32nd EDRN Steering Committee Meeting
September 12-14, 2017
Renaissance Hotel Seattle
Seattle, Washington

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Meeting Attendees:

EDRN Members:

Denise Aberle, Karen Anderson, Kristen Anton, Daniel Barnett, Robert Bast, Surinder Batra, Randall Brand, Dean Brenner, Robert Bresalier, Daniel Chan, Arul Chinnaiyan, Maureen Colbert, Kenneth Cole, Daniel Crichton, Jackie Dahlgren, Gerard Davis, Brenda Diergaarde, Charles Drescher, Steven Dubinett, Ziding Feng, Pierluigi Gasparini, Sandra Gaston, Robert Gillies, William Grady, William Graves, Kishore Guda, Brian Haab, Hua-Jun He, Kirk Herman, James Herman, Tony Hu, Ying Huang, Qihong Huang, Maneesh Jain, Wenqing Jiang, Sukhwinder Kaur, Heather Kincaid, Thomas Kislinger, Joshua LaBaer, Paul Lampe, Robin Leach, Marc Lenburg, Christopher Li, Dan Lin, Michael Liss, Guodong Liu, Tao Liu, Carolina Livi, Anna Lokshin, Gary Longton, David Lubman, Anirban Maitra, Royce Malnik, Sanford Markowitz, Jeffrey Marks, Tracey Marsh, Pierre Massion, Martin McIntosh, Dale McLerran, Lisa Newcomb, Hans Nielsen, Julius Nyalwidhe, Stephanie Page-Lester, Harvey Pass, Amanda Paulovich, Katherine Pellegrini, Margaret Pepe, Gyorgy Petrovics, David Ransohoff, Suzanna Reid, Kristin Rodgers, Karin Rodland, Mack Ruffin, Martin Sanda, Matthew Schabath, Robert Schoen, Ananda Sen, Ie-Ming Shih,Tujin Shi, Javed Siddiqui, Louise Showe, Javed Siddiqui, Steven Skates, Lori Sokoll, Shiv Srivastava, Sanford Stass, Deanna Stelling, Cha-Mei Tang, Nabihah Tayob, Jason Terrell, Mark Thornquist, Scott Tomlins, Missy Tuck, Tian-Li Wang, Tza-Huei (Jeff) Wang, Brandy Weaver, John Wei, David Wong, Haining Yang, Matthew Young, Hui Zhang, Zhen Zhang, Yingye Zheng

NCI/NIH Staff:

Jacob Kagan, Karl Krueger, Guillermo Marquez, Christos Patriotis, Jo Ann Rinaudo, Lynn Sorbara, Sudhir Srivastava

Guests:

Claus Linbjerg Andersen, Garnet Anderson, Jonathan Bibliowicz, Edwin Diaz, Carly Garrison, Richard Ivey, Jon Ladd, Lawrence LaPointe, Kristy Lastwika, Stanley Liu, Edward Lo, Uddin Hoti Naser, Hoifung Poon, Chirayu Shah, Matthew Snyder, Lotte Steuten, Riana Wernick

Tuesday, September 12, 2017

Opening of Steering Committee Meeting and Approval of March, 2017 EDRN Steering Committee Meeting Minutes

Joshua LaBaer, M.D., Ph.D., Arizona State University/The Biodesign Institute

Dr. LaBaer welcomed everyone to the meeting and thanked them for their attendance.

Welcome

Garnet Anderson, Ph.D., Senior Vice President and Director, Public Health Sciences, Fred Hutchinson Cancer Research Center

Dr. Anderson welcomed everyone to Seattle and to Fred Hutch, home to 2,700+ staff, including 223 scientific faculty, located on 15 acres in Seattle's vibrant South Lake Union neighborhood. She described the structure of Fred Hutch as follows:

Five Scientific Divisions:

- Clinical Research
- Basic Sciences
- Public Health Sciences
- Human Biology
- Vaccine and Infectious Diseases

Interdivisional initiatives

- Hutchinson Institute for Cancer Outcomes Research (HICOR)
- Immunotherapy Integrated Research Center (IRC)
- Pathogen-Associated Malignancies IRC

Public Health Sciences

Our objective: To identify strategies that will ultimately reduce the incidence of and mortality from cancer and other diseases.

