathogenic mechanisms. Genetic studies have revealed an association between CP and mutations in the cationic trypsinogen gene (PRSS1), the serine protease inhibitor Kazal type 1 gene (SPINK1), and the cystic fibrosis transmembrane conductance regulator gene (CFTR), chymotrypsin C gene (CTRC), and a triplication of the trypsinogen. We have previously shown that the IVS3+2T>C (c.194+2T>C) mutation in the SPINK1 gene is frequent in Japanese patients with pancreatitis. Additionally, we have examined the mRNA sequence of the SPINK1 gene and revealed the IVS3+2T>C mutation causes skipping of the whole of exon 3. Here we present the association of SPINK1 mutations with chronic pancreatitis in Japan, and whether the disease course is different between mutation-positive and -negative patients. Furthermore, we examined lower limit of serum PSTI level to identify the SPINK1 IVS3+2T>C mutation. This study was approved by the Ethics Committee of Tohoku University School of Medicine. Genomic DNA was prepared from 258 Japanese patients with CP and 527 healthy controls. The etiologies of CP in this study were as follows: hereditary (n=11 from 11 families), familial (n=15 from 15 families), idiopathic (n=78), alcoholic (n=124), and autoimmune (n=30). Mutational analysis of the SPINK1 gene was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis and direct sequencing. Serum PSTI concentrations were measured by a commercial radioimmunoassay kit (Ab Bead PSTI; Eiken Chemical Co., Tokyo, Japan). The p.N34S mutation was present in 16 patients with CP. The IVS3+2T>C and was present in 16 patients with CP. The prevalence of p.N34S and IVS3+2T>C were significantly higher in patients with familial pancreatitis (40 % and 13 %, respectively) and with idiopathic CP (10% and 12%) than normal subjects (0.4 % and 0 %). Patients with the p.N34S mutation presented with earlier symptom onset and more dilatation of the main pancreatic duct, followed by more frequent surgical and/or endoscopic intervention and pancreatic cancer development than those without SPINK1 mutations. Low levels of serum PSTI (<6.0 ng/ml) showed sensitivity of 78% and specificity of 95% in the differentiation of IVS3+2T>C carriers from patients without SPINK1 mutation.

**Conclusions:** SPINK1 mutations were associated with idiopathic and familial CP. The IVS3+2T>C mutation in the SPINK1 gene formed a unique genetic background in Japan. Patients with the SPINK1 p.N34S mutation presents more severe clinical courses, implying that genetic risk assessment might be useful to identify individuals who are likely to develop severe CP. Additionally, measurement of serum PSTI level might be useful to detect IVS3+2T>C mutation in the SPINK1 gene.
The Revised Japanese Diagnostic Criteria for Chronic Pancreatitis: Definition of Early Chronic Pancreatitis and Clinical Feature

Shimosegawa T #1, Hirota M #1 and The Committee for Revision of Japanese Clinical Diagnostic Criteria for CP supported by the Research Committee of Intractable Pancreatic Diseases of Japan (RCIPD), the Japan Pancreas Society (JPS) and the Japanese Society of Gastroenterology (JSGE).

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In Japan, for the diagnosis of chronic pancreatitis (CP), we had used the 2001 JPS (Japan Pancreas Society) criteria that were revised by the addition of magnetic resonance cholangiopancreatography (MRCP) findings to the 1995 JPS criteria. Since the criteria were set for diagnosing advanced CP, they were not helpful to improve the patients' prognoses. In addition, they were unsuitable for the current clinical practice because exocrine pancreatic function tests, which had been obsoleted in our country, were included in the diagnostic factors. For these reasons, the Research Committee of the Intractable Pancreatic Diseases supported by the Ministry of Health, Labour and Welfare of Japan (RCIPD), the JPS and the Japanese Society of Gastroenterology (JSGE) have revised the 2001 JPS diagnostic criteria for CP in 2009. The revised criteria (JDCCP 2009) are characterized by many unique trials. The JDCCP 2009 classified CP into alcoholic and nonalcoholic types, two categories with distinct clinico-pathological characteristics. In addition, to make application of the criteria simple and convenient, they were designed to enable a diagnosis of CP when definite or probable findings in the imaging or histological factors are found. At the same time, the criteria were devised to detect alcoholic pancreatic injury in the early stages by including clinical signs related to heavy drinking. The most prominent feature of the revised criteria was the introduction and definition of early CP by employing EUS findings.

To clarify the clinical feature of early CP, a multicenter clinical study was conducted. Clinical records of 814 CP patients from 10 institutes who had been classified either definitive, probable or possible CP by the 2001 JPS criteria were re-evaluated by the revised JDCCP 2009. The results demonstrated that 53 of 814 CP patients (5.3%) were classified to the early CP by the revised criteria. The early CP patients were characterized by the followings: they were male predominant (M/F 3.44), 10 years younger compared with the average age of definitive and probable CP patients, 57% of them were moderate to heavy drinkers, 60% complained intermittent abdominal pain, 47% had a relatively short clinical history of less than 5 years after the disease onset. It is important to determine and clarify the clinico-pathological outcome of early CP patients by prospective long-term follow-up studies.

The Prevalence of Hepatitis C Virus Infection in Japanese Patients with Chronic Pancreatitis

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Introduction: It was reported that the prevalence of hepatitis C virus (HCV) infection in Japanese was approximately 1 to 3 %. Chronic HCV infection is considered a major cause of liver disease, as chronic hepatitis (CH), liver cirrhosis (LC), or hepatocellular carcinoma (HCC). The prevalence and influence of HCV infection in the patients with chronic pancreatitis are unknown. We assessed the prevalence of
HCV infection, the influence of HCV infection on the nutritional status and glycemic control, and the mortality in the Japanese patients with chronic pancreatitis.

**Method:** The patients with chronic pancreatitis (compensated and decompensated state, N=88) were selected. The prevalence of HCV infection was evaluated (HCV positive group and HCV negative group). And the number of patients (male : female), age, height, weight, body mass index (BMI), the prevalence of liver diseases (CH, LC, HCC) and diabetes (DM) were determined in two groups. The treatment of chronic pancreatitis (the amount of digestive enzyme), the nutritional status (serum total protein (TP), albumin (Alb), hemoglobin (Hb) and total cholesterol (TC)) and the index of glycemic control (HbA1c) were also evaluated. Results were mean+/−SD, and P value >0.05 was significant difference.

