

## Pancreatic Cancer in Mice and Man: The Penn Workshop 2004

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### Abstract

**A three-day conference cosponsored by the National Cancer Institute Mouse Models of Human Cancer Consortium and the Abramson Cancer Center of the University of Pennsylvania was convened on December 1, 2004, in Philadelphia, Pennsylvania. The purpose of the conference was to compare the histopathologic changes in murine models of exocrine pancreatic cancer to human disease and to discuss potential preclinical applications of these models. The participants of this international meeting included over 100 physicians and scientists with expertise in pancreatic cancer pathology, therapy, detection, and biology, and they were organized accordingly into working groups. The format of the meeting was a series of short presentations by individual participants followed by working group breakout sessions. The working groups presented their reports on the final day of the conference, and highlights of selected individual presentations and working group recommendations are summarized here and in an accompanying pathology consensus report. (Cancer Res 2006; 66(1): 14-7)**

### Pancreatic Cancer: Introduction and Rationale to Develop Animal Models

Pancreatic ductal adenocarcinoma (PDA) is the most lethal malignancy by anatomic site, with 32,180 new cases and 31,800 deaths expected in the United States in 2005 (1). The extreme mortality of PDA is attributable to a lack of effective early detection methods and the poor efficacy of existing therapies for advanced disease. Even among the 10% to 20% of all patients diagnosed with surgically resectable PDA, most ultimately die of recurrent and metastatic disease (2). This grim situation prompted a previous conference to convene in Park City, Utah, in 1999 to discuss the biology and therapy of human pancreatic cancer and prioritize areas for further research. One of the primary recommendations emanating from the Park City conference was that relevant animal models of pancreatic cancer are needed to accelerate the pace of discovery (3). The 5 years following the Park City conference have witnessed dramatic progress in the creation of genetically engineered mutant mouse models (GEM) of exocrine pancreatic

cancer. However, the lack of standard histologic and pathologic criteria for the analysis and classification of these GEMs has caused much confusion and has limited their dissemination and proper utilization. The opportunity to clarify the characterization of these GEMs and validate them as models of human PDA could not occur at a more opportune time.

### Overview of Pancreatic Cancer Biology and Therapy

The hypothesis that pancreatic intraepithelial neoplasms (PanIN) represent a preinvasive form of PDA (3) has been largely embraced by the community following the Park City conference. Ralph Hruban (Johns Hopkins University, Baltimore, MD) presented additional clinical, molecular, and histologic evidence that supports the PanIN to PDA progression, including tissue obtained from asymptomatic individuals with an elevated risk of pancreatic cancer who underwent surveillance biopsies for early pancreatic neoplasia (4). PanINs were initially thought to originate from mature intralobular ductal epithelial cells that undergo metaplasia and progressive dysplasia, and Steve Leach (Johns Hopkins University) presented an alternative hypothesis that PanINs may be derived from rare tissue progenitor or stem cells, such as centroacinar cells, or facultative stem-like cells that await identification. Countering the popular misconception that pancreatic neoplasia is an uncommon malignancy, Scott Kern (Johns Hopkins University) commented that early-grade PanINs may actually be one of the most common neoplasms found in elderly individuals at autopsy (5), and Gloria Petersen (Mayo Clinic, Rochester, MN) projected that the incidence of PDA in the United States would double over the next two decades due to the aging of the population.

Surgical resection remains the predominant approach for operable PDA, and Jeff Drebin (University of Pennsylvania, Philadelphia, PA) suggested that neoadjuvant approaches should be pursued to increase resectability and provide tissue for the pharmacodynamic assessment of investigational agents. Although the role of radiation therapy is controversial in the adjuvant and palliative setting for PDA patients, Stephen Hahn (University of Pennsylvania) countered that carefully controlled studies show benefit. Furthermore, he presented data that radiation therapy could act synergistically with farnesyl transferase inhibitors in preclinical and clinical trials (6). James Abbruzzese (MD Anderson Cancer Center, Houston, TX) reported little progress toward improving upon the limited efficacy of gemcitabine in advanced pancreatic cancer, and he urged the investigation of rationally targeted agents. However, he cautioned that *ex vivo* activity does not translate to *in vivo* efficacy using the example of paradoxical disease progression in a clinical trial of PDA patients treated with