142 Faculty organized into 5 programs

- Translational Research
- Epidemiology
- Cancer Prevention Research
- Biostatistics
- Computational Biology

Carotene and Retinol Efficacy Trial

18,000 men and women at high risk for lung cancer

Biospecimens:

- Serial pre-diagnosis serum, plasma, whole blood, blood spots
- Lung cancer tissues

Information: www.compass.fhcrc.org/caretWeb/

Contacts:

- Mark Thornquist (mthornqu@fredhutch.org)
- Gary Goodman (gary.goodman@swedish.org)

PCPT: 18,880 men; SELECT: 34,887 men

- Serial prediagnostic bloods
- Prostate biopsy and prostatectomy specimens

Information:

- www.swog.org/Visitors/PCPT.asp
- www.SWOG/visitors/SELECT

Contacts:

- Cathy Tangen (ctangen@fredhutch.org)
- Phyllis Goodman (pgoodman@fredhutch.org)

WHI Life and Longevity After Cancer Study (LILAC)

- 161,000 post-menopausal women
- Biospecimens

  - Serum, plasma, buffy coat collected at 2 time points
  - FFPE tumor specimens for selected solid tumor sites (LILAC)

Information: www.whi.org/researchers

Contacts:

- Garnet Anderson (garnet@whi.org)
- Charles Kooperberg (CLK@fredhutch.org)
- Lisa Johnson (ljohnson@whi.org)

Accessing these cohorts

- All are eager to have their repositories support high quality research
- All have well-defined mechanisms to evaluate requests for specimens
- All require
  - Written scientific proposal with preliminary data
  - Collaboration
  - Funding

State of the EDRN

Sudhir Srivastava, Ph.D., M.P.H., National Cancer Institute

Major Programs Addressing Early Detection

- Early Detection Research Network; www.cancer.gov/edrn
- Alliance of Glycobiologists: www.glycomics.cancer.gov
- Chronic Pancreatitis, Diabetes, Pancreatitis and Cancer Consortium (CPDPC): http://cscpdpc.mdanderson.org/
- Molecular and Cellular Characterization of Early Lesions
- Pancreatic Cancer Detection Consortium (PCDC)
- Cancer Imaging and Biomarker Consortium (CIB)
- Liquid Biopsy Consortium (RFA is out)
- Consortium on Translational Research on Liver Cancer (RFA is out)
- International Activities

The Early Detection Research Network
(EDRN)

EDRN Program Objectives

- Establish an investigator-initiated infrastructure to support development and validation of early detection biomarkers and biomarkers of progression
- Foster interaction between academic, clinical and industrial leaders
- Standardize biomarker validation criteria
- Develop a quality assurance program
- Bring biomarkers to clinical use

Partnering Organizations

- National Institute of Standards and Technology
- Center for Prostate Disease Research , DOD
- Pacific Northwest National Laboratory, DOE
- Jet Propulsion Laboratory, NASA
- Canary Foundation of America
- Lustgarten Foundation N.Y.
- International collaborations
- Industry (15 active)
- Associate Members (more than 200)
-

EDRN Milestones:

From Structure to Process to Outcomes:

Moonshot Program - Overall Goals of the Cancer Moonshot

- Accelerate progress in cancer, including prevention & screening
- From cutting edge research to wider uptake of standard of care
- Encourage greater cooperation and collaboration •
- Within and between academia, government, and private sector
- Enhance data sharing

Lung Collaborative Group
Lynn Sorbara, Ph.D., Karl Krueger, Ph.D.
NIH/NCI/Division of Cancer Prevention
Team Projects
LTP 2 – Indeterminate Nodules
Goal – to test/validate biomarkers that may help distinguish benign vs. malignant small nodules seen on CT.

- An ongoing project - the accrual of blood, bronchial brushes, from patients with small indeterminate nodules.
- Samples are blinded; eventually will be distributed to all sites for testing of their various biomarkers.
- The total number of patients to be accrued is 300 with 140 so far completed.
- Two new sites for accrual have been added recently (University of Pittsburgh and Moffitt Cancer Center).
- Updated protocols are completed; funding for acquisition of additional patients has been approved.

LTP3 – Biomarkers of Tumor Behavior after Resection of Clinical Stage I Lung Cancer
Goal – to improve the management of early adenocarcinoma by developing biomarkers predictive of tumor behavior.
Aims:

- To derive and refine molecular and imagining candidate biomarkers diagnostic of early lung ACC;
- To select molecular candidate diagnostic and predictive of aggressive lung ACC behavior; (similarly, will select candidates of indolent behavior);
- To understand whether behavior of lung cancers have specific anatomic characteristics using clinical CT images;
- To test how a predictive model integrating clinical, molecular and imaging biomarkers may best inform behavior and diagnosis in early stage disease

Future Team Projects
LTP4 – Pre-invasive Lesions ATLAS
Goal – To Discover Biomarkers Predictive of Disease Development

Specimens to be used/collected:

- Bronchial dysplasia (bronchoscopy or surgical specimens), and
- Ground glass opacities, atypical alveolar dysplasia or adenocarcinoma in situ

Currently in process:
Assembly of lists of specimens available with appropriate annotation.