**Result:** The number of HCV positive group were 5(3 males) and HCV negative group were 83(58 males), therefore the prevalence rate was 5.7%. There was one patient with LC in HCV positive group. The prevalence of DM was approximately 80 % ( HbA1c: 6.6+/−0.8 %) in HCV positive group and approximately 60% ( HbA1c: 7.0+/−1.9%) in HCV negative group; there was no significant difference in two groups. The amount of digestive enzyme (Berizym®) was 8.3+/−5.1 g/day in HCV positive group and 8.2+/−3.0 g/day in HCV negative group. TP level was 6.8+/−0.6 g/dl in HCV positive group and 7.2+/−0.6 g/dl in HCV negative group. Alb level was 3.7+/−0.7 g/dl in HCV positive group and 4.0+/−0.5 g/dl in HCV negative group. Hb level was 11.5+/−2.1 g/dl in HCV positive group and 13.0+/−1.8 g/dl in HCV negative group. TC level was 138.0+/−79.1 mg/dl in HCV positive group and 170.7+/−47.3 mg/dl in HCV negative group; there were no significant differences in two groups.

**Conclusion:** Our result indicated that the prevalence of HCV infection in Japanese patients with chronic pancreatitis was higher than that of recent studies in Japanese. No significant differences were observed in the glycemic control and the nutritional status in HCV positive group and HCV negative group. We also report the mortality and the cause of death in each group.

#12 Investigation of the SPINK1 N34S Mutation in Alcoholic Chronic Pancreatitis in Romania: A Clinical Analysis Based on the Criteria of the MANNHEIM Classification

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**Background and Aims:** The N34S mutation in the serine protease inhibitor Kazal type I (SPINK1) gene has been associated with chronic pancreatitis. Clinical data about the phenotypic expression of alcoholic chronic pancreatitis with the N34S variant are limited. The prevalence of the N34S mutation in patients with chronic pancreatitis and healthy individuals from Eastern Europe is unknown. We studied Romanian patients with chronic pancreatitis and investigated the clinical presentation in patients with N34S mutation.

**Methods:** The SPINK1 N34S variant was analyzed in 80 patients with chronic alcoholic pancreatitis and 96 healthy controls by an allele specific PCR method and a restriction fragment length polymorphism method. For comparison of the clinical courses of the disease, patients with alcoholic chronic pancreatitis and a SPINK1 mutation were age of onset- and sex-matched with alcoholic chronic pancreatitis patients without the mutation, and were also matched according to the disease duration. The clinical course of alcoholic pancreatitis was evaluated according to the severity criteria of the MANNHEIM classification system of chronic pancreatitis.

**Results:** A heterozygous N34S mutation was found in n=1/96 healthy individuals (1%) and in n=4/80 patients (5%) with alcoholic chronic pancreatitis (p=0.301). We sex-matched 2 male N34S positive
patients with alcoholic chronic pancreatitis with available N34S negative patients according to the age at onset and to disease duration. In summary, we did not observe a more severe course of the disease in patients carrying the N34S variant. In the other two patients with N34S mutation, matching was not possible because one patient was lost to follow-up, and, for the female patient with N34S mutation, no further female patients fulfilling the required criteria were available.

**Conclusion:** The N34S mutation is associated in 5% of the patients with alcoholic chronic pancreatitis from Romania, but the mutation is of minor relevance for the clinical course of the disease.

#13 Comparison of the Mannheim Diagnostic Criteria of Autoimmune Pancreatitis with Other Diagnostic Criteria Systems

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**Background:** Different diagnostic criteria for autoimmune pancreatitis (AiP) have been developed in various centers from Europe, USA and Asia. We recently developed the Mannheim AiP Diagnostic Criteria. A consensus about the different diagnostic systems has not been reached.

**Objectives:** To compare the Mannheim AiP Diagnostic Criteria with other diagnostic systems.

**Methods:** Patients with non-alcoholic pancreatitis from our clinic (1997-2009) were studied. “Mannheim Definite AiP” is diagnosed in patients with negative pancreatic cancer work-up and fulfilling Mayo HISORt or Asian AiP Criteria; or simultaneously presenting with either typical imaging findings (CT or MRI), elevation of serum IgG4 or other autoantibodies, and disease response to steroids; or simultaneously presenting with idiopathic pancreatic disease, other autoimmune disease or features suggestive of AiP (e.g. Sjögren’s syndrome), and disease response to steroids. In patients with “Mannheim Definite AiP”, we compared the Mannheim AiP Diagnostic Criteria with Japan-, Korean-, Asian-, Mayo HISORt-, Revised Mayo HISORt- and Italian-Criteria.

**Results:** We detected “Mannheim Definite AiP” in n=21 patients. In n=5/21 patients, pancreatic histology was obtained by surgery. In only these patients, diagnosis of AiP could be established by any diagnostic system. In n=8/21 patients, the diagnosis of AiP was only achieved with the Mannheim AiP Diagnostic Criteria. In this cohort of patients, all individuals responded to steroid medication.

**Conclusions:** The Mannheim AiP Diagnostic Criteria allow the diagnosis of AIP in atypical forms of the disease.

#14 Validation of the Mannheim Classification System of Chronic Pancreatitis by Clinical Categorization of 523 Patients

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**Introduction:** The M-ANNHEIM classification represents a new classification of chronic pancreatitis (CP). It stratifies multiple (M) etiological risk factors (Alcohol (A), Nicotine (N), Nutrition (N), Heredity (H), Efferent duct factors (E), Immunity (I), Miscellaneous factors (M), differentiates various disease stages and defines different degrees of disease severity with a clinical scoring system. A validation of this system has not yet been performed.

**Aims:** To determine whether the M-ANNHEIM classification provides a meaningful clinical disease description, is useful in monitoring disease courses, and allows prognostic evaluation of disease activity.
**Methods:** Retrospective and partial prospective categorization of patients according to the MANNHEIM classification (1997 until 2007, Dept. Medicine II. Mannheim, exclusion of biliary pancreatitis).

**Results:** We identified n=523 patients (n=137 with MANNHEIM possible CP, n=386 with probable or definite CP). The MANNHEIM stages of disease significantly correlated with disease duration (p<0.0001) and the MANNHEIM severity index (p<0.0001, Spearman correlation coefficient=0.72). The MANNHEIM classification was applied to patients with autoimmune pancreatitis (n=6) at comparable points in time and was useful in monitoring disease activity. Necessity of pancreatic surgery was not significantly associated with MANNHEIM stages of disease (p=0.06), but significantly correlated with the MANNHEIM severity index (n=22 patients with indications for pancreatic surgery; MANNHEIM severity index A and B (< 11 points) in n=8/416 (2%); severity index D and E (> 15 Punkte) in n=5/28 (19%); p<0.0001).

