The American Pancreatic Association/Hirshberg Foundation Symposium Celebrating the 10th Anniversary of the Seed Grant Program

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The Hirshberg Foundation for Pancreatic Cancer Research was founded by Agi Hirshberg in 1997, following her husband Ronald Hirshberg's 8-month, 7-day battle with the disease. (<http://www.pancreatic.org/>). At the time, the Hirshberg family viewed pancreatic cancer as the "unknown enemy" (http://www.pancreatic.org/site/c.htJYJ8MPIwE/b.8276835/k.66CB/Agi_Hirshbergs_Story.htm). In the years since Ronald Hirshberg's death, Agi Hirshberg has made it her mission to unmask the unknown for others facing the same enemy.

When patients present with a new diagnosis of pancreatic cancer, most are already in an advanced stage of the disease. Pancreatic cancer is resistant to most chemotherapeutic agents and radiotherapy. These factors combine to make pancreatic cancer a highly lethal disease, and according to the National Cancer Institute (NCI) 2013 estimates, pancreatic cancer was diagnosed in approximately 45,220 US citizens, and some 38,460 died of the disease.¹ The Annual Report to the Nation on the Status of Cancer, 1975–2009, shows that overall cancer death rates continued to decline in the United States among both men and women, among all major racial and ethnic groups, and for all of the many common cancer sites, including lung, colon and rectum, female breast, and prostate. However, this trend does not hold true for pancreatic cancer. The report demonstrates that death rates continued to increase during the latest time period (2000 through 2009) for pancreatic cancer.² Only about 23% of patients with cancer of the pancreas will still be living 1 year after diagnosis, and only about 4% will survive 5 years. Even for those diagnosed with pancreatic cancer that has not spread to other organs or systems, the overall 5-year survival rate approaches only 15%.

The Hirshberg Foundation's vision is to advance pancreatic cancer research and provide information, resources, and support to pancreatic cancer patients and their families. The mission of the Foundation is as follows:

- To find a cure for pancreatic cancer in honor of Ron Hirshberg and the thousands of people who are diagnosed with this disease each year.
- To create a premier Pancreatic Cancer Center where all needs of pancreatic cancer patients can be met in one location with the most advanced treatment options.

- To be recognized as a patient support reference source for pancreatic cancer patients and their families.
- To fund projects and programs designed to improve patient care, treatment, and, ultimately, pancreatic cancer survival rates.
- To integrate and unite generations, young and old, through physical fitness participation, while creating public awareness and raising money to find a cure for pancreatic cancer.³

The Foundation began by funding 2 key research projects at the David Geffen School of Medicine at the University of California, Los Angeles (UCLA). The Foundation first established the Ronald S. Hirshberg Translational Pancreatic Cancer Research Laboratory under the directorship of Dr Howard Reber and then created the Ronald S. Hirshberg Chair in Translational Pancreatic Cancer Research, which was awarded to Prof Enrique Rozengurt. Since then, the Foundation has continued its research commitment to the pancreatic cancer research program at UCLA.

HIRSHBERG FOUNDATION SEED GRANT PROGRAM

In 2003, the Foundation launched its national Seed Grant program. Ms Agi Hirshberg stated that the Hirshberg Foundation's efforts have "significantly raised awareness and understanding of the biology, treatment, and prevention of pancreatic cancer, a disease that continues to be one of the most fatal and least funded of all cancer types."⁴ The NCI spent only 0.9% (\$42,300,000) of its approximate \$4.6 billion cancer research budget on pancreatic cancer in 2003, when the program began.⁵ The aim of the program is to increase interest and investment in research by recruiting established scientists and encouraging new investigators to enter the field. The seed grants are designed to allow researchers in all fields to develop the preliminary data necessary to pursue additional funding from the National Institutes of Health (NIH) and other funding agencies. The Foundation established a national Scientific Advisory Board chaired by Vay Liang W. (Bill) Go, with Laszlo G. Boros, MD; Murray Korc, MD; Laurence J. Miller, MD; Stephen J. Pandol, MD; Howard A. Reber, MD; Lee S. Rosen, MD; and Diane M. Simeone, MD. Ashok Saluja, PhD and Anirban Maitra, MD, joined the board in 2014.

The number of seed grant applications has quadrupled since the initial 12 applications received in 2003. The Scientific Advisory Committee reviews and assigns priority scores for all submitted seed grant applications based on the NIH review and funding guidelines, and the Foundation makes the final award decision. The seed grant recipients are listed in Table 1. To date, the program has provided more than \$5 million in seed grants to research programs involving the molecular mechanism of pancreatic cancer, early diagnosis, surgical and chemotherapeutic management, psychosocial approaches, and prevention strategies. The yearly recipients are invited to present their research at the annual Agi Hirshberg Symposium on pancreatic cancer research held at the UCLA Faculty Center. Previous

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presentations and videos are available on the Hirshberg Foundation Web site. The Hirshberg Seed Grant recipients have contributed significantly to pancreatic cancer research and have generated more than \$45 million in additional grant support from the NIH and other funding agencies to support their research programs to date.

The Foundation has funded Seed Grant Award recipients at such institutions as Beth Israel Medical Center; City of Hope; Columbia University; Dana-Farber Cancer Institute; Duke University Medical Center; Fox Chase Cancer Center; Harbor-UCLA Medical Center; Harvard Medical School; Johns Hopkins University; Loma Linda University; Massachusetts General Hospital; Mayo Clinic and Mayo College of Medicine; MD Anderson; Memorial Sloan-Kettering Cancer Center; Tel Aviv University; Thomas Jefferson University; University of Arizona Cancer Center; University of California, Los Angeles; University of California, San Diego; University of Southern California; University of Minnesota; University of Pittsburgh; and Vanderbilt Medical School. Highlights of some supported studies include genetic markers in saliva associated with pancreatic cancer, the potential role of fructose in helping pancreatic cancer cells to multiply, the role of the antidiabetic drug, metformin, on inhibiting pancreatic cancer cell growth, key molecular and cellular pathways that control pancreatic cancer cell growth and survival, the microenvironment for pancreatic cancer cell growth and the interaction with inflammatory processes, the efficacy of phytonutrients to inhibit pancreatic cancer development and growth in preclinical models and human clinical trial studies, and the role of dietary factors, obesity, and diabetes mellitus in pancreatic cancer prevention. The Seed Grant program has fulfilled its mission by bringing new investigators into the field and also promoting collaboration between investigators from different disciplines. The Seed Grant program has been an important part of this effort during the last 10 years and will continue to provide an essential contribution to pancreatic cancer research in the years to come.