GU Collaborative Group
Jacob Kagan, Ph.D., Richard Mazurchuk, Ph.D.
NIH/NCI/Division of Cancer Prevention

GU Collaborative Group
Proposed 3 Core fund studies:

- Expansion of the Tissue Upgrading Study – Robin Leach, Martin Sanda
- Clinical Utility of multiplex biomarkers for high grade prostate cancer: A cost effective – John Wei and Daniel Chan
- MRI Pre-Validation Study – John Wei

Expansion of the Tissue Upgrading Study
The overall goal is to improve the identification of men at risk of upgrading of their prostate cancer so that curative interventions can be more specifically directed, avoiding over-treatment of indolent prostate cancers.
This study combines patient accrual efforts from ten clinical sites to build a unique specimen reference set for both pre-validation and validation testing of biomarkers from six EDRN research labs.

Aim 1: Collect tissue, blood, and urine specimens to obtain a total of 440 subjects under the URS protocol
Aim 2: Evaluate the target urine biomarker assays in the pre- validation set
Aim 3: Evaluate the performance of urine biomarkers that pass pre- validation testing in the validation set.

Clinical Utility of Multiplex Biomarkers for High-grade Prostate Cancer

Aim 1: Expand the current EDRN's unique pre-diagnostic reference set with RNA sequencing of pre-biopsy urine.

Will apply the uMIPS RNA sequencing chip on banked urine samples from the PCA3 cohort in order to conduct head to head comparison of urinary biomarkers for the detection of high grade prostate cancer on needle biopsy. This will be evaluated on those who underwent initial and repeat prostate biopsies.
Aim 2: Extend the EDRN PCA3 reference set through a 5 year cohort follow up of those who underwent an initial prostate biopsy. Perform an unbiased comparative analyses of biomarkers (blood, urine and tissue) in their ability to predict high grade prostate cancer detection within 5 years of initial biopsy.
For tissue analysis will validate the MiPC_v2 RNA tissue sequencing chip.

MRI Pre-Validation Study
The goal of this study is to evaluate if the addition of prostate MRI to a panel including PSA, PCA3, TMPRSS2:ERG will significantly improve specificity for high-grade prostate cancer by 10%.

Aim 1: Create an optimal panel of urine and blood biomarkers that will select those cases most likely to benefit from a MRI targeted biopsy
Aim 2: Directly compare PSA and urinary biomarkers with MRI to determine which ones are value added in the setting of initial biopsy
Aim 3: Evaluate changes in these biomarkers and MRI to determine if longitudinal changes predict subsequent high-grade prostate cancer
Aim 4: Optimize MRI imaging to improve test performance. Importantly, this study will create a unique, prospective, cohort that will become the foundational reference set for of a range of future biomarker studies

Think Tank Meeting: a Cancer Biomarker Data Commons (CBDC)

- A modifiable interoperable data system to support biomarker development
  - Biomarker knowledgebase and forum
  - Public ad private data collections
  - Cloud-based computing platform
  - Crowd-sourcing competitions
  - Machine learning tools

Meeting logistics

- February 8-9, 2018 in Rockville, MD
- Organizers: NCI and JPL/NASA
- 20-30 invited speakers

Breast/GYN Collaborative Group
Christos Patriotis, Ph.D., Sharmistha Ghosh-Jangijian
NIH/NCI/Division of Cancer Prevention

Breast/GYN Cancers Collaborative Group-Ovarian Cancer Collaborative Projects

1. Circulating Autoantibody Biomarkers for Early Detection of Ovarian Cancer

- Clinical Aim: Early detection of OC using a regular blood test in normal risk postmenopausal women
- Lead-PI: Robert Bast, MDACC
- Other Participating PIs: Karen Lu (MDACC); Karen Anderson (ASU); Charles Drescher (FHCRC); Steven Skates (MGH); Zhen Zhang (JHU)

2. tDNA in Uterine Lavage and Serum Proteins in Early Detection of Ovarian Cancer
   - Aim: Develop a prospective biospecimen resource of blood and uterine lavage tDNA to investigate the possible complementarity uterine lavage tDNA mutational biomarkers and serum protein biomarkers to increase the sensitivity and specificity of early detection of ovarian cancer
   - Lead-PI: Steven Skates, MGH
   - Other Participating PIs: Robert Bast (MDACC); Charles Drescher (FHCRC); Ie-Ming Shih (JHU), Daniel Chan (JHU), Bethan Powell (Kaiser-Permanente), Lucy Gilbert (McGill U.), Paul Speiser (U. Vienna), Rosana Risques (UW), Jesse Salk (TwinStrand BioSciences)