**Conclusion:** The MANNHEIM classification unifies clinical disease description, represents a useful tool for monitoring disease activity, and allows prognostic evaluation of necessity of pancreatic surgery.

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**#15 Pancreatic Duct Compliance Following Secretin Stimulation: A Novel EUS Diagnostic Tool for Chronic Pancreatitis**

**Timothy B. Gardner, Edward D. Purich, Stuart R. Gordon**

**Background & Aim:** The EUS diagnosis of chronic pancreatitis (CP) historically relies on standard morphologic criteria, although uniform consensus does not exist as to how these criteria should be applied. We performed dynamic EUS evaluation of pancreatic duct compliance following secretin-stimulation (sEUS) along with EUS morphologic examination (EUS) and duodenal bicarbonate measurement (ePFT) in one endoscopic session. Our aims were to evaluate the feasibility of the combined examination and compare dynamic EUS measurements of pancreatic ductal compliance with duodenal [HCO₃⁻] for the diagnosis of CP.

**Methods:** Patients with suspected CP were referred for combined EUS, sEUS and ePFT examination. All patients underwent EUS morphologic examination using standard criteria and duodenal fluid [HCO₃⁻] measurement at 15, 30 and 45 minutes following secretin-stimulation. The pancreatic duct diameter was measured in the head, body and tail at baseline, and at t₂, t₄, t₆, t₈, t₁₀ minutes following secretin administration. Ductal compliance was defined as the percent change from baseline of the maximum ductal diameter following secretin stimulation. Duodenal fluid [HCO₃⁻] <80 mEq/L was considered diagnostic for CP.

**Results:** 24 patients underwent combined examination; 7 were male, the mean age was 45 years (19-73) and conscious sedation was used for all procedures. There were no complications from the combined procedures and all were completed in one endoscopic session. Linear regression analysis demonstrated fair correlation between maximum change in ductal diameter and duodenal [HCO₃⁻] (r²=0.29; see figure). Ductal measurement in the head, body and tail independently yielded similar results (r²=0.21, 0.13, and 0.22, respectively; p=0.11). Baseline ductal dilatation positively correlated with [HCO₃⁻] <80 mEq/L in the head, body and tail (pooled r²=0.10).

**Conclusions:** EUS measurement of ductal compliance following secretin stimulation correlated with duodenal fluid [HCO₃⁻] measurement. Although prospective study is warranted to further define its test characteristics, sEUS is simple to perform, and has the advantage of rapidly providing both a
The Impact of Prior Biliary Stenting on the Accuracy and Complication Rate of EUS-FNA for Diagnosing Pancreatic Adenocarcinoma

Jessica M. Fisher MD, Stuart R. Gordon MD, Timothy B. Gardner MD

Background & Aims: Patients with pancreatic head or neck masses frequently present with obstructive jaundice and may undergo biliary stent placement prior to tissue diagnosis by endoscopic ultrasound fine needle aspiration (EUS-FNA). Our aims were to determine if the presence of a biliary stent at the time of EUS-FNA made obtaining a positive diagnosis of adenocarcinoma more difficult and/or increased the EUS-associated complication rate.

Methods: A retrospective chart review of patients who underwent EUS-FNA and were diagnosed with pancreatic head or neck adenocarcinoma at our institution since 2000 was performed. Patients were stratified into three groups—those with biliary stent placed >24 hours prior to EUS-FNA, those without a stent at the time of EUS-FNA, and those with a stent placed immediately prior to EUS-FNA (same day). The primary outcomes were diagnostic yield of EUS-FNA and procedure-related complications. Groups were compared using two-tailed and ANOVA comparative testing.

Results: A total of 170 patients without stents and 98 patients with stents at the time of EUS-FNA were identified. Patients with stents were divided into those placed >24 hours prior to EUS-FNA (n=87) and those placed the same day as EUS-FNA (n=11). The groups did not differ in gender, age, size or location of mass, number of passes, needle size, stent size or type, or presence of a cytopathologist.
In patients without stents, the rate of tissue diagnosis via EUS-FNA was 92.4% (157/170), compared with a rate of 88.5% (77/87) in those with stents placed >24 hours prior to EUS-FNA (p=0.36). However, patients with stents placed immediately prior to EUS-FNA were more likely to have indeterminate results from the EUS-FNA (36.4% atypical, non-diagnostic, or suspicious for malignancy) than patients without stents (p<0.02) and patients with stents placed >24 hours prior to EUS-FNA (p<0.05). Complication rates were the same among all three groups (0.0-3.4%) and most commonly included pancreatitis (2.2%), perforation (0.7%), and bile leak (0.4%).

**Conclusion:** Prior stenting of biliary obstruction due to pancreatic adenocarcinoma does not influence the rate of tissue diagnosis if performed more than 24 hours before EUS-FNA. Lack of immediate EUS access should not preclude stent placement in appropriate patients with malignant biliary obstruction who will undergo EUS-FNA. However, stenting prior to EUS for staging is not recommended based on previous research which indicates that biliary stents decrease the accuracy of staging.

#17 Interobserver Agreement for Pancreatic EUS Determined by Back-to-Back Examinations

**Stuart R. Gordon and Timothy B. Gardner**

**Background & Aims:** Morphologic EUS examination is often considered the gold standard for the diagnosis of chronic pancreatitis (CP), although there are concerns about lack of interobserver agreement (IOA). We performed same day back-to-back EUS examinations on patients without known hepatopancreaticobiliary disease to determine IOA between experienced endosonographers for pancreatic morphology.

**Methods:** Prior to the study, participating endosonographers (each perform >500 EUS procedures annually) reached consensus on the definition of the specific EUS morphologic criteria to diagnosis CP. Patients without a personal or family history of hepatopancreaticobiliary disease referred for EUS then underwent back-to-back same day EUS examinations. Standard parenchymal and ductal evaluation was performed using a radial echoendoscope by each endosonographer, and each was blinded to the findings of the other. Cohen's kappa scores were calculated for each parenchymal and ductal feature.

**Results:** 44 patients underwent back-to-back examination. 24 were male and indications for EUS included staging of esophageal malignancy (10), submucosal mass (9), lymphadenopathy (7), GIST (4), and other (14). Both endosonographers agreed that 32% (14/44) had hyperechoic strands, 30% (13/44) had hyperechoic duct walls, 16% (7/44) had hyperechoic foci, 14% (6/44) had a dilated main pancreatic duct, 9% (4/44) had parenchymal lobularity, and 5% (2/44) had parenchymal cysts. Cohen's kappa scores are shown in the enclosed table and demonstrated "good" or better correlation only for the presence of hyperechoic strands and parenchymal cysts.