THE AMERICAN PANCREATIC ASSOCIATION/ HIRSHBERG FOUNDATION SYMPOSIUM CELEBRATING THE 10TH ANNIVERSARY OF THE SEED GRANT PROGRAM

The 10th Annual Agi Hirshberg Symposium was presented in 2 parts. The scientific portion occurred at the 44th annual meeting of the American Pancreatic Association (APA) in Miami, Fla, from October 30 to November 2, 2013. The patient support portion was held at the UCLA Faculty Center on February 8, 2014, in collaboration with the UCLA Center for Pancreatic Diseases. The patient support symposium focused on the current state of the art in pancreatic cancer management.

The 2013 APA annual meeting started with the Hirshberg Foundation Key Note Symposium on October 30, 2013, which focused on paraneoplastic metabolic complications of pancreatic cancer and followed with the celebration of the 10th anniversary of the Foundation seed grant program on November 2, 2013. Previous seed grant awardees were selected to present their scientific contributions on the mechanics of carcinogenesis, therapeutic advances, and the early detection and prevention of pancreatic cancer.

HIRSHBERG FOUNDATION KEYNOTE SYMPOSIUM PARANEOPLASTIC METABOLIC COMPLICATIONS OF PANCREATIC CANCER

The 2013 APA annual meeting started with the Hirshberg Foundation keynote symposium on paraneoplastic metabolic

complications of pancreatic cancer. Agi Hirshberg began the proceedings by outlining the history of the Foundation with an emphasis on the important partnerships with the APA, European Pancreatic Club, and the Japan Pancreas Society. Suresh Chari, MD, of the Mayo Clinic spoke on paraneoplastic diabetes in pancreatic cancer followed by Michael J. Tisdale, PhD, from Aston University, Birmingham, United Kingdom, on the mechanism and treatment of cachexia in patients with pancreatic cancer and concluded with F. Charles Brunicaudi, MD, PhD, from UCLA on the vertically integrated translational studies of pancreatic and duodenal homeobox 1 (PDX1) as a therapeutic target for pancreatic cancer via a novel bifunctional RNAi platform. Here are the highlights of their respective presentations.

Paraneoplastic Diabetes in Pancreatic Cancer by Suresh T. Chari, MD, Mayo Clinic College of Medicine, Rochester, Minn

There is growing evidence that pancreatic cancer causes diabetes. About 50% to 67% of pancreatic cancer patients have diabetes, and another 35% have impaired glucose tolerance. Diabetes usually manifests in the 3 years before pancreatic cancer diagnosis. New-onset diabetes in pancreatic cancer resolves with cancer resection. Conversely, the risk of pancreatic cancer in new-onset diabetes is about 8-fold higher than in the general population. Epidemiologic and experimental evidence strongly suggests that diabetes in pancreatic cancer is a paraneoplastic phenomenon caused by tumor secreted products. β Cell dysfunction and peripheral insulin resistance are seen in type 2 diabetes as well as pancreatic cancer-associated diabetes. However, in contrast to type 2 diabetes, onset and progression of glucose intolerance in pancreatic cancer-induced diabetes occur paradoxically in the face of ongoing, often profound, weight loss. Recently, adrenomedullin, which is overexpressed in pancreatic cancer, was reported as a potential mediator of β -cell dysfunction. Selective loss of subcutaneous adipose tissue with preservation of visceral adipose tissue may explain lack of improvement in diabetes despite weight loss. We believe that the extremely high prevalence of hyperglycemia in pancreatic cancer is not accidental or incidental, but rather an adaptive mechanism developed by the tumor to counter a hostile tumor microenvironment low in oxygen and glucose. Understanding the mechanism and mediators of pancreatic cancer-induced diabetes mellitus could lead to identification of novel biomarkers for early diagnosis of the cancer. However, to cost-effectively screen for pancreatic cancer, the cohort of new-onset diabetes will need further enrichment for pancreatic cancer. In a Mayo study (EXamination of the PANcreas in New Diabetes [EXPAND]), we will identify 1000 subjects older than 50 years with new-onset diabetes ("first sieve"), of whom 100 with weight loss and/or elevated CA-19-9 ("second sieve") will be screened for pancreatic cancer using endoscopic ultrasound. To rapidly bring this strategy to clinical practice, additional studies better defining the first and second sieves are urgently needed.

Mechanism and Treatment of Cachexia in Patients With Pancreatic Cancer by Michael J. Tisdale, PhD, School of Life and Health Sciences, Aston University, Birmingham, United Kingdom

Eighty-five percent of patients with pancreatic cancer experience a wasting syndrome called cachexia, with loss of adipose tissue and skeletal muscle and eventually death when the weight loss reaches 30%. There are several proposed mediators of the cachectic process including cytokines (tumor necrosis factor α , interleukin 6, interleukin 1, and interferon γ) and tumor catabolic products such as a lipid-mobilizing factor and

proteolysis-inducing factor (PIF). Our own studies have concentrated on the latter factors.