- Identification of Biomarkers for Non-Triple Negative Breast Cancer
  - Clinical Applications: 1) to inform women with (-) mammogram timing of subsequent screening (6 mos vs. 1 yr); 2) to inform continuation of mammographic screening among 75-79 yo women
  - Lead-PI: Christopher Li, FHCRC
  - Other Participating PIs: Karen Anderson (ASU); Jeffrey Marks (Duke U); Amanda Paulovich (FHCRC); Michael Lewis (Baylor U)
- Combined Imaging and Blood Biomarkers for Breast Cancer Diagnosis
  - Aim: Develop a retrospective and a prospective cohort of mammographic images and blood to be used in biomarker model development and testing
  - Lead-PI: Jeffrey Marks, Duke U.
  - Other Participating PIs: John Heine (Moffitt CC), Cha-Mei Tang (Creatv Microtech)
- Combining Avatar mice and Targeted Mass Spectrometry to Identify Blood Protein Biomarkers for Early Detection of Breast Cancer
  - Aims: 1) N-glycopeptide enrichment in plasma of PDX-bearing mice to detect human proteins; 2) perform LC-MS/MS runs on Thermo Scientific Orbitrap Fusion Lumos Tribid mass spectrometer for deeper detection of human proteins in mouse plasma
  - Lead-PI: Amanda Paulovich, FHCRC
  - Other Participating PIs: Michael Lewis (Baylor U.), Richard Smith, Karin Rodland, Tao Liu (PNNL)
- Evaluation of Biomarkers of BC in Women Diagnosed with Benign Breast Disease
  - Aim: Pre-validation of biomarkers that could predict likelihood of invasive BC following a tissue diagnosis of benign breast pathology
  - Lead-PI: Andrew Godwin, KUMC
  - Other Participating PIs: Jeffrey Marks (Duke U)

Colon, Pancreas and Other GI Cancers Collaborative Group
JoAnn Rinaudo, Ph.D., Matt Young, Ph.D.
NIH/NCI/Division of Cancer Prevention

Collaborative projects for the Colon Cancer Team

1. ctDNA and Aberrant Methylation in Colorectal Cancer Screening and Monitoring
   - Kenneth Kinzler, Johns Hopkins University
   - Robert Schoen, University of Pittsburgh
   - Sandord Markowitz, Case Western Reserve University
   - William Grady, Fred Hutchinson Cancer Research Center
2. Steps Towards Validation of Plasma Biomarkers for the Detection of Colorectal Adenoma and Cancer
   - Paul Lampe, Fred Hutchinson Cancer Research Center

- Robert Bresalier, MD Andersen Cancer Research Center
- Ziding Feng, MD Andersen Cancer Research Center
- Hans Jørgen Nielsen and Ib J Christensen, University of Copenhagen/Hvidovre Hospital
- Dean Brenner, Regents of the University of Michigan

Collaborative projects for the Pancreatic Cancer

Aim 1 – Compare the performance of several candidate biomarkers for discriminating resectable PDAC from benign pancreatic disease both alone and in combination with CA19-9

Aim 2 – Test the value of novel combinations of biomarkers to identify candidates for further testing using the reference set.

Biomarkers include –

- MUC5AC (two different glycoforms); sTRA, other sialyl-Lewis X family member
- MUC4, MUC5AC, CA19.9, disialyl-LewisA, sialyl-LewisA, disialyl-LewisX and sialyl-LewisX on the carrier mucin MUC4 and MUC5AC and evaluation of MUC4 levels (using novel mAbs)
- LRG1, TIMP1, four autoantibodies and two metabolites
- CEA, TIMP1, TIMP4, and ApoA1

Specimens (from U Pittsburgh, MD Anderson, and Mayo Clinic) –

Stage I, II, III, and IV pancreatic cancers
Metastatic PDAC
Neuroendocrine tumors
Ampullary carcinoma
Chronic DM
Benign Biliary Obstruction
Chronic Pancreatitis
"Healthy" controls – nonsmokers
"Healthy" controls - smokers
Resectable PDAC with DM within 3 Years
Resectable PDAC with Chronic DM
Resectable PDAC with No DM
Pseudocysts
Serous cysts
IPMNs
MCNs
Cholangiocarcinoma