**Note:** D.A. Tuveson is a Rita Allen Foundation Scholar.

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the rapamycin derivative CCI-779.<sup>7</sup> An exciting preclinical finding presented by Scott Kern (Johns Hopkins University) was the correlation between germ line mutations in Fanconi anemia genes, such as the *BRCA2* gene, and an increased sensitivity to the alkylating agent mitomycin C (7). According to Gloria Petersen (Mayo Clinic), *BRCA2* gene mutations account for as much as 17% of the familial aggregation of PDA (8); thus, this finding could be clinically relevant to a sizable number of patients and should be rigorously evaluated in clinical trials.

### Classification and Further Development of GEMs of Exocrine Pancreatic Cancer

Although the “call to arms” from the Park City meeting likely played a role, the recent productivity in model development was still surprising because *Mus musculus* had not previously been reported to develop PanIN or PDA spontaneously or in response to carcinogen treatment. Eleven investigators presented 13 GEMs of exocrine pancreatic dysplasia and neoplasia, including a new model of chronic pancreatitis. Due to time constraints, GEMs of pancreatic endocrine tumors were not analyzed in this conference. Before the meeting, tissue from each model was processed for histologic and immunohistochemical analysis at a National Cancer Institute (NCI) core facility, and sets of slides were provided to the working group of human and veterinary pathologists. The pathology panel, directed by Ralph Hruban (Johns Hopkins University), was charged with systematically evaluating each model and attempting to relate each to the established classification schemes for human pancreatic cancer. In this manner, a consensus opinion was formulated delineating standard methods to characterize and describe GEMs of exocrine pancreatic cancer (9). Importantly, some models recapitulated certain aspects of human PanIN and PDA quite closely and each of the models was felt to have meritorious features for specific applications.

A breakout group led by Chris Wright (Vanderbilt University, Nashville, TN), Ray MacDonald (University of Texas Southwestern, Dallas, TX), Charles Murtaugh (University of Utah, Salt Lake City, UT), and Gloria Su (Columbia University, New York, NY) discussed the creation of improved GEMs for pancreatic cancer. The committee strongly supported cross-disciplinary collaborations between developmental biologists and cancer geneticists to address pertinent scientific issues and to organize their respective efforts in a mutually beneficial fashion. The characterization of the cell lineages responsible for normal and neoplastic ductal differentiation was viewed as a top priority. Suggested approaches included the identification of ductal-specific markers to enable the engineering of Tamoxifen-inducible Cre recombinase alleles for lineage-tracing studies within specific developmental contexts, and the rigorous investigation of centroacinar cells as candidate exocrine stem cells. The development of models of cystic pancreatic neoplasms, including intraductal papillary mucinous neoplasms, was also discussed as an unmet need in the field. The creation of a pancreatic cancer consortium community that integrates pancreatic developmental biologists and cancer geneticists was proposed as a means to increase recognition and funding for this area. Furthermore, the pancreatic cancer consortium community would establish committees to document and advertise available reagents and protocols,

create a GEM tissue bank, and organize educational efforts, such as regular meetings and joint training fellowships. In particular, a specific and often updated website as a focus point for information sharing was proposed.

### Therapeutic Applications of Exocrine Pancreatic Cancer GEMs

A group co-chaired by Howard Crawford (State University of New York, Stony Brook, NY), Channing Der (University of North Carolina, Chapel Hill, NC), Matthias Hebrok (University of California San Francisco, San Francisco, CA), Andrew Lowy (University of Cincinnati, Cincinnati, OH), and Margaret Tempero (University of California San Francisco) addressed the potential value of GEMs for the preclinical evaluation of novel therapies. The panel discussed issues pertinent to the optimal integration of GEMs into the drug development process, elaborated upon the infrastructural requirements for this endeavor, and finally deliberated whether the GEMs could uniquely assist in the prioritizing of potential therapeutic targets.