Most investigators agree that loss of body fat in cachectic subjects is due to an increase in lipolysis. Extracts of the MAC16 tumor, which produces cachexia in mice, as well as urine from patients with pancreatic cancer showed evidence for a lipid-mobilizing factor, and on purification, this was shown to be zinc- α 2-glycoprotein (ZAG), a known protein with unknown function. Zinc- α 2-glycoprotein is produced not only by cachexia-inducing tumors, but also by adipose tissue (both white [WAT] and brown [BAT]), and mRNA for ZAG is increased up to 10-fold in animals and cancer patients with cachexia. In contrast, expression is low in obese humans. Zinc- α 2-glycoprotein mRNA shows a negative correlation with body mass index, but a positive correlation with weight loss. In obese mice, ZAG produces selective loss of body fat by an increased heat generation through increased expression of uncoupling proteins (UCP-1 and UCP-3) in BAT and skeletal muscle.

Loss of skeletal muscle in cachexia arises from an increase in protein degradation and/or a decrease in synthesis. Studies on the MAC16 tumor in mice identified PIF, which is also present in the urine of patients with pancreatic cancer and lung tumors of patients with cachexia. In mice, PIF causes loss of lean body mass through an increase in protein degradation through the ubiquitin-proteasome pathway and a decrease in synthesis. Clones of a human breast tumor expressing short hairpin RNA (shRNA) to the PIF core peptide showed no expression of PIF and no loss of body weight when transplanted into nude mice, whereas the vector control produced progressive weight loss.

Eicosapentaenoic acid attenuates PIF-induced weight loss in mice and weight reduction in both experimental animals and pancreatic cancer patients with cachexia. Clinical studies show this increase in body weight is due to an increase in lean body mass.

Vertically-Integrated Translational Studies of PDX1 as a Therapeutic Target for Pancreatic Cancer Via a Novel Bifunctional RNAi Platform by F. C. Brunicardi, MD, PhD, UCLA David Geffen School of Medicine, Los Angeles, Calif

Pancreatic and duodenal homeobox 1 regulates embryonic pancreatic development, β -cell maturation, normal β -cell function, and most recently pancreatic cancer. Short hairpin RNA is synthetic molecules that mimic endogenous siRNA, involved in gene silencing. Mouse and human bifunctional (bi)-shRNAPDX1 was developed using mouse and human PDX1 gene sequences accessed via GenBank. Human pancreatic cancer cell lines, PANC1 and MIA PaCa2, as well as PDX1 overexpressing HEK293 cells were used to form xenograft tumors in SCID mice. Murine models of islet hyperplasia and insulinoma, SSTR1/5(−/−) and SCID mice with implanted β TC-6 cells, were also studied. Study mice received 3 intravenous infusions of bi-shRNAPDX1. Yucatan miniature pigs were administered a single intravenous infusion of Good Medical Practice-quality bi-shRNAPDX1 to evaluate safety in a biorelevant large animal model. Systemic infusion of human bi-shRNAPDX1 ablated the xenograft tumors in mice and led to increased survival. Systemically delivered mouse bi-shRNAPDX1 in SSTR1/5(−/−) mice and mice with insulinoma resulted in mild, temporal hyperglycemia and decreased insulin secretion, followed by return to baseline levels. Single infusion of Good Medical Practice-bi-shRNAPDX1 was well tolerated in pigs with premedication with no signs of acute toxicity. Bifunctional shRNAPDX1 suppressed PDX1

expression in pig islets but did not alter circulating insulin and glucose levels. Bifunctional shRNAPDX1 successfully treats human xenograft tumors in mice and exhibits minimal off-target effects on glucose metabolism. Future toxicology studies are planned to justify a phase I clinical trial.

APA/HIRSHBERG FOUNDATION SYMPOSIUM CELEBRATING 10 YEARS OF THE SEED GRANT PROGRAM

Agi Hirshberg welcomed the participation of the APA in the celebration of the 10th anniversary of the seed grant program and on its accomplishments. The presenters were all former awardees of the seed grant program. The symposium included keynote speeches by Anirban Maitra, MD, Ashok Saluja, PhD, and Diane Simeone, MD. The presentations covered mechanism and carcinogenesis, therapy and early detection, prevention, and stratification of pancreatic cancer.

Pancreatic Cancer—Making Mountains Out of Genomic Molehills by Anirban Maitra, MD, Sheikh Ahmed Bin Zayed Al Nahyan Center for Pancreatic Cancer Research University of Texas MD Anderson Cancer Center

The sequencing of the pancreatic cancer genome has identified somatic mutations of KRAS as the most preponderant alteration in this neoplasm, but unlike lung cancer or melanomas, no major subsets have emerged in terms of actionable mutations. Therapeutic targeting of mutant KRAS (mtKRAS) per se has not been successful in the clinic, although significant research efforts are underway to develop novel targeting strategies, including a concerted federally funded project at the NCI. In the interim, blocking proximate downstream effectors of oncogenic KRAS, particularly as combination therapies to account for compensatory pathway activation, remains a tractable choice that is being explored in the clinic. While mtKRAS remains the big “enchilada” in pancreatic cancer, parsing the genomic data does identify other minor subsets (“genomic molehills”) with potential for therapeutic intervention. The best cited example is biallelic loss-of-function mutations of the Fanconi anemia genes, which render the neoplastic cells exquisitely sensitive to poly (ADP-ribose) polymerase inhibitors or platinum agents. Other minor subsets include tumors with activating mutations of *ERBB2*, tumors that are wild type for KRAS, and those with alterations of the *PIK3CA* pathway. Given the relative infrequency of these alterations, it is unlikely that one can conduct pancreatic cancer-centric clinical trials that would require a priori documentation of any given somatic mutation. Instead, a more pragmatic approach might be to conduct “basket” trials that are tumor agnostic and enroll by mutational profile. An unexplored dimension in pancreatic cancer pertains to the significant subset (25%–30%) of tumors that harbor loss of function mutations of chromatin modifying genes, such as *MLL3*, *MLL2*, *ARID1A*, *KDM2B*, and so on. Genetically engineered models recapitulating these mutations should provide a platform for co-clinical trials, to identify therapeutic targets that are synthetic lethal to these loss of function mutations.