Approved Core Fund Projects

Group	PI	Project Title
Breast/Gyn	Skates	tDNA in uterine lavage and plasma proteins in BRCA1 and BRCA2 carriers at RRSO
GI	Brand	Alliance for PDAC - BM verification study in cyst fluid
GI	Brenner	GLNE 010
GI	Maitra	New-onset Diabetes and Pancreacitc Cancer Study
Lung	Massion	Validation of Biomarkers of Risk for the Early Detection of Lung Cancer
Lung	Pass	Chile Study
Lung	Spira	Biomarkers to discern malignancy in indeterminate nodules LTP2
Lung	Massion	Biomarkers of Recurrence after Resection of Clinical Stage I Lung cancer - LTP3
Prostate	Leach	San Antonio Biomarkers of Risk for Prostate Cancer - Upgrading Reference Set Phase II
Non-EDRN Investigators		
Breast/Gyn	Godwin	Evaluating Predictive Biomarkers of Breast Cancer in Women Diagnosed with Benign Breast Disease
GI	Fan *	Nanoplasmic quantification of turmor-derived circulating extracellular vesicles
Lung	Willey *	Validation of lung cancer Risk Test (LCRT) Biomarker
Prostate	Wei	Clinical Utility of multiplex biomarkers for high grade prostate cancer: A cost effective…
Prostate	Wei	MRI Prevalidation Study
* Associate Members		

New Activities
CBDC's role in a Cancer Data Ecosystem
Support for the entire biomarker development pipeline

Liver Cancer Initiatives
Receipt Date – October 24, 2017
RFA-CA-17-025 – Consortium on Translational Research in Early Detection of Liver Cancer: Translational Research Centers (U01) – up to 5 awards
RFA-CA-17-028 – Consortium on Translational Research in Early Detection of Liver Cancer: Data Management and Coordinating Center (U24) – 1 award

Liquid Biopsy RFA

Precompetitive Collaboration on Liquid
Biopsy for Early Cancer Assessment (U01)

Release date:
August 2, 2017
Open Date (Earliest Submission Date)
September 24, 2017
Letter of Intent Due Date(s)
30 days before application due date
Application Due Date(s)
October 24, 2017 and January 23, 2018, by 5:00 PM local time of applicant organization. All types of non-AIDS applications allowed for this funding opportunity announcement are due on these dates.

Need for 3D and 4D Atlases of Premalignant Lesions for Prevention and Early Detection

- Histologic features of premalignant lesions are not adequate for developing precision risk stratification and intervention strategies.
- There is lack of comprehensive knowledge of microenvironment and genomic and epigenomic alterations that drive cancer progression at its earliest stages.
- Systematic efforts to longitudinally collect and perform molecular and cellular profiling of premalignant lesions as they progress towards frank malignancy or regress are lacking.
- Integration with quantitative imaging data is necessary.

PreCancer Atlas

- RFA is likely to be out by October 2017
- Requires multi-PI, multi-disciplinary application

Site Task Reminder
55 EDRN PIs

- Only 5 investigators entered their Publications into the Secure Website in 2017 (Daniel Chan, Sanford Stass, Pierre Massion, Christopher Li and Ziding Feng)
- Only 13 investigators have completed the specimen survey (Dean Brenner, Jeffrey Marks, John Semmes, William Grady, Sanford Markowitz, Harvey Pass, Daniel Chan, Paul Lampe, Christopher Li, Surinder Batra, Robert Schoen, Louise Showe and Ziding Feng)
- Only 21 investigators have entered their research interests (Dean Brenner, Jeffrey Marks, Ian Thompson, John Semmes, Randall Brand, William Grady, Brian Haab, Sanford Markowitz, Harvey Pass, Arul Chinnaiyan, Daniel Chan, Sanford Stass, Karen Anderson, Tao Liu, Paul Lampe, Christopher Li, Surinder Batra, Robert Schoen, Steven Skates, Louise Showe and Ziding Feng)
- Out of 54 e-mails inquiring about new Protocols IDs for this cycle only 13 people responded which resulted in 2 Protocols for Matthew Schabath and 1 Protocol for Joshua LaBaer/Karen Anderson

Japan-EDRN NCI Annual Meeting
March 18-10, 2018

- The 5th Annual Meeting is scheduled for March 18-19, 2018

- Japan's AMED is sponsoring the meeting and willing to pay for accommodations for a select EDRN investigators
- Please indicate Royce of your willingness to travel and attend

EDRN Workshop
March 6-8, 2018
Planning Committee Meeting Tomorrow At Noon
There Will Be Joint Meeting With MCL Consortium As Well

Deep Learning for Precision Medicine
Hoifung Poon, Ph.D., Microsoft Research
Deep Learning for Precision Medicine

- Overview
- Precision medicine
- Deep learning
- Project Hanover
- EDRN opportunities

Medicine Today Is Imprecise
Top 20 drugs 80% non-responders
Wasted
1/3 health spending
$1 Trillion/year

Disruption: Big Data
Accenture study: 93% of US doctors using EMRs

Why We Haven't Solved Precision Medicine?