#### Integration of GEMs into Preclinical Evaluation

The availability of GEMs of PanIN and PDA are welcomed additions to preclinical therapeutic investigations, as GEMs seem to recapitulate cardinal features of human PanIN and PDA, such as genetic heterogeneity and cellular diversity in the tumor microenvironment. However, it is currently unproven whether GEMs will more closely predict therapeutic efficacy in pancreatic cancer patients than the current Food and Drug Administration standard of tumor xenografts. Indeed, the failure of farnesyl transferase inhibitors serves as a poignant reminder that efficacy assessments in GEMs can be extremely misleading, particularly when there are major discrepancies between the disease and pathways being modeled in the GEMs and those present in human patients [i.e., overexpressed oncogenic *HRAS* in a mouse mammary tumor model (10) compared with physiologically expressed oncogenic *KRAS* in a patient's pancreatic or colorectal tumor]. To avoid this pitfall in the future, GEMs that best recapitulate the genetic and biological features of PanIN/PDA will likely be the most informative for these efforts. Additionally, because it is controversial whether genes that contribute to the *ex vivo* transformation of murine cells will have the same importance in human neoplasms (11), tumor dependency pathways in GEMs will ideally be identified by *in vivo* genetic and pharmacologic approaches. An additional concern is the oftentimes unpredictable pharmacokinetic properties of compounds in mice compared with humans and the lack of necessary expertise for pharmacologic measurements in most academically based laboratories. Finally, the identification of pharmacodynamic variables in GEMs for translation to the clinic requires exquisite cross-species similarities in response to target inhibition.

#### Infrastructural Needs

For these preclinical therapeutic efforts in GEMs to be successful, core facilities and services similar to those necessary for the proper conduct of investigational human clinical trials will be required. Specifically, six areas were identified as critical components for a successful program. First and foremost, a high-throughput murine pathology core is needed for uniform tissue processing, banking, and analysis in coordination with a trained veterinary pathologist (or human pathologist with specialty training with murine pancreatic cancer GEMs). Second, cutting edge pharmacodynamic monitoring will require the availability of

<sup>7</sup> J. Abbruzzese, personal communication.

molecular diagnostics including murine-specific cytogenetics, genomics, and proteomics. Third, accessibility of a pharmacokinetics laboratory is crucial for the rapid assessment of drug and metabolite levels obtained from murine pancreatic tissues. Fourth, a small animal imaging facility with instruments capable of detecting pancreatic tumors by anatomic or functional modalities will enable the appropriate enrollment of mice into prevention or intervention trials and facilitate noninvasive disease monitoring. Fifth, a preclinical therapeutics core with chemical biology and some medicinal chemistry expertise can coordinate the *ex vivo* identification of chemical compounds that target the cell autonomous compartment. Finally, formal interactions with biostatisticians will be necessary for proper trial design and data interpretation. Importantly, institutional and national financial commitment will be needed to establish such an infrastructure for the scientific community.

### Targets to Evaluate with GEMs

Although many potential molecular targets for therapeutic intervention exist in PDA, no inhibitors have yet been described that show marked efficacy in patients. Oncogenic *KRAS* remains an intractable target despite extensive efforts by the academic and private sectors. Alternatively, the kinase suppressor of Ras seems to be required for *KRAS* oncogenicity and can be inhibited by antisense approaches in preclinical studies, according to Richard Kolesnik (Memorial Sloan-Kettering Cancer Center, New York, NY; ref. 12). Recent interest has focused on the inhibition of the developmental pathways Notch (13) and Hedgehog (14, 15) in PDA, and several agents are likely to begin clinical assessment shortly. Inhibition of mitogenic receptor tyrosine kinases, including epidermal growth factor receptor, Her2/neu, and vascular endothelial growth factor receptor (VEGFR), with small molecules or ligand-sequestering antibodies is under clinical evaluation. Additionally, Douglas Hanahan (University of California San Francisco) presented data that concomitant blockade of pericytes with platelet-derived growth factor receptor inhibition and endothelial cells with VEGFR inhibition could synergistically inhibit angiogenesis and tumor progression in an islet cell carcinoma model (16). Matrix metalloproteinases were previously considered attractive targets before negative data in several clinical trials (17) and the future investment in this target seems uncertain. Notably, many of these putative targets exist in the described GEMs of pancreatic cancer, which should allow clarification of their importance before clinical assessment.