Acetylation of Mutant KRAS Lysine 147 Is an Oncogenic Posttranslation Modification Directed by SIRT2 by David Gius, MD, PhD, Department of Radiation Oncology, Robert Lurie Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, Ill

KRAS activating mutations are present in up to 35% of lung adenocarcinomas; however, as is observed in other human

malignancies, additional aberrant genetic and/or biochemical events are required for carcinogenesis. In addition, it has been shown that mtKRAS activity in lung cancer cells, whether mouse or human, is highly elevated, as compared with adjacent, untransformed cells with similar mtKRAS levels. These observations point to the importance of mtKRAS enzymatic activity in lung and pancreas adenocarcinoma and imply that modulating enzymatic activity influences carcinogenesis. Thus, our results suggest that cells lacking Sirt2, or exhibiting decreased SIRT2 activity, will have increased aberrant mtKRAS acetylation and enzymatic activity, and this plays a role in the establishment of an adenocarcinoma permissive phenotype. A significant percentage of human lung and pancreas adenocarcinomas contain mtKRAS protein, and mice lacking SIRT2 develop lung adenocarcinomas suggesting a potential mechanistic oncogenic connection. SIRT2 interacts with, deacetylates, and directs mtKRAS enzymatic activity. Mass spectrometry and an anti-KRASAc-K147 antibody showed that SIRT2 deacetylates KRAS lysine 147, and a substitution mutant (KRAS^{G12D} to KRAS^{G12D-K147Q}) that mimics an acetylated lysine, exhibited increased enzymatic activity and a more in vitro transformed phenotype. Sir2^{-/-} Kras^{G12D} mice exhibited lung and pancreas tumors earlier than the Kras^{G12D} mice. Finally, anti-KRAS-Ac-K147 antibody staining tumors showed increased mtKRAS acetylation in the murine Sirt2^{-/-} Kras^{G12D} and Kras^{LSL} tumors and human lung and pancreas adenocarcinoma samples. These results suggest that mtKRAS contains a potential oncogenic acetyl-lysine post-translation modification.

The Increasingly Important Roles of Posttranscriptional Regulation in Pancreatic Cancer by Isidore Rigoutsos, PhD, Jefferson Medical College, Thomas Jefferson University

Pancreatic cancer remains one of the most lethal types of cancer despite tremendous progress in elucidating the molecular events of pancreatic tumorigenesis. Over the years, research efforts focused largely on the genetic aspects of the disease. By comparison, the contributions beyond genetic and transcriptional events remained underexplored.

MicroRNAs (miRNAs) are short noncoding RNAs approximately 22 nts in length that are potent mediators of post-transcriptional regulation. MicroRNAs target messenger RNAs in their untranslated and amino acid coding regions in a sequence-dependent manner. MicroRNAs also bind pseudogenes and other noncoding RNAs. Posttranscriptional regulation by miRNAs exhibits tissue- and cell-state-dependent characteristics and is critical to many fundamental biological processes. Links to miRNAs have been demonstrated in development, neurodegeneration, immunity, aging, many cancers, and so on. Consequently, “signatures” composed of dysregulated miRNAs have been determined for many of these contexts including pancreatic cancer.

We and others have been generating evidence for several years, in both human and mouse, that the “canonical model” of miRNA targeting is incomplete and that a far more complex “expanded model” is at work: according to the latter, miRNAs regulate many more genes than originally believed and do so through more complex and intricate interactions. Recent technological and methodological advances now make it possible to begin improving our mechanistic understanding of the rules that govern miRNA regulation of the transcriptome in pancreatic cancer and in other settings. Using a combination of newly developed computational methods and Ago CLIP-seq (=deep sequencing of RNA after UV cross-linking and immunoprecipitation with an Ago antibody) in pancreatic cancer cell

lines, we uncovered unexpected facets of miRNA activity with far-reaching implications about the roles of miRNAs. Our findings confirm that the “expanded model” is in effect in pancreatic cancer cells and provide a solid foundation for an in-depth exploration of these posttranscriptional aspects of the disease process and their relationships to chemotherapeutic efficacy and resistance mechanisms.

An Emerging Novel Therapy Against Pancreatic Cancer: Minnelide by Ashok K. Saluja, PhD, University of Minnesota

Pancreatic cancer is one of the most lethal human malignancies, with an all-stage 5-year survival of less than 5%, a number that highlights the urgent need for more effective therapeutic strategies. Our studies have shown that triptolide, a diterpenoid, is effective against pancreatic cancer cells in vitro as well as in vivo. However, triptolide is poorly soluble in water, limiting its clinical utility. We have recently synthesized a water-soluble prodrug of triptolide, Minnelide, which has shown tremendous promise in preclinical studies.

Since its synthesis, Minnelide has been extensively evaluated in preclinical studies. In orthotopic mouse models with very aggressive cell lines, the Minnelide-treated mice showed a definite regression and reduction in tumor burden. A survival study analysis showed that untreated mice had a median survival of 36 days, whereas mice treated with Minnelide continued to live for a year. In the same study, the tumors did not recur even when treatment with Minnelide was discontinued.

Similar studies using human tumor xenografts implanted and propagated in mice also showed a dramatic decrease in tumor volume when treated with Minnelide. Tumors as large as 1 cm³ in volume regressed on treatment with Minnelide. Our studies also showed that prolonged Minnelide treatment did not result in any obvious toxicity in the animals, and the tumors did not recur even when treatment was stopped.

Although Minnelide has been extremely efficient in inducing pancreatic cancer cell death, the signaling pathways leading to this are still unclear. Previous studies have demonstrated that Minnelide down-regulates the prosurvival protein heat shock protein 70 (HSP70). We have now unraveled the relationship between several pro-proliferation pathways that are targeted by triptolide leading to down-regulation of HSP70, eventually leading to cell death.

Our studies have shown that triptolide induces endoplasmic reticulum (ER) stress and inhibits nuclear factor κ B (NF- κ B), thereby affecting 2 pathways that play a prominent role in carcinogenesis. Furthermore, our data suggest that triptolide affects these 2 pathways by down-regulating specificity protein 1 (Sp1). Specificity protein 1 is a transcription factor that is not expressed in terminally differentiated pancreatic cells but is overexpressed in pancreatic cancer cell lines. Both NF- κ B and ER stress proteins are regulated by transcriptional activity of Sp1. Triptolide inhibits the activity of Sp1 leading to down-regulation of NF- κ B on one hand and induction of ER stress on the other hand. This leads to down-regulation of HSPs and results in lysosomal membrane permeabilization, which culminates in cell death.