Knowledge Bottleneck

- Big data: Hard to interpret
  - E.g.: Tumor mutations
- Rich knowledge: Hard to curate
  - E.g.: PubMed, clinical trials, EMRs
- Wanted: Machine reading

Machine Reading

- Reasoning Bottleneck
- Small training data
- Complex features
- Rich knowledge
- Wanted: Knowledge-rich machine learning
  - Machine Learning
  - Machine Learning
  - Machine Learning

Example: Personalize Drug Combos

- Targeted drugs: 149
- Pairs: 11,026
- Tested: 102 (in two years)
- Unknown: 10,924

Knowledge-Rich Machine Learning
Example: Personalize Drug Combos
Targeted drugs: 149
Pairs: 11,026
Tested: 102 (in two years)

Unknown: 10,924

Project Hanover
Challenge: Long Tail of Variations
negative regulation
532 inhibited, 252 inhibition, 218 inhibit, 207 blocked, 175 inhibits, 157 decreased, 156 reduced, 112 suppressed, 108 decrease, 86 inhibitor, 81 Inhibition, 68 inhibitors, 67 abolished, 66 suppress, 65 block, 63 prevented, 48 suppression, 47 blocks, 44 inhibiting, 42 loss, 39 impaired, 38 reduction, 32 down-regulated, 29 abrogated, 27 prevents, 27 attenuated, 26 repression, 26 decreases, 26 down-regulation, 25 diminished, 25 downregulated, 25 suppresses, 22 interfere, 21 absence, 21 repress ……

Challenge: Complex Relations
Deep Learning

- Key idea: distributed representation
- Non-linear dimension reduction
- Automate feature learning

Neural Unit
Neural Network
Deep Learning
Promise: Representation Learning
Promise: Multi-Task Learning
Annotation Bottleneck
Deep learning requires many labeled examples
Hire experts to label: Not scalable
Crowdsource: Lack domain expertise
Learning with Indirect Supervision
Unsupervised learning
Statistical relational learning
Situated learning
Distant supervision
Incidental learning
Grounded learning
Grounded Learning

Project Hanover: Grounded Learning
Entity Linking (a.k.a. Normalization)
Entity Linking
Entity Linking

Entity Linking: Grounded Learning
Distant supervision: UMLS ontologies
Positive examples: Mentions matching lexicon
Reduce noise: Filter short mentions
Negative examples: Random noun phrases

Entity Linking: Grounded Learning
Deep learning dominates feature-engineered
Transfer learning: PMC $\rightarrow$ Clinical trials, EMRs
Grounded learning substantially outperforms cTAKES etc.

Relation Extraction
Relation Extraction: Grounded Learning
Distant supervision: Manually curated databases
Positive examples: Co-occurring w. known relations

Reduce noise: Minimal span

Negative examples: Random co-occurring tuples
Graph LSTM
Recurrent Neural Network
Recurrent Neural Network
Long Short-Term Memory (LSTM)
Why Graph?
Relation Extraction: Grounded Learning
Deep learning dominates feature-engineered
Multi-task learning improves accuracy
Cross-sentence triples extraction yield
Grounded learning: 70 X manual curation
Knowledge Base Embedding
Knowledge Base Embedding
Reasoning: Efficient
Recall on held-out: + 20 points
Learned features for other predictions
Example: Personalize Drug Combos
Targeted drugs: 149
Pairs: 11,026
Tested: 102 (in two years)
Unknown: 10,924
Personalize Drug Combinations
Patient: Transcriptome
Drug: Gene targets
Gene embedding → key features
Learning Interpretable Model
Learning Interpretable Model

New: Chronic Disease Modeling
EDRN Opportunities
Literature: Prior knowledge on biomarkers
EMR: Patient information
Clinical trial: Structured eligibility criteria
Big data mining: Omics, EMRs, images
Mechanism discovery: Serial biomarker

Collaborators
Knight: Brian Drucker, Jeff Tyner
Chicago: Andrey Rzhetsky
Wisconsin: Mark Craven
Microsoft Research: Chris Quirk, Kristina Toutanova, Scott Yih, Bill Bolosky, David Heckerman, Lucy Vanderwende, Ravi Pandya
Interns: Maxim Grechkin, Ankur Parikh, Victoria Lin, Sheng Wang, Stephen Mayhew, Daniel Fried, Violet Peng, Hao Cheng, Hai Wang

Selected Highlights of EDRN Progress
Session Moderator: Jo Ann Rinaudo, Ph.D., National Cancer Institute
[CVCs will report on their validation projects.]