**Conclusions:** Back-to-back same day EUS examinations demonstrated a wide variation in interobserver agreement for standard pancreatic morphologic findings. These results suggest the need for improvements in the current EUS classification system of CP.

**Inter-Observer Agreement Between Endoscopists for EUS Criteria:**

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Unweighted Kappa Score</th>
<th>95% CI</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperechoic Foci</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.386</td>
<td>(0.071 to 0.700)</td>
<td>Fair</td>
</tr>
<tr>
<td>Head</td>
<td>0.229</td>
<td>(-0.088 to 0.546)</td>
<td>Fair</td>
</tr>
<tr>
<td>Body</td>
<td>0.356</td>
<td>(0.062 to 0.650)</td>
<td>Fair</td>
</tr>
<tr>
<td>Tail</td>
<td>0.399</td>
<td>(0.108 to 0.689)</td>
<td>Fair</td>
</tr>
<tr>
<td>Hyperechoic Strands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.624</td>
<td>(From 0.388 to 0.860)</td>
<td>Good</td>
</tr>
</tbody>
</table>
Comparison of EUS and CT in Assessing Loco-Regional Lymph Node Involvement, Vascular Invasion, and Resectability of Pancreatic Cancer: A Meta-Analysis

Haq Nawaz, Yi-Fan Chen, Asif Khalid, Michael K Sanders, James A Moser, Douglas Landsittle, Georgios Ioannis Papachristou

**Background:** Accurate pre-operative staging is required in patients with pancreatic cancer to optimize selection for resection. Endoscopic ultrasound (EUS) is currently utilized to assess loco-regional spread of tumor and to obtain tissue diagnosis.

**Purpose:** The aim of this systematic analysis is to assess accuracy of EUS in determining loco-regional lymph node staging (NS), vascular invasion (VI) and resectability (R) of pancreatic cancer.

**Methods:** MEDLINE and EMBase database were searched to identify published studies that assessed pre-operative NS, VI and R of pancreatic cancer using EUS. Only studies that provided data to determine sensitivity, specificity, positive (PPV) and negative predictive value (NPV) were selected. When available, data on computerized tomography (CT) accuracy were also extracted from the selected studies. The bivariate generalized linear random effects model was used to estimate the pooled summary of each outcome and 95% confidence intervals (95% CI). For evaluating the heterogeneity among studies, Q statistic and I-squared statistic were used.

**Results:** Thirty studies published from 1988 to 2008 met the inclusion criteria (Table 1). Curvilinear echo endoscopes were used in 8 studies; CT data were available in 15 studies. The study results were heterogeneous.

**Table 1. Summary of Pooled Estimates and 95% CI for EUS and CT**

<table>
<thead>
<tr>
<th></th>
<th>NS</th>
<th>VI</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pooled estimates</td>
<td>95% CI</td>
<td>Pooled estimates</td>
</tr>
<tr>
<td><strong>EUS</strong></td>
<td>n=502</td>
<td>0.50-0.84</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>n=904</td>
<td>0.68-0.86</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>n=377</td>
<td>0.71-0.87</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>n=247</td>
<td>0.55-0.74</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>CT</strong></td>
<td>n=222</td>
<td>0.69</td>
<td>0.79</td>
</tr>
</tbody>
</table>

"Intraductal stones, irregular duct caliber and dilated side branches not included because n<2

"Not enough positive findings to warrant reporting Kappa score separately in head, body and tail

"Dilation defined as > 3 mm in head, 2 mm in body and 1 mm in tail
Conclusion: Pooled estimates show that EUS has a high PPV and NPV for diagnosing VI and R of pancreatic cancer. EUS appears to be more sensitive than CT in the detection of both NS and VI. This meta-analysis confirms that EUS is an accurate modality in assessing loco regional spread in patients with pancreas cancer and should be considered standard of care.

#19 Long-Term Success of Endoscopic Management of Smoldering Pancreatitis (SP): A Single Tertiary Center Experience.

Haq Nawaz, Dhiraj Yadav, Michael Sanders, Adam Slivka, Georgios I. Papachristou

Background: SP refers to persistent pancreatic inflammation following acute pancreatitis (AP) lasting for ≥10 days. Previous studies reported promising results following ERCP with pancreatic duct (PD) stenting (>90% symptom resolution).

Purpose: To report our experience in the management of SP patients using pancreatic rest with TPN and/or NJ-tube insertion and PD stenting.

Methods: SP patients were identified through a retrospective review of University of Pittsburgh Medical Center’s endoscopic records from 2005 to 2009. Inclusion criteria included patients with discrete episode of AP followed by all of the following lasting for >10 days from onset of AP - 1) abdominal pain requiring daily narcotics, 2) food intolerance associated with weight loss, 3) persistent serum amylase and lipase elevation, and 4) ongoing pancreatic inflammation on CT scan. Exclusion criteria included presence of large fluid collections (>5 cm), PD disruption, pancreatic necrosis, multi-organ failure and chronic pancreatitis. Success was defined by complete resolution of symptoms, lab and radiographic abnormalities.

Results: 15 patients met inclusion criteria (8M; 7F) with mean age 40.4 yrs (range 12-73 yrs) and idiopathic etiology in 8/15 (53%) cases. Five patients had a sentinel attack; while the remaining 10 patients had history of recurrent AP. Nine patients were initially treated with either NJ or TPN. The mean duration of symptoms before starting NJ or TPN was 22 days and the mean duration of either modality was 36 days. ERCP with PD stenting was performed in all patients, 8 of which also underwent pancreatic sphincterotomy. The mean duration for which PD stenting remained in place was 25 days (range 1-56 days). No ERCP complications occurred. The mean duration of follow-up was 46.5 weeks (range 9-104 wks). Long-term symptom resolution occurred in 9/15 patients (60%).

Conclusion: PD stenting can lead to symptom resolution in 60% of patients with SP by relieving functional obstruction in the pancreatic duct. This approach appears to be more successful in patients with sentinel attack of acute pancreatitis.

