

EDRN Pancreas Working Group Meeting

Executive Summary

Overview

Members of the EDRN Pancreas Working Group met in Denver, Colorado on January 5 and 6, 2006. The meeting was organized by Dr. Tony Hollingsworth of the University of Nebraska. The purpose of this meeting was to bring together members of different EDRN Biomarker Developmental Laboratories (BDL), Clinical Epidemiology and Validation Centers (CEVC), Biomarker Reference Laboratories (BRL), Associate Members, NCI Program Staff, and members of Patient Advocacy Organizations with interests in the detection of early pancreatic cancer. The goals were to inform members of this working group of the current research being undertaken in the various laboratories, to establish collaborative studies, to discuss potential validation studies for biomarkers of early pancreatic cancer, and to discuss the formation of EDRN Pancreatic Cancer Reference Sets.

Individual Presentations

Dr. Anna Lokshin, PI of an EDRN BDL at the University of Pittsburgh, presented her work on the use of the Luminex multianalyte profiling platform to identify panels of serum proteins that identify pancreatic cancer with high sensitivity and specificity. The Luminex platform uses antibodies attached to color-coded beads to quantitate the levels of multiple proteins in a low-volume, sensitive assay. Drs. William Bigbee and Xuemei Zeng, also from the University of Pittsburgh, described the use of various mass spectrometry approaches, including MALDI-TOF MS, to profile serum proteins of patients with pancreatic cancer.

Dr. Ann Killary, PI of an EDRN BDL at the University of Texas M.D. Anderson Cancer Center, presented her work to discover biomarkers to detect pancreatic adenocarcinoma using genomic strategies to investigate pathways involving loss of function of tumor suppressor genes. This research focuses on chromosomes 3p and 1p. Her groups has completed the sequencing of candidate cDNAs that were obtained from a suppression subtraction library and which represent genes in the 3p12 pathway. She also presented Affimetrix array experiments to identify which of these pathway genes are also differentially expressed in pancreatic tumor cell lines. A list of candidate biomarkers are being examined for differential expression in pancreatic tumor/normal samples. Genes that are differentially expressed across these platforms will be examined further for validation studies in collaboration with the other pancreas BDL laboratories.

Dr. Subrata Sen, co-PI of the BDL at the University of Texas M.D. Anderson Cancer Center, described his work to identify over-expressed genes in human pancreatic cancer by performing detailed genetic profiling of chromosomes 20q and 12p amplicons. He presented data on two amplicons in four well characterized pancreatic cancer cell lines (BxPC-3, Capan-2, MIA PaCa-2, PANC-1) utilizing Agilent CGH and expression microarray platforms. The analysis employs Agilent CGH Analytics and Feature Extraction software. For fine mapping the amplicon boundaries along the length of each chromosome additional statistical analysis with the Circular Binary Segmentation (CBS) method is being performed. Utilizing these approaches, a list of candidate biomarkers located on the common minimal amplicon intervals is being generated, which will then be taken forward for validation on a test set of tumor samples.

Dr. Marsha Frazier, also a co-PI of the BDL at the University of Texas M.D. Anderson Cancer Center, is examining the role of SNPs in candidate genes identified by the BDL as early detection markers and is developing methylation assays for candidate genes from Dr. Killary's project.

The overall goal of this M.D. Anderson Cancer Center BDL is to develop a panel of biomarkers for the early detection of pancreatic cancer utilizing locus-specific DNA based fluorescent in situ hybridization (FISH) and

polymerase chain reaction (PCR) assays, protein specific immunochemical detection assays, as well as SNPs that predict early onset, methylation assays and assays for microsatellite instability.

Tony Hollingsworth, PI of an EDRN BDL at the University of Nebraska, presented his work to improve the utility of the CA19-9 for detecting pancreatic cancer by adding to the test a determination of the core protein on which this carbohydrate antigen is detected. This proposal is based upon recent discoveries about the molecular nature of different mucin core proteins that are expressed by different adenocarcinomas. Surinder Batra, co-Investigator of the University of Nebraska BDL, described the development of antibodies against the MUC4 protein to detect early pancreatic cancer. He also reported that MUC4 mRNA expression is elevated in peripheral blood mononuclear cells of patients with pancreatic cancer. Michel Ouellette, co-Investigator of the University of Nebraska BDL, described the development a normal human pancreatic cell lines that are at different stages of transformation. These cells are immortalized by transfection with human telomerase and sequential addition of other genetic insults (mutations to K-Ras, knockout of p16/p53, addition of SV40 small T), and will be used to identify novel biomarkers for the development of pancreatic cancer.

David Tuveson of University of Pennsylvania and Sunil Hingorani of the Fred Hutchison Cancer Research Center discussed the use of mouse models to discover novel biomarkers of pancreatic cancer. David Tuveson presented preliminary proteomic data on sera from an oncogenic K-Ras knock in-model, and Sunil Hingorani described new mouse models for distinct types of pancreatic cancer. Kenneth Yu from the University of Pennsylvania described the characterization of proteins in human pancreatic cancer serum using differential gel electrophoresis and tandem mass spectrometry.