Urinary RNA, Serum Biomarkers, and Prostate Cancer-Specific PET Imaging: A Trifecta to Enhance Prostate Cancer Detection
Martin Sanda, M.D., Emory University
Urinary RNA, Serum Biomarkers, and Axumin PET Imaging:

A Trifecta to Better Detect Aggressive Prostate Cancer

Martin G. Sanda, M.D.
PI, Emory-Harvard-University of Washington EDRN CVC
Professor and Chairman, Department of Urology
Emory University School of Medicine & Winship Cancer Institute

Serum Biomarkers, Urinary RNA, Imaging to Better Detect Aggressive Prostate Cancer

- Serum
  - Prostate Health Index: phi (D Chan, L Sokoll et al)
- Urinary RNA
  - PCA3 (J Wei et al)
  - TMPRSS2:Erg (S Tomlins, A Chinnayian et al)
  - Multiplex urinary RNA (K Pellegrini et al)
- Combining Urinary RNA and Serum Biomarkers
  - Serum phi and urinary PCA3 (E Huang et al)
- Imaging
  - Mets: Axumin-PET(D Schuster, M Alemozaffar et al)

Serum: Prostate Health Index (phi)

Phi = [-2]pro-PSA / free PSA * √ total PSA

Less impact of prostate size/BPH (than total PSA)

Complementary Collaboration between NCI & Industry:

- EDRN Studies
  - Retrospective prediction of biopsy (Sokoll et al J Urology 2008)
  - Prospective validation (Sokoll et al Ca Epi Bio Prev 2011)
  - Gleason score 7+ on initial biopsy (de la Calle 2015)
- Beckman pivotal trial
  - Catalona et al J Urology 2011
  - Improved accuracy over PSA and %free PSA
  - Any Prostate Ca on Initial or Repeat Biopsy

Prostate Health Index (phi) to Detect Aggressive Prostate Cancer: Results

- PHI specificity for aggressive Pca higher than PSA, %free PSA
- Using PHI ≥ 24 to select men for biopsy can:
  - Avoid unnecessary biopsies in 36% of biopsy candidates
  - Avoid over-detection of Gleason 6 cancer in 26%

Current Status:

- FDA-approved, commercially available clinical assay
- Awaiting CMS reimbursement code/policy (in process)
- Clinical adoption underway - slowly

Serum Biomarkers, Urinary RNA, Imaging to Better Detect Aggressive Prostate Cancer

- Urinary RNA
  - PCA3 (J Wei et al)
  - TMPRSS2:Erg (S Tomlins, A Chinnayian et al)
  - Multiplex urinary RNA (K Pellegrini et al)

Urinary RNA: PCA3 J Wei et al, EDRN PCA3 Validation Trial, J Clin Onc 2014

- DD3: a non-coding RNA associated with PCA tissue (Bussemakers et al 1999)
- Detected in post-DRE urine sediment (Hessels et al 2003)
- Adopted to clinical grade assays in post-DRE whole urine (Genprobe/ Hologics)
- FDA approval to select men for repeat prostate biopsy (2012)

Urinary RNA Testing to Refine Prostate Ca Detection

- TMPRSS2:ERG (A Chinnayian, University of Michigan BDL)
  - Gene rearrangement (deletion or translocation) in ~ half of prostate cancers (Tomlins et al, Science, 2005)
  - TMPRSS2-Erg RNA detected in post-DRE urine sediment by RT-PCR (Laxman et al 2006)
  - Possible combination with PCA3 suggested (Hessels et al 2007)

Urinary T2:Erg and PCA3 RNA Testing to Refine Prostate Cancer Detection
M Sanda et al JAMA Oncology 2017

Study Objectives
- To determine if combined T2:erg and PCA3 RNA assay can improve specificity for detecting 'aggressive' PCA (Prostate biopsy Gleason score $\geq$ 7)
  - Reduce unnecessary biopsy and 'over-diagnosis' of cancers with Gleason score = 6
- To model the lifetime cost impact if urinary T2:Erg and PCA3 was used to identify men for prostate biopsy