Our study shows for the first time that triptolide-induced cell death in pancreatic cancer is mediated by down-regulation of prosurvival pathways (like ER stress, HSP70, and NF- κ B) controlled by Sp1. This molecular analysis of the mode of action of triptolide or Minnelide is of great usefulness in delineating its biological function as it is undergoing phase I clinical trials since August 2013.

Insights Into the Translation of HSP Inhibitors to the Treatment of Pancreatic Cancer by Gabriela Chiosis, PhD, Memorial Sloan-Kettering Cancer Center, New York, NY

The development of a malignant phenotype is associated with dysregulation of multiple pathways and molecules. To adapt, cells co-opt HSPs to help maintain a functional cellular state under the transforming pressure. Among the major HSPs are HSP90 and HSP70, proteins that act in an interconnected but also distinct fashion to regulate and maintain the disease phenotype. In pancreatic cancer, these HSPs have a major role in maintaining the altered signaling pathways and the elevated antiapoptotic threshold characteristic of these tumors. Consequently, approaches that target these HSPs are especially promising for inclusion in the pancreatic cancer treatment regimens. This talk will present some of our efforts in the design, development, and clinical translation of HSP inhibitors and in the rational selection of pancreatic patients who are more likely to draw a benefit from such drugs.

Novel Therapeutic Targets (EphB4, Axl, GRP78) in Pancreatic Cancer by Parkash Gill, MD, University of Southern California, Los Angeles, Calif

Locally advanced or metastatic pancreatic carcinoma is primarily treated with cytotoxic chemotherapy. Most recent entries to these agents include Abraxane and Erbitux. Understanding the genetic basis and pathways activated in pancreatic carcinoma provides opportunities to develop novel therapeutics. We are investigating 2 novel targets, which include receptor kinases EphB4 and GRP78.

EphB4 receptor kinase is aberrantly expressed in tumor cells, and its ligand EphrinB2 is expressed in the tumor endothelial cells. EphB4-EphrinB2 interaction leads to bidirectional signaling promoting tumor cell survival and progression. We are investigating the induction of EphB4 in pancreatic ductal adenocarcinoma (PDAC) in spontaneous tumor model in which *mtKRAS* is induced, and *p53* is deleted. Preliminary data support the hypothesis. Second, we are investigating if the deletion of EphB4 in the same cells will reduce the development of PDAC. Once proven, we will investigate the efficacy of blocking EphB4-EphrinB2 interaction and signaling using decoy soluble EphB4 receptor extracellular domain (sEphB4) fusion protein containing full-length human serum albumin (sEphB4HSA). Albumin fusion promotes drug delivery into the tumor and markedly enhances pharmacokinetics. sEphB4HSA has potent antitumor activity as a single agent and when combined with gemcitabine in orthotopic models. Studies will become the basis for the conduct of clinical investigation in patients with pancreatic carcinoma.

GRP78, a stress response protein, is highly induced in pancreatic cancer. GRP78 also translocates from the ER to the tumor cell surface where it interacts with several membrane proteins and activate PI3K pathway. Genetic deletion of GRP78 attenuates several genetically defined spontaneous tumor models. We are thus targeting surface GRP78 as a potential therapeutic agent. We have identified a monoclonal antibody that binds surface GRP78 with high affinity, induces endocytosis and degradation, and inhibits PI3K signaling. We plan to investigate the surface GRP78 expression in spontaneous PDAC tumor model in which *mtKRAS* is induced and *p53* is deleted. Next, we will investigate the efficacy of GRP78-specific antibody as a single agent and in combination with gemcitabine and Albutaxol. These data will provide the Foundation for go-no-go decision to conduct human

clinical trial in pancreatic carcinoma using GRP78-specific humanized antibody.

Targeting Histone Demethylases in Pancreatic Cancer by Alexandros Tzatsos, MD, PhD, Massachusetts General Hospital/Harvard

By using a systems biology approach, we have identified members of the Jumonji-domain containing histone demethylase family as important oncogenes in pancreatic cancer. Gain- and loss-of-function experiments coupled to genome-wide gene expression and chromatin immunoprecipitation studies showed that histone demethylase KDM2B subverts cellular differentiation and sustains metabolic homeostasis in pancreatic cancer. Given the reversibility of epigenetic changes, ongoing efforts to target KDM2B may provide new approaches for the treatment of pancreatic cancer.

Advances in Early Detection of Pancreatic Cancer by Diane Simeone, MD, University of Michigan

Pancreatic cancer is a complex heterogeneous disease, and much effort has been put forth to try to improve outcomes in patients who develop it. This is particularly relevant as a recent report has found that if outcomes are unchanged, pancreatic cancer will become the second leading cause of cancer death in the United States by 2030. Approaches to improve outcomes in pancreatic cancer focus on 3 areas: improved treatment strategies, early detection, and prevention. Although the largest research effort has been directed to developing new therapeutics, ultimately we will gain greater headway if we develop methods to detect pancreatic cancer at its earliest stages. Although more than 2000 studies have been published on early detection biomarkers for pancreatic cancer, progress has been slow. Most studies are done in late-stage rather than early-stage samples and lack proper disease controls, including chronic pancreatitis, diabetes, and biliary obstruction. An effective biomarker test for pancreatic cancer will need to have a very high sensitivity and specificity to be useful as a screening tool. Despite these obstacles, collaboration among high-volume centers in early-stage sample collection and in biomarker development and validation, partnered with technological advances in measurements in circulating DNA and tumor cells, may lead to acceleration in the pace of finding an early detection strategy for the disease.