Hemant Roy, PI of an EDRN BDL at Evanston Northwestern Healthcare Research Institute, described the use of spectral markers taken from the histologically normal mucosa to risk stratify for pancreatic cancer. His team has found a striking alteration in spectral slope in the mucosa of pancreatic cancer patients. Randall Brand, an EDRN Associate Member from Evanston Northwestern Healthcare Research Institute, discussed his collaboration with EXACT Science evaluating a DNA Integrity Assay in the pancreatic juice and stool for the early detection of pancreatic cancer.

Diane Simeone, an EDRN Associate Member from the University of Michigan, presented her work on CEACAM1, a serum marker for detecting pancreatic cancer, which suggests that these antibodies against this protein could be added to a panel of antibodies to improve upon the performance of CA19-9.

Henry Lynch, PI of an EDRN CEVC at Creighton University, provided an update on hereditarily pancreatic cancer. He discussed research on hereditary pancreatic cancer in general, and in particular, its strong p16 causal connection with the familial atypical multiple mole melanoma (FAMMM) syndrome.

William Grizzle, PI of the EDRN BRL at the University of Alabama at Birmingham, presented his work on molecular markers for both early detection and prognosis of pancreatic lesions. He also described his experiences in EDRN-sponsored validation trials in for other cancers and what lessons could be drawn from these trials that could be applied to potential validation trials for makers for early pancreatic cancer.

Paul Wagner, a Program Director with National Cancer Institute, described the EDRN validation process, the criteria the EDRN uses to evaluate proposals for validation trials, and how investigators can apply for funds to conduct validation trials. He used the ongoing EDRN-sponsored validation trial for hepatocellular carcinoma to illustrate the process.

Two members of the patient advocacy community, Paula Kim of Translating Research Across Communities and Liz Thompson of the Pancreatic Cancer Action Network (PanCAN), attended and actively participated in group discussions.

Pancreatic Cancer Reference Set

A concern common to most the participants is the lack of access to specimens from patients with early stage pancreatic cancer. Most studies rely on samples of convenience that consist largely of specimens from patients with late stage cancers. Meg Mandelson discussed her ongoing specimen collection at the Center for Health Studies Group Health Cooperative, and Ann Killary discussed her collaborative specimen collections with the NCI SPORE at University of Texas M.D. Anderson Cancer Center. These presentations were followed by a group discussion that included, Tony Hollingsworth, Diane Simeone, Randal Brand, Michelle Anderson (University of Michigan) and Judy Anderson (University of Nebraska). The Working Group concluded that the assembly of EDRN Pancreatic Cancer Reference Sets was essential to accelerate the validation of biomarkers for early stage pancreatic cancer.

The following working groups for EDRN Pancreas Reference Set were formed.

1. **Common Data Elements.** Randal Brand (Chair), Meg Mandelson, Tony Hollingsworth, and Sunil Hingorani.

This group will draft at least 2 sets of uniform data elements to be ascertained at subject enrollment and through follow-up.

- ◆ **Core:** These data elements would comprise a "lowest common denominator" approach of data collection that would be collected on everyone.
- ◆ **Expanded:** These would consist of additional data collected by subsets of investigators with capacity, and interest, in collecting additional data. Examples of expanded data may include more detailed information on risk factors such as smoking, alcohol and history of chronic conditions. Expanded data might also include follow-up (e.g. survival among cases, cancer and benign outcomes among controls).

2. **Eligibility Criteria.** Diane Simeone (Chair), Randal Brand, Meg Mandelson, Tony Hollingsworth, and Sunil Hingorani.

This group will draft detailed eligibility criteria for (1) cases, (2) diseased controls, and (3) healthy controls. Potential matching criteria to cases may be considered by this group.

1. **Cases.** Criteria for early disease.
 2. **Diseased controls.** Includes eligible conditions and associated clinical data (e.g. chronic pancreatitis, newly diagnosed other cancers identified as consequence of diagnostic evaluation for possible pancreatic cancer).
 3. **Healthy controls.** Includes criteria for age and possible exclusions based on health history (e.g. prior EUS/ERCP, jaundice and impaired liver function, DM and glucose intolerance).
3. **Biospecimens.** Bill Grizzle (Chair), Ann Killary, Michelle Anderson, Meg Mandelson, and Bill Bigbee.

This group will draft protocols for specimen collection, handling, processing, and storage. The entire group will contribute to a two-page draft proposal, including a set of specific aims, that will be further refined at the March 2006 EDRN Steering Committee Meeting. Group members will work together in a series of conference calls and by email to complete this document.

Collaborative and Validation Studies

The PIs of the three EDRN BDLs agreed to continue their recently established collaboration to identify and evaluate novel biomarkers for pancreatic cancer. Two EDRN Associate Members Brian Haab, Van Andel

Research Institute, and Craig Logsdon, University of Texas M.D. Anderson Cancer Center are also participating in this collaboration. The goal of this collaboration is to identify biomarkers for early pancreatic cancer to be used in an EDRN-sponsored validation trial.

A fourth working group, consisting of the PI of four EDRN Pancreatic BDL, Anna Lokshin, Ann Killary, Bill Bigbee and Tony Hollingsworth (Chair), was formed to select biomarkers, assays and platforms to move forward into a validation trial. This Group will be comprised of the BDL leaders. This group will coordinate markers and help determine who may have a marker or panel of markers to move forward to test using the proposed EDRN Pancreatic Cancer Reference Sets.