<table>
<thead>
<tr>
<th>SP</th>
<th>Mean Age (yrs)</th>
<th>Male Sex</th>
<th>Etiology (N)</th>
<th>Mean Symptom Duration Before ERCP (days)</th>
<th>Abnormal PD on ERCP</th>
<th>Short-term symptom resolution (%)</th>
<th>Mean Duration of Follow-up (wks)</th>
<th>Long term Symptom Resolution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sentinel Attack</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=5</td>
<td>41</td>
<td>4</td>
<td>Idiopathic (4) Alcohol (1)</td>
<td>63</td>
<td>2</td>
<td>4 (80%)</td>
<td>51</td>
<td>4 (80%) *</td>
</tr>
<tr>
<td>Recurrent Attack</td>
<td>40</td>
<td>4</td>
<td>Idiopathic (4) Cystic</td>
<td>81</td>
<td>1</td>
<td>6 (60%)</td>
<td>44</td>
<td>5 (50%) *</td>
</tr>
</tbody>
</table>
**Leptin Levels Decrease in Acute Pancreatitis**

Anna Evans, Jessica Larusch, Michael O’Connell, Georgios Papachristou, David Whitcomb

**Background:** Acute pancreatitis (AP) is an acute inflammatory event that originates within the pancreas. Most patients develop mild acute pancreatitis (MAP). However, approximately 10%-20% of patients experience an exaggerated inflammatory response that results in severe acute pancreatitis (SAP) and can lead to systemic inflammation, organ failure, and death. Obesity (defined as BMI≥30) is a known risk factor for developing SAP. Adipokines, including leptin, are released from adipose tissue and contribute to the inflammatory response to injury. As an adipokine, leptin levels are increased in obesity. An increase in serum leptin during an episode of SAP would suggest that leptin may not only be part of the proinflammatory cascade and worsen severity, but that it may mediate the effects of obesity in SAP. Suppression of leptin levels during SAP would suggest that the suppression or suspension of the biological effects of leptin occurs during systemic inflammation.

**Objective:** Determine if serum leptin levels are affected by an acute mild or severe inflammatory condition such as Acute Pancreatitis, and to determine if changes are associated with genetic polymorphisms.

**Methods:** 230 consecutive consenting patients with AP were enrolled into the SAPS/PROOF study upon admission to three medical centers in the Pittsburgh area. Additionally, 448 patients with recurrent AP (RAP) and 383 controls were selected from the NAPS2 study, which uses similar ascertainment tools. Blood samples were collected for DNA, serum and plasma. Serum and plasma leptin levels were measured using standard Luminex assay. TaqMan primers were developed for 9 functional leptin gene-associated SNPs selected from HapMap CEU including rs7799039 A>G, rs2167270 G>A, rs4731427 C>T, rs1137101 A>G, rs17151919 G>A, rs1800583 A>G, rs13306517 G>A, rs28954113 T>C, and rs1800564 A>G. Genotypes were constructed, evaluated for haplotypes, and correlated with patient data.

**Results:** Of the 644 patients with AP, there were 487 with RAP, 131 with MAP, 43 with SAP, and 23 with AP of undetermined severity. No associations between any individual SNP and AP susceptibility were found. Examination of the four most common haplotypes (GGTG, AGTA, AATA, and AGTG respectively) showed no significant difference in susceptibility. 68 patients had serum leptin levels available for the early phase of AP; these were compared to 26 controls. Serum leptin levels increased with BMI in the control patients (p<0.00078). Serum leptin levels in pancreatitis patients (mean: 18.09, SD: 19.32, range: 0.13-125.5) were significantly lower than control patients (mean: 28.54, SD: 20.91, Range: 3.08-87.44) (p=0.012). Of note, the BMI for pancreatitis patients with serum leptin levels was significantly higher (Mean 30.14) than that of control patients (Mean 25.1) (p=0.0003, 95% CI = -7.97, -2.05).

**Discussion:** Leptin is an important pro-inflammatory adipokine. Functional leptin polymorphisms do not appear to alter the risk of developing AP. Serum leptin levels were shown to increase with BMI in control patients, and the pancreatitis patients had higher BMI levels than the control patients. However, serum leptin levels were shown to decrease in pancreatitis patients compared to controls. Leptin levels appear to be suppressed during severe acute pancreatitis.
#21 Evaluation of Peroxiredoxin-4 as a Severity Marker for Acute Pancreatitis

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¹Department of Medicine A, Ernst-Moritz-Arndt-University Greifswald, Germany
²Brahms AG, Hennigsdorf, Germany

Introduction: Acute pancreatitis (AP) is associated with a highly variable clinical course during which 10-20% of patients develop severe disease with single or multiple organ failure. Discriminating between mild and severe cases on hospital admission would allow for appropriate triage to an intensive care unit or early treatment. Here we tested whether Peroxiredoxin-4 (Prdx4), an oxidative-stress-marker elevated in sepsis and other types of inflammation, can predict severity in acute pancreatitis.

Methods: We measured levels of Peroxiredoxin-4 in serum samples of patients suffering from mild (n=106) or severe (n=16) acute pancreatitis and in 138 healthy blood donors. Blood was drawn on admission and daily throughout the first 3 days of hospitalization and labelled with reference to the day of pain onset. Prdx4 was analysed by an immunoluminometric assay.

Results: At day 1 after disease onset Prdx4 was already significantly elevated in pancreatitis patients in comparison to controls (3.92 vs. 0.83 U/L). A significant difference in Prdx4 levels between mild and severe acute pancreatitis was not detectable until day 3 after the onset of pain. CRP levels, in comparison, could discriminate between mild and severe pancreatitis as early as 48h after the onset of pain. At later time points (days 3 to 5) Prdx4 and CRP performed equally well in discriminating between mild and severe acute pancreatitis.

Conclusions: Peroxiredoxin-4 levels rise significantly during acute pancreatitis. Discrimination between mild and severe pancreatitis using Peroxiredoxin-4 levels is possible - but 24h later than with CRP, an already established severity marker for acute pancreatitis.

#22 Asparagine-Linked Glycosylation of Human Chymotrypsinogen C (CTRC)

Melinda Bence, Miklós Sahin-Tóth

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Background and Aims: Mutations in the digestive proenzyme chymotrypsinogen C (CTRC) increase the risk for chronic pancreatitis. The mutations cause misfolding and diminished secretion, thereby decreasing the protective trypsin-degrading activity of CTRC in the pancreas. In addition, misfolding of CTRC mutants can cause endoplasmic reticulum (ER) stress, which may contribute to acinar cell damage through induction of apoptosis. The aim of the present study was to determine whether or not human CTRC undergoes Asn-linked glycosylation and to examine the role of this modification in CTRC folding and function.

Methods: Potential sites of Asn-linked glycosylation (Asn-Xaa-Ser/Thr) in human CTRC were eliminated by mutating the Asn residues to Ser individually or in combination. CTRC mutants were expressed in HEK 293T and AR42J cells. PNGase F and endoglycosidase H were utilized to determine the glycosylation state of CTRC mutants.