Salivary Biomarker Development for Pancreatic Cancer Detection by David Wong, DMD, DMSc, UCLA

In recent years, sparked by initiatives from the National Institute of Dental & Craniofacial Research, saliva has attracted widespread interest as a diagnostic medium for rapid, point-of-care testing. The advantages of using saliva for disease diagnostics include ease of access, noninvasive sample collection, increased acceptance by patients, and reduced risks of infectious disease transmission.

Lack of detection technology for early-stage pancreatic cancer invariably leads to a typical clinical presentation of incurable disease at initial diagnosis. New strategies and biomarkers for early detection are sorely needed. We conducted a prospective sample collection and retrospective blinded validation to evaluate the performance and translational utilities of salivary extracellular RNA and microbial biomarkers for the noninvasive detection of early-stage resectable pancreatic cancer.

Two biomarker discovery technologies were used to profile transcriptome and microflora in saliva. The Affymetrix HG U133 Plus 2.0 Array was used to discover altered gene

expression in saliva supernatant. The Human Oral Microbe Identification Microarray was used to investigate microflora shift in saliva pellet. Biomarkers selected from both studies were subjected to clinical validation using an independent sample set of 30 early-stage pancreatic cancer, 30 chronic pancreatitis, and 30 control subjects.

Two panels of salivary biomarkers, including 12 messenger RNA biomarkers and 2 microbial biomarkers, were discovered and validated. The logistic regression model with the combination of four biomarkers (*KRAS*, *MBD3L2*, *ACRV1*, and *DPM1*) could differentiate pancreatic cancer patients from subjects with no cancer (chronic pancreatitis and control subjects), yielding a receiver operating characteristic plot are under the curve value of 0.971 with 90.0% sensitivity and 95.0% specificity.

The salivary biomarkers possess discriminatory power for the detection of early-stage pancreatic cancer, with high specificity and sensitivity. These reports provided the proof of concept of multiplex salivary biomarkers for the noninvasive detection of a systemic cancer and pave the way for prediction model validation study followed by pivotal clinical validation.

Obesity, Inflammation, and Pancreatic Cancer Development by Kathleen Hertzner, MD, PhD, Los Angeles, Calif, Presenting for Guido Eibl, MD, UCLA, Los Angeles, Calif

There is epidemiologic evidence that obesity increases the risk of several human cancers. Various underlying mechanisms, including inflammation and insulin resistance, are proposed. However, the driving mechanisms in pancreatic cancer are poorly understood. The goal of our study was to develop a model of diet-induced obesity and pancreatic cancer development in a state-of-the-art mouse model, which resembles important clinical features of human obesity, for example, weight gain and metabolic disturbances. Offspring of Pdx-1-Cre and LSL-KrasG12D mice were allocated to either a diet high in fats and calories (HFCD; ~4535 kcal/kg; 40% of calories from fats) or control diet (~3725 kcal/kg; 12% of calories from fats) for 3 months. Compared with control animals, mice fed the HFCD significantly gained more weight and developed hyperinsulinemia, hyperglycemia, hyperleptinemia, and elevated levels of insulinlike growth factor 1. The pancreas of HFCD-fed animals showed robust signs of inflammation with increased numbers of infiltrating inflammatory cells (macrophages and T cells), elevated levels of cytokines and chemokines, increased stromal fibrosis, and more advanced pancreatic intraepithelial neoplasia (PanIN) lesions. Our results demonstrate that a diet high in fats and calories leads to obesity and metabolic disturbances similar to humans and accelerates early pancreatic neoplasia in the conditional KrasG12D mouse model. This model and findings will provide the basis for more robust studies attempting to unravel the mechanisms underlying the cancer-promoting properties of obesity as well as to evaluate dietary and chemopreventive strategies targeting obesity-associated pancreatic cancer development.

Epithelial-to-Mesenchymal Transition and Stemness in Pancreatic Cancer by Mouad Edderkaoui, PhD, UCLA, Los Angeles, Calif

Smoking is a major risk factor for pancreatic cancer. We developed novel mouse models of pancreatic cancer induced by smoking. We used 2 pancreatic cancer mouse models induced by overexpression of *KRAS* with 2 different degrees of

disease complexity. In the first model, we injected EL-Kras mice with smoking compound NNK, and in the second model, we exposed Pdx1-Cre;LSL-Kras mice to cigarette smoke in smoking chambers. We measured the effect of smoking on PanIN lesion formation, fibrosis, proliferation, death pathways, epithelial-to-mesenchymal transition (EMT), and stemness. Epithelial-to-mesenchymal transition is a critical step for cancer cells metastasis and leads to the acquisition of cancer stem cell features including resistance to treatments.

We found that smoking caused stimulation of PanIN lesion formation in both models. Smoking significantly stimulated fibrosis, cell proliferation and inhibition of apoptosis, and, in a lesser extent, stimulated inflammation. Importantly, we found that smoking stimulated EMT even in early PanIN lesions in mice pancreas. The levels of vimentin and transcription factor Zeb1 were increased in the pancreas of mice exposed to smoking, whereas E-cadherin level was decreased. Stem cell markers CD133 and sox2 levels were increased by smoking. Inhibition of Zeb1 using specific siRNA prevented EMT and stemness in pancreatic cancer cells MIA-PaCa-2 and AS-PC1. Combination of Zeb1 inhibition and gemcitabine induced cell death and prevented EMT and acquisition of cancer stem cells features in pancreatic cancer cells.

This data indicate that EMT and stemness occur at early stage of pancreatic cancer development and suggest the combination of gemcitabine with EMT inhibitor to prevent metastasis and development of resistance to treatment.