### Early Detection of Exocrine Pancreatic Cancer Using GEMs

A working group led by Teri Brentnall (University of Washington, Seattle, WA), Michael Goggins (Johns Hopkins University), Tony Hollingsworth (University of Nebraska, Omaha, NE), and David Whitcomb (University of Pittsburgh, Pittsburgh, PA) discussed the role of GEMs in the establishment of biomarkers for early pancreatic cancer. Biomarker discovery efforts using PDA patient serum has yet to produce an effective screening test and similar studies in high-risk patients are difficult because patients with PanIN are uncommonly identified prospectively. Furthermore, because PanIN-1A are present in most people, and only a fraction of patients progress to more advanced PanINs and PDA, biomarkers of the most advanced form of PanIN (PanIN-3), representing the carcinoma *in situ* stage of PanIN, should be a priority for the field. Fortunately, certain GEMs show the entire

spectrum of PanINs and the uniform genetic background and controlled environmental conditions of GEMs are also advantageous to biomarker discovery. Preliminary work using serum SELDI showed the feasibility of detecting PanIN “proteomic signatures” in these models (18), although no specific biomarkers have been reported.

The panel enthusiastically recommended the collection of multiple tissues (blood, pancreatic tissue, and pancreatic juice) from GEMs of advanced PanIN to pursue proteomic, genetic, and epigenetic biomarkers across all available platforms. Potential drawbacks to investigating biomarkers in GEMs include the genetically engineered bias inherent to the models, the possible lack of correlation between the mouse and human samples, and the potential contribution of pancreatic fibrosis and pancreatitis in the murine samples. These caveats notwithstanding, the panel suggested five areas of investigation with respect to biomarker discovery in GEMs. (a) Prospective biomarkers of PanIN-3 and PDA in mice would be validated in parallel human PDA specimens using appropriate reagents. (b) Adult onset diabetes mellitus is occasionally noted in patients before the diagnosis of PDA (19); therefore, GEMs should be evaluated for hyperglycemia during the progression of PanIN and onset of PDA to determine if diabetes correlates with the presence of specific biomarkers. (c) Anatomic and functional imaging of disease progression should be coordinated with biomarker discovery efforts to ensure proper disease classification and facilitate the identification of biomarkers of disease evolution. (d) Assessment of biomarkers of disease response should be pursued by genetically reversing the underlying state *in vivo* and/or using efficacious pharmacologic strategies. (e) Information obtained from the GEMs could be used to develop mathematical models that correlate disease pathogenesis with the presence of markers.

### Summary

The accomplishments of this NCI-Penn workshop extended far beyond our primary objective of producing a consensus pathologic nomenclature for exocrine pancreatic cancer GEMs. The dynamic interactions fostered within and between the various breakout groups directly resulted in the elaboration of specific proposals and goals that should shape the future direction of this community and ensure its success. Although any findings in these GEMs will need to be verified in PDA patients, the accurate murine models of PanIN and PDA should spur innovation and discovery of novel detection methods and new therapeutics for patients suffering from this malignancy.

### Appendix A. Pancreatic Cancer in Mice and Man: The Penn Workshop 2004

#### Speakers

Keynotes:	Douglas Hanahan (University of California San Francisco) Raul Urrutia (Mayo Clinic) Murray Korc (Dartmouth)
Overviews:	Scott Kern (Johns Hopkins University) Ralph Hruban (Johns Hopkins University)

Gloria Petersen (Mayo Clinic)  
 Jeff Drebin  
 (University of Pennsylvania)  
 Stephen Hahn  
 (University of Pennsylvania)  
 James Abbruzzese  
 (MD Anderson Cancer Center)  
 Ray MacDonald  
 (University of Texas Southwestern)  
 Steve Leach  
 (Johns Hopkins University)  
 Richard Kolesnick  
 (Memorial Sloan-Kettering  
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