Results: Human CTRC contains a single Asn-linked glycan on Asn52. Mutation of Asn52 (N52S) has no effect on CTRC activity but reduces CTRC secretion about 10-fold. Decreased secretion is probably due to misfolding and degradation of unglycosylated CTRC in the ER, as overexpression of the N52S CTRC mutant elicits ER stress in AR42J acinar cells. Despite its critical role, Asn52 is not conserved in
rat CTRC, which is glycosylated on Asn90. Introduction of Asn90 into the N52S human CTRC mutant restored full glycosylation of CTRC but increased secretion only by 4-fold.

**Conclusion:** Asn-linked glycosylation of human CTRC is required for efficient folding and secretion, however, the Asn-linked glycan is unimportant for activity or inhibitor binding. Comparative studies with rat CTRC revealed that the position of the Asn-linked glycan is critical for optimal folding, and it may vary among the otherwise highly homologous mammalian CTRC sequences.

#23 Hypertriglyceridemia Independent Propofol-Induced Pancreatitis

**Thiruvengadam Muniraj MD, Sabitha Vignesh MD, Sudha Thiruvengadam MD, Prathab Devaraj MD, Mona Parikh MD, Steven Ganchuk BPharm**

UPMC Mercy Hospital - Pittsburgh - PA 15219

**Introduction:** The following describes a case of propofol associated acute pancreatitis in an intensive care patient without any elevations of serum triglyceride levels. The acute pancreatitis resolved with discontinuation of propofol and reoccurred with its reintroduction.

**Case:** 71-year old hypertensive male was found to have an acute right middle cerebral artery stroke. After appropriate intervention with a right carotid artery stent, the patient was started on eptifibatide drip, but developed hemothysis and inability to protect his airway, requiring an intensive care unit admission, intubation, and a mechanical ventilator. Propofol infusion was started for sedation and continued for 6 days to a total dose of 8.8 g. On the fifth day, patient developed epigastric tenderness and abdominal distension. Serum amylase and lipase were elevated at 1062 and 1911 units/dL with a normal serum triglyceride level of 102 mg/dL. On day 7, the patient was extubated and amylase and lipase levels normalized to 107 and 46 units the next day.

<table>
<thead>
<tr>
<th>Day</th>
<th>Amylase</th>
<th>Lipase</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>55</td>
<td>33</td>
<td>82</td>
</tr>
<tr>
<td>5</td>
<td>1062</td>
<td>1911</td>
<td>102</td>
</tr>
<tr>
<td>7</td>
<td>145</td>
<td>83</td>
<td>88</td>
</tr>
<tr>
<td>9</td>
<td>212</td>
<td>133</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>108</td>
<td>51</td>
<td>72</td>
</tr>
<tr>
<td>15</td>
<td>572</td>
<td>767</td>
<td>80</td>
</tr>
<tr>
<td>17</td>
<td>107</td>
<td>46</td>
<td>101</td>
</tr>
</tbody>
</table>

Due to persistent hypertension and agitation, propofol was carefully reintroduced. His amylase and lipase increased to 572 and 767 mg/dl within 24 hours after restarting propofol, but triglyceride level continued to remain normal at 80mg/dl. Propofol was discontinued and the enzyme levels normalized to 107 and 46 units the next day.

**Discussion:** Drug-induced pancreatitis is rare but should be considered in patients who present with idiopathic pancreatitis. Around 100 drugs have been reported to cause via various mechanisms. Propofol has been speculated to cause pancreatitis indirectly by inducing hypertriglyceridemia, which leads to an increase in pancreatic lipase in capillaries, resulting in lipolysis, ischemia, capillary damage, and microthrombi. Further release of lipase perpetuates the inflammatory cycle. However, Propofol-associated pancreatitis has been reported to occur in the absence of hypertriglyceridemia, especially after a single bolus during induction of anesthesia. In this patient, both the initial and second bout
following re-challenge of propofol was not associated with significant increase in serum triglycerides. This suggests that propofol could cause pancreatitis by mechanisms other than hypertriglyceridemia.

#24 Glycemic Characteristics in Continuously Monitored Patients with Pancreatic Diabetes Treating with Pancreatic Enzyme Replacement Therapy

Yusuke Tando¹, Yuki Matsuhashi¹, Akihito Kon¹, Shinji Chikazawa¹, Atsufumi Matsumoto¹, Eri Sato¹, Miyuki Yanagimachi¹, Toshihiro Suda¹, Teruo Nakamura²

¹Department of Endocrinology and Metabolism, Hirosaki University Graduate School of Medicine
²Healthcareunit, Hirosaki University School of Medicine

Despite recent advances in therapy, achieving stable glycemic control may be difficult for patients with pancreatic diabetes.

Objectives: To investigate daily patterns of glycemic excursions in patients with pancreatic diabetes monitored continuously for a maximum period of 3 days, and to clarify the relationship between glucose profiles and pancreatic enzyme replacement therapy on these patients.

Methods: Eight patients with pancreatic diabetes attached continuous glucose monitoring (CGM) system (MiniMed) and data derived from CGM system for 72 h were compared to fingerstick glucose measurements (4-6 times a day). During continuous monitoring, patients documented the timing of food intake, insulin injections and hypoglycemic events. Moreover, serial glucose measurements were divided into periods of euglycemia (70–140 mg/dl), hyperglycemia (>140 mg/dl), and hypoglycemia (<70 mg/dl). The proportions of time patients were hypoglycemic, euglycemic, and hyperglycemic and the total areas under the curves (AUCs) were determined.

Results: All patients (46-84 y.o) were treated by insulin (14-54 U/day) and pancreatic enzyme (3-12 g/day). Patients remained in the euglycemic range for ~42% of the total day, were hypoglycemic 20%, and were hyperglycemic 38%. Hypoglycemia was more prevalent nocturnally and hyperglycemia postprandial. The mean time of undetected hyperglycemia was 82 +/- 6 min/day. Six patients had nocturnal hypoglycemia on at least one of the three nights of monitoring. A change in insulin dosage was made in some patients on the basis of the data provided by continuous glucose monitoring. Increasing in dose of pancreatic enzyme supplement prolonged time to reach a maximum glucose level in postprandial.

Conclusion: Continuous glucose monitoring is helpful for monitoring patients with pancreatic diabetes and for adjusting diabetes therapy. It can accurately detect not only high postprandial blood glucose levels but also time to reach a maximum glucose level affected by pancreatic enzyme supplements. Moreover, it is also able to detect nocturnal hypoglycemic events that may go unrecognized by intermittent blood glucose monitoring.