SIDMAP Contributions to Cancer Metabolomics, Vay Liang W. Go, MD, UCLA Center for Excellence in Pancreatic Diseases, Los Angeles, Calif

One of the key contributions made by Agi Hirshberg to industry and academia was her founding of SiDMAP, a company that is focused on using tracer-based metabolomics technology in drug development research and in investigation of cancer metabolism. SiDMAP's analytical approach is designed to investigate metabolic pathways in vitro, in vivo, or in human subjects using a single of ^{13}C -labeled tracer such as [1,2- ^{13}C]-glucose or [U- ^{13}C]-palmitate. The metabolism of these ^{13}C -labeled substrates introduces ^{13}C carbons into a multitude of metabolic intermediates that are central to glucose and TCA cycle metabolism. SiDMAP through the use of proprietary technology determines in key metabolites the number of ^{13}C incorporated and their relative positions within the molecules (isotopomer distribution), which are reported in the form of a heat map (SiDMAP Array) for easy visual interpretation of metabolic fluxes. Thus, the SiDMAP Array depicts the overall system effect of diseases, drugs, age, and individual variations on metabolic pathways.

Unlike other metabolomics technologies, SiDMAP technology, with the use of specifically chosen ^{13}C or ^2H labeled substrates, provides information on metabolic fluxes of energy/substrate-producing pathways. The optimal utilization of chemical energy and production of carbohydrates, amino acids, and fatty acids, by the cell define the homeostasis of the cell, which is an important parameter of a biological system only measured by SiDMAP technology. With this cutting-edge technology, SiDMAP has provided analytical and consultative services to a wide range of pharmaceutical companies in their development of anticancer, anti-inflammatory, or anti-diabetes treatments. It has also aided academia in understanding cancer metabolic phenotypes in tumors carrying specific mutations such as isocitrate dehydrogenase

TABLE 1. Seed Grant Recipients

Year	Name	Project
2012–2013	Alexandros Tzatsos, MD, PhD Massachusetts General Hospital-Cancer Center	“Validation of the histone demethylase KDM2B as a new therapeutic target in pancreatic cancer”
	Anne Coscarelli, PhD UCLA	“Providing optimal psychosocial support to patients and families through the journey of pancreatic cancer”
	Ido Wolf, MD Tel Aviv Sourasky Medical Center	“The hormone klotho: a novel regulator of the ERK1/2 cascade in pancreatic adenocarcinoma”
	Isidore Rigoutsos, PhD Thomas Jefferson University	“New vistas for gene regulation in pancreatic cancer”
	Mouad Edderkaoui, PhD Brentwood Biomedical Research Institute, Los Angeles, Calif	“Targeting EMT pathway as a promising strategy for pancreatic cancer treatment”
	Parkash Gill, MD University of Southern California	“Role of EphB4 in Kras mutant PDAC”
	Qing- Yi Lu, MD, PhD UCLA	“The phytonutrient core of the UCLA Center for Excellence in Pancreatic Diseases”
	Pamela Itkin-Ansari, PhD University of California, San Diego	“Restoring balance to the bHLH/Id3 Axis as a potential therapy for pancreatic cancer”
	Andrea Bullock, MD Beth Israel Deaconess Medical Center; Harvard Medical School	“Mitogenic signaling after loss of tumor suppressor genes BRCA1 or BRCA2 in pancreatic cancer”
	Stephan D. Kendall, MD Duke University Medical Center	“Using genetically engineered mouse models to evaluate the role of macrophages in pancreatic tumorigenesis”
2011	David Gius, MD, PhD Vanderbilt Medical School	“Sirt2 regulation of KRAS is an early event in pancreas carcinogenesis”
	Emmanuelle Meuillet, PhD The University of Arizona Cancer Center	“Mechanistic and translational studies of the inflammatory response in pancreatic cancer”
	Yung-Ya Lin, PhD UCLA	“An integrated synergistic approach for early detection and targeted therapy of pancreatic cancers by magnetic resonance molecular imaging and nano medicine”
	Andrea Viale, MD Dana-Farber Cancer Institute	“Role of oncogenic K-RAS in self-renewal and maintenance of cancer stem cells in pancreatic tumor”
	Ying Ma, PhD University of Texas MD Anderson Cancer Center	“Mast cell blockade as immunotherapy for pancreatic cancer”
	Larry Karnitz, PhD Mayo Clinic	“An innovative drug combination for pancreatic cancer”
	Carmen Visus, PhD University of Pittsburgh	“Combinatorial strategy for targeting pancreatic cancer stem cells”
	Gabriela Chiosis, PhD Molecular Pharmacology & Chemistry and Medicine	“Inhibition of molecular chaperones as a potential therapy for pancreatic cancer”
	Shen Hu, PhD UCLA	“Tracer-based proteomics of pancreatic cancer”
	Timothy Donahue, MD UCLA	“Identifying the malignant signatures associated with the development of human pancreatic cancer”
2010	Jonathan Cheng, MD Fox Chase Cancer Center	“Tumor stromal fibroblasts as a therapeutic target”
	Wai-Nang Paul Lee, MD LABiomedical Research Institute Harbor-UCLA Medical Center	“Proteomics of pancreatic cancer: determination of protein turnover”
	Shuping Vincent Wu, PhD VA, Greater Los Angeles Healthcare System	“Stress and pancreatic cancer: role of corticotropin releasing factor (CRF) system”

(Continued on next page)

TABLE 1. (Continued)