#25 Pancreatic Enzyme Products: A Regulatory Overview

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Purpose: Until recently, pancreatic enzyme products (PEPs) were marketed without formal FDA review under a new drug application (NDA). FDA became aware of bioavailability problems with these products which led to concerns about the potential for under- and over-treatment of patients. As a result, FDA used its authority to regulate PEPs and educate manufacturers on how to obtain agency approval for their products.
Methods: In July 1991, FDA proposed that all pancreatic extract drug products are new drugs, requiring an approved NDA for marketing. A final rule in this regard was published in the Federal Register of April 24, 1995 (60 FR 20162).

To ensure that all PEPs sold in the United States meet FDA’s standards for safety, effectiveness, and product quality, the FDA notified PEP manufacturers in 2004 that these products were required to have an approved NDA in order to continue to be sold (April 2004 Federal Register notice). Manufacturers were informed that they had four years to obtain FDA approval of their PEP products. In 2006, the FDA issued a guidance to assist manufacturers in preparing and submitting applications for approval. In 2007, FDA extended the deadline to April 28, 2010 for the makers of unapproved pancreatic enzyme products to stop manufacturing and distributing unapproved products (October 2007 Federal Register notice).

Results: As of May 2010, FDA approved three PEPs because they meet the regulatory standards for quality, safety, and effectiveness. Creon and Zenpep were approved for marketing in 2009 and Pancreaze was approved in April 2010. All approved PEP products have drug labels with important information for healthcare professionals and Medication Guides that explain the product’s risks and benefits for patients.

Conclusion: Despite FDA’s attempts to provide technical assistance to all manufacturers of pancreatic enzyme products, some manufacturers did not receive FDA approval prior to the April 28, 2010 deadline. Currently, the FDA is working closely with manufacturers of such products to ensure that the products meet FDA standards. The Center for Drug Evaluation and Research and the Office of Special Health Issues (OSHI) are working together to maintain active communication with the exocrine pancreatic insufficiency disease community. OSHI has received ongoing communications from patients, families, advocates, and health professionals expressing both concerns and reassurances regarding diminishing access to currently-unapproved PEPs and the ability of patients to transition to the approved PEPs. Supplies of approved PEPs are expected to meet demand. Protecting patients’ health is a priority for FDA, and the agency will continue to facilitate the successful transition of unapproved PEPs to approved status.

#26 Pathologic Protease Activity is Reduced in Calcineurin-A beta Knockout Mice

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The premature activation of digestive proenzymes, specifically proteases, within the pancreatic acinar cell is an early and critical event during acute pancreatitis. Our previous studies demonstrate that this activation requires a distinct pathologic rise in cytosolic Ca\(^{2+}\). Further, we have previously shown in pharmacological studies that a target of aberrant Ca\(^{2+}\) in acinar cells is the Ca\(^{2+}\)/calmodulin-dependent phosphatase calcineurin (CN). CN inhibition reduced the severity of pancreatitis both in vitro and in vivo.

In this current study, we hypothesized that CN within acinar cells is responsible for the initiation of the pancreatitis response. To test this hypothesis, we obtained mice deficient in the pancreas-predominant isoform -A beta and examined whether acinar cells from these mice were protected from pancreatitis. Acinar cells were freshly isolated from calcineurin-A beta (CN-A\(\beta\)) deficient mice. PCR was performed on RNA extracts. Live cells were stimulated for 1 hr with high concentrations of the Ca\(^{2+}\)-activating and pancreatitis-inducing secretagogues caerulein (100 nM; CCK analog) and carbachol (1 mM; Ach analog). Pancreatic enzyme activity and total content were measured. Acinar cells lacking CN-A\(\beta\) exhibited a 60% and 93% reduction in chymotrypsin activity when stimulated with caerulein and carbachol, respectively (n=3; P<0.05). This reduction was seen even at 2 hr post-induction. No significant differences in amylase secretion were observed. Interestingly, total enzyme content per volume of cells was reduced as well as zymogen granule area in pancreatic tissue sections. Nevertheless, chymotrypsin
activity relative to total chymotrypsin content was still significantly less in the CN-Aβ knockout mice compared with wildtype. These data suggest that the acinar cell predominant CN isoform, CN-Aβ, mediates pathologic protease activity during pancreatitis and may serve as an important target of the aberrant acinar cell Ca\(^{2+}\) rise associated with this disease.

#27 Synergistic Role of TRPV1 and TRPA1 in Pancreatic Pain and Inflammation

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Recent studies have shown that the pancreas receives innervation from a select population of primary afferents that express receptors from the transient receptor potential (TRP) family of nonselective ion channels. These receptors, TRPV1 and TRPA1, are important not only because they appear to be required for inflammatory hyperalgesia, but because they may also be responsible for regulating efferent properties of primary afferents that initiate neurogenic inflammation. To induce inflammation, caerulein (50 µg/kg) was injected intraperitoneally (i.p.) hourly for 8 hours. Alexa Fluor-conjugated cholera toxin B (CTB-488) was injected into the head of the pancreas of adult C57BL/6 male mice to label neurons retrogradely in both dorsal root (DRG) and nodose (NG) ganglia. CTB-positive DRG (T9-T12) and NG neurons were examined for responses to application of TRPV1 and TRPA1 agonists capsaicin and mustard oil (MO) and antagonists respectively, using calcium imaging. Single cell PCR and RT-qPCR was used to verify the presence of these molecules in pancreatic DRG and NG neurons. In addition, the present study shows that in a caerulein model of pancreatitis, pancreatic afferents arising from both the NG and DRG exhibit changes in membrane properties indicative of increased excitability (decreases in resting potential and rheobase). In addition, there is an increase in the number of neurons expressing functional TRPV1 and TRPA1 channels, and the response of these receptors to their specific ligands is enhanced. That these changes in afferent phenotype contribute to pancreatitis is demonstrated by the ability of TRPV1 and TRPA1 antagonists to block caerulein-induced inflammation. These antagonists are effective at blocking inflammation individually and also exhibit synergy when combined. Not only can these antagonists block pancreatic inflammation, but they also reverse changes in pain-like behavior in a manner similar to low dose morphine. These results suggests TRPV1 and TRPA1 antagonists may be effective at blocking as well as efficacious in preventing or reversing the inflammatory processes that drive pancreatitis.

Acknowledgements: Supported by T32 DK063922 and ROIs NS019912 (GFG), NS050758 (BMD), and NS033730 (KMA)

#28 Neuroplastic Changes in a Mouse Model of Pancreatic Ductal Adenocarcinoma (PDAC).