Year	Name	Project
2007	Ashok K Saluja, PhD University of Minnesota	“The inhibition of HSP70 expression sensitizes pancreatic cancer cells to TRAIL-induced apoptotic cell death”
	Frank A. Sinicrope, MD Mayo Clinic and Mayo College of Medicine	“BH3 mimetic reverses apoptosis resistance in human pancreatic cancer cells”
	Hongxiang Hui, MD, PhD UCLA	“Glucagon-like peptide-1 is a promising anti-pancreatic cancer stem cell agent by cell-differentiation induction and growth inhibition”
	Edward Garon, MD UCLA	“Clinical trial of gemcitabine with genistein or placebo after resection for pancreatic adenocarcinoma”
	David W. Dawson, MD, PhD UCLA	UCLA Pancreatic Tissue Bank and Data System
	Harold Frucht, MD Columbia University	“Pancreatic physiology in the screening and early detection of human pancreatic adenocarcinoma”
	Diane M. Harris, PhD UCLA	“Fatty acid synthase inhibitors in pancreatic cancer”
2006	Joseph Kim, MD City of Hope National Medical Center and Beckman Research Institute	“Targeting chemokine receptor CXCR4 to prevent pancreatic cancer”
	Krisztina Kisfalvi, MD, PhD UCLA	“Role of protein kinase D (PKD) in the proliferation of pancreatic cancer cells”
	Christopher Wolfgang, MD, PhD Johns Hopkins University	“Targeting lipid mediations of pancreatic cancer growth”
	Nathan R. Wall, PhD, MS, BS Loma Linda University	“Surviving regulation of apoptosis in pancreatic cancer”
2004–2005	Anna Gukovskaya, PhD UCLA	“Sensitization of pancreatic cancer to gemcitabine chemotherapy by inhibiting anti-apoptotic XIAP with herb-derived embelin”
	Howard A. Reber, MD UCLA	Continuing funding for Hirshberg Translational Pancreatic Cancer Research Laboratory at UCLA
	Stephen J. Pandol, MD Med-VA Greater LA Healthcare System	“Regulation of DNA synthesis pathways by reactive oxygen species in pancreatic cancer”
	Sarah M. Dry, MD UCLA	Funding for the UCLA Pancreatic Tissue Bank

and fumarate hydratase. SiDMap has been successfully validated in a vast array of human diseases, a nonexclusive list is provided on their Web site, <http://www.sidmap.com>.

HIRSHBERG AWARD FOR BEST ABSTRACTS IN PANCREATIC CANCER PRESENTED AT THE 2013 APA ANNUAL MEETING

The Foundation has been awarding the best basic and clinical abstracts presented at the annual meeting of the APA for over a decade. Previous award winners are posted on the Foundation's Web site and the APA Web site (<http://www.american-pancreatic-association.org/>). The abstracts are published in *Pancreas*. The 2013 awardees are Noboru Ideno, PhD, from Kyushu University, Fukuoka, Japan, and Junpei Yamaguchi, MD, PhD, from Harvard Medical School, Boston, Mass. Both presented their work at the symposium. Their abstracts were published in the November 2013 issue of *Pancreas*.

“Clinical Significance of *GNAS* Mutation for Invasive Pancreatic Carcinoma Distinct From/Derived From Intraductal Papillary Mucinous Neoplasm (IPMN) of the Pancreas.” Ideno N,¹ Ohtsuka T,¹ Tamura K,¹ Aso T,¹ Ohuchida K,¹ Takahata S,¹ Oda Y,² Mizumoto K,¹ Tanaka M.¹ Departments of ¹Surgery and Oncology and ²Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.⁶

“Loss of TFF2 in Pancreatic Duct Glands (PDG) Results in the Formation of IPMN Suggesting TFF2 May Function as a Tumor Suppressor.”

Yamaguchi J,¹ Mino-Kenudson M,² Liss AS,¹ Lillemoe KD,¹ Fernández-del Castillo C,¹ Warshaw AL,¹ Thayer SP.¹ Warshaw Institute and Departments of ¹Surgery and ²Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA.⁷

The celebration was concluded by Vay Liang W. Go, MD, who thanked Agi Hirshberg and her Foundation for their continued support of pancreatic cancer research. He conveyed the heartfelt gratitude of the scientific community for the Foundation's vision, leadership, and stewardship.

HIRSHBERG FOUNDATION PATIENT AND FAMILY SUPPORT PROGRAM

The Hirshberg Foundation endeavors to maintain the connection between the researchers working on the disease and the patients who may eventually benefit from their work. To that end, the Foundation provides patient information and support through its Web site (www.pancreatic.org), its biannual newsletter, and through the annual Hirshberg patient symposium at UCLA. The patient symposium is free for participants and has become an important think-tank for pancreatic cancer. Each

year, the event is attended by more than 200 researchers, clinicians, patients, survivors, and families struggling with the disease. Past symposia have covered topics including oncology, surgery, psychosocial management, nutrition and integrative medicine, genetics, basic science, and radiology. The 2014 Agi Hirshberg Symposium, in collaboration with the UCLA Center for Pancreatic Diseases, featured a panel of pancreatic cancer survivors as well as presentations by Agi Hirshberg; Vay Liang Go, MD, UCLA; Howard Reber, MD, UCLA; Bennett Roth, MD, UCLA; Lee Rosen, MD, UCLA; Carolyn Katzin, MS, CNS, MNT, UCLA; and Barbara Clerkin, MPH, RN, UCLA.

The Foundation builds strong relationships with the pancreatic cancer patient community and with the families and friends of those who have passed. The passion of this group helps spur the Foundation's many fundraising efforts, including the annual LA Cancer Challenge, the Tour de Pier, and other events. In the end, the goal is to advance the mission of the Foundation—one that is shared by the researchers, the patients, the family support group, and supporters of the Foundation.

CONCLUSIONS

The Hirshberg Foundation for Pancreatic Cancer Research Seed Grant program proudly marked its 10th anniversary in 2013. The Foundation has been involved in the annual meeting of the APA over the last decade. It was very appropriate that the 10th anniversary celebration was held at the 2013 meeting of the APA. The APA/Hirshberg symposium celebrating 10 years of the Hirshberg Seed Grant program highlighted the research work of previous recipients and included accomplishments in research in the areas of pancreatic carcinogenesis, diagnosis and therapy, and prevention. Video highlights of the seed grant recipients' presentations are posted on the Foundation's Web site (http://www.pancreatic.org/site/c.htJYJ8MPIwE/b.8867159/k.A425/APAScientific_Symposium_2013.htm). The Foundation continues in its mission to improve the lives and outcomes of those who encounter the enemy that is known as pancreatic

cancer. Looking back at the years of progress, we are proud of what the Foundation has accomplished, but we also recognize that we have not yet achieved the mission of the Foundation and our national goal of increasing the pancreatic cancer survival rate. We know that continuing to fund promising research will improve the outcomes in the management of pancreatic cancer, as it has for other cancers. We look forward to resuming our seed grant program in 2014.

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