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Abstract Body: Perineural tumor invasion of intrapancreatic nerves and extrapancreatic nerve plexuses is a key feature of pancreatic malignancies and is thought to play an important role in
pancreatic cancer-related pain and cancer spread. Here we provide a preliminary description of changes in pancreatic innervation in a mouse model of PDAC that will allow us to examine the mechanisms underlying cancer-induced pathophysiology of the peripheral nervous system. Several neurotrophic factors, including nerve growth factor (NGF) and members of the glial cell line-derived neurotrophic factor (GDNF) family of growth factors, and their receptors can be found in the pancreas and are increased in human pancreatic tumors. These growth factors are capable of inducing neuronal outgrowth and may contribute to sprouting and hypertrophy of pancreatic nerves, which is one of the hallmarks of human PDAC. We hypothesized that Artemin, a member of the GDNF family of growth factors, and NGF play a central role in this cross-talk between cancer cells and pancreatic nerves. Experiments were performed with transgenic mice with pancreas-specific expression of the mutated Kras oncogene and heterozygous deletion of the p53 tumor suppressor (Kras). Animals were sacrificed at time points ranging from 13.9-17.4 weeks and tissue was retrieved for histological analysis and assessment of growth factor expression using real time PCR. All Kras mice had developed multifocal pancreatic cancer (by week 13) as evidenced by large masses, obstruction of the biliary tree (by week 17) and a strong desmoplastic reaction. Normal pancreatic innervation was altered with hypertrophied nerves running along blood vessels and extending into the pancreatic parenchyma and tumor nodules, but not into the fibrous tissue. Immunohistochemical experiments showed prominent expression of tyrosine hydroxylase and the Artemin receptor GFRα3 on nerve fibers. Compared to wildtype mice, the expression of NGF and its receptor trkA was increased 3.22-fold and 8.85-fold, respectively, in cancer animals. While not statistically significant, a 1.99-fold increase in Artemin expression was measured in the Kras animals. Finally, a 2.27-fold decrease in the expression of GFRα3 in cancer animals was observed. These data indicate significant neuroplastic changes occur in the Kras model of pancreatic cancer and these changes correlate with altered expression of growth factors, mimicking data obtained in human resection specimens. Thus, this animal model will enable us to systemically study the impact of changes in trophic factor signaling on cancer growth and animal behavior.

#29 Significant Association between ABO Blood Group and Pancreatic Cancer

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Background: Pancreatic adenocarcinoma is a rapidly fatal disease with few established risk factors and no effective methods of screening or prevention. Recent evidence from case-control and prospective studies suggests that the ABO blood group may be associated with the risk of developing pancreatic cancer.

Aims: In an effort to further discern how pancreatic cancer and ABO blood group are associated, we compared the serologically-determined ABO blood group of pancreatic cancer patients with the ABO group of Central Blood Bank (CBB) donors in Pittsburgh.

Methods: The donor database of the CBB in Pittsburgh, Pennsylvania was queried for the ABO group of individual donors. The CBB collects blood mainly in southwestern Pennsylvania. Between 1979 through 2009 there were 708,842 unique blood donors. Pancreatic cancer patients from the University of Pittsburgh’s affiliated hospitals who provided informed consent to be part of their pancreatic cancer research registry were included in this study. Of 359 eligible pancreatic cancer patients in the registry, 274 had a historical or current ABO blood group available after querying the Centralized Transfusion
Service’s (CTS) database, which includes all patients who have a type and screen performed at Pittsburgh-area hospitals. FDA approved manual and automated ABO grouping methods and reagents were employed at the CBB and at the CTS. Proportions of ABO blood groups for pancreatic cancer patients and blood donors were compared using Chi-squared analysis.

Results: The frequency of group A was statistically significantly higher amongst pancreatic cancer patients compared to its frequency amongst the regional blood donors [odds ratio (OR) = 1.43 (P = 0.004)]. Conversely, the frequency of group O was significantly lower amongst pancreatic cancer patients relative to community blood donors (OR =0.60; P = 0.00007). There were limited group B (n = 38) and AB (n = 17) pancreatic cancer patients; the overall P trend value comparing patient to donor blood groups was 0.001.

Summary/Conclusion: Our study demonstrates that blood group A individuals in southwestern Pennsylvania are significantly more likely to develop pancreatic cancer, and those with blood group O significantly less likely to develop pancreatic cancer, than expected by chance alone. In a prospective cohort study of 107,503 United States residents, individuals with blood group A, B, and AB, had an elevated risk of pancreatic cancer compared to participants with blood group O. Notably, blood group was self-reported in that prospective study. In our study, the presence of serologically-determined blood group information from the blood bank was an entry criterion; thus, there was no possibility of recall bias influencing our results. ABO blood group is genetically-determined and therefore is not a modifiable risk factor for pancreatic cancer like cigarette smoking, body mass index, diet or lifestyle. Combinations of multiple risk factors, including ABO blood group, could be used to calculate whether some individuals are at high enough risk to warrant counseling for risk reduction strategies or inclusion in screening trials. Although the mechanisms of pancreatic cancer oncogenesis have not been fully deciphered, the association of ABO blood group with pancreatic cancer risk has been confirmed.

#30 Elimination of Cancer Stem Cells in Pancreatic Cancer Cell Lines by Combinatorial Therapy

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Key words: cancer stem cells, ALDH, CTL, pancreatic cancer

*This work was supported by the Hirshberg Foundation

Abstract: Cancer Stem Cells (CSC) are tumor initiating cells that are frequently resistant to chemotherapy and radio therapies. ALDH bright cells have been shown to be a marker of Cancer Stem Cells in different tumors including pancreatic adenocarcinoma. Hedgehog (Hh) signaling pathway plays a critical role in invasion and metastasis in pancreatic cancer. Recent evidence suggests that Hh may be specifically overexpressed in ALDHbright cells. In the past, we have been able to generate ALDH1A188-96 specific T cells. Now we are able to target pancreatic cancer stem cells with ALDH1A188-96 peptide-specific T cells in combination with cyclopamine, an inhibitor of Hh signaling pathway. We have used FACS analysis to identify and sort ALDHbright cells in different pancreatic cell lines. We characterized stem/progenitor cells in pancreatic cell lines. We found that ALDHbright cells were highly tumorigenic in NOD/SCID mice. The reactivity of the ALDH1A188-96 peptide-specific T cells was tested “in vitro” in flow-based cytotoxicity assays. ALDH bright cells were targeted in vitro for Immunotherapy with ALDH1A188-96 peptide-specific T cells and cyclopamine. We are able to target ALDH bright cells for immunotherapy utilizing ALDH1A1 specific T cells in combination with the Hh inhibitor cyclopamine and this may result in control of tumor growth and metastasis in pancreatic tumors.