Urinary T2:Erg and PCA3 RNA Testing to Refine Prostate Cancer Detection
Combining Urinary T2:Erg and PCA3 RNA Testing Improves Specificity for Predicting Gleason $\geq$7 PCa
Urinary T2:Erg and PCA3 Testing at Age = 55-64 Yrs Would Reduce Lifetime Health Care Costs
Targeted Sequencing of Prostate Ca-Associated RNAs in Extracellular Vesicles From Post-DRE Urine (K Pellegrini et al, ongoing)

Serum Biomarkers, Urinary RNA, Imaging to Better Detect Aggressive Prostate Cancer
- Combining Urinary RNA and Serum Biomarkers
  - Serum phi and urinary PCA3 (E Huang et al)

Combining FDA-Approved Clinical Assays: phi and PCA3 RNA Testing to Refine Prostate Cancer Detection

Combining Serum Prostate Health Index with post-DRE Urine PCA3 and T2Erg Clinical Assays to Predict Aggressive Prostate Ca (E Huang et al, ongoing)
Serum Biomarkers, Urinary RNA, Imaging
to Better Detect Aggressive Prostate Cancer
- Imaging
  - Mets: Axumin-PET(D Schuster, M Alemozaffar et al)

Detection of Prostate Cancer Micrometastatic Disease by FACBC-PET
M Goodman, Dept of Radiology & Chemistry; D Schuster, Dept of Radiology, et al

Imaging: Axumin PET
Current Status:
- FDA-approved, widely available
- CMS reimbursement to detect metastases in PSA recurrence following primary treatment
- New clinical use: detect metastases at initial diagnosis of high risk disease to guide primary treatment

Clinical Trial: Axumin-PET prior to superextended lymphadenectomy in high risk, localized prostate cancer
Serum Biomarkers, Urinary RNA, Imaging to Better Detect Aggressive Prostate Cancer
- Serum
  - Prostate Health Index: phi (D Chan, L Sokoll et al)
- Urinary RNA
  - PCA3 (J Wei et al)
  - TMPRSS2:Erg (S Tomlins, A Chinnayian et al)
  - Multiplex urinary RNA (K Pellegrini et al)
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  - Serum phi and urinary PCA3 (E Huang et al)
- Imaging
  - Mets: Axumin-PET(D Schuster, M Alemozaffar et al)

Validation of Colon Cancer Biomarkers

Dean Brenner, M.D., University of Michigan

GLNE 010: Aims in 2007

1. Estimate the sensitivity and specificity for
    a. colorectal adenocarcinoma
    b. screen relevant neoplasms (SRN) (colorectal adenocarcinoma, high grade dysplasia, and adenomas ≥1cm) for stool (vimentin methylation) and circulating (galectin-3 ligand, ctDNA, other future) biomarkers
2. Determine whether stool vimentin methylation sensitivity >FIT same specificity of stool vimentin methylation test.

3a. Estimate sensitivity and specificity of the above individual binary biomarkers in combination with FI.

3b. Determine if sensitivity of stool vimentin methylation + FIT > FIT

4. Establish an highly quality FDA GCP biosamples for future validation and discovery.

Study Design "GLNE 010"

Registrations, Evaluable,

Case Rates

- Registrations: 5,154
- Evaluable Completions: 4,669 (88%)
- Number of Events to Date: 29
  - Cancers: 10 (0.2%)
  - High Grade Dysplasia: 19 (0.4%)
- Case Proportion: 0.6%
- Projected Case Proportion: 1.5%
  - Large adenoma case proportion: 14%

This was complete in 2013

We recruited 4,669 eligible in 18 months.

Project Activation Steps 1

1. Finalize protocol
    - Required 7 months of internal EDRN review
2. Submit, negotiate Core Funds, Set aside funding
3. IRB Review
    - 8 EDRN Institutions, 3 new institutions
    - Alliance
4. CDAs, MTAs
    - Negotiate, execute with three different companies

Project Activation Steps 2

5. Subcontracts
    - Execute Subcontracts with Each Institution
    - 3 Separate Funding pools—Overhead problems
6. Training
    - Site Visit
7. Procuring Supplies
    - Manufacturing 1000s of kits for shipment

GLNE 010 Current Status

GLNE 010: Aims in 2007

It has been a decade!

Science marches on!

Exact Sciences Panel – Sensitivity by Stage Histology

Issues with Stool DNA and Fecal Occult Blood Tests

- FIT test x 1 only. Serial testing may alter FIT performance.

- High cost
- Complex collection and shipping procedures
- Universal FIT Screening Programs: 40% Refuse the Test
- Exact test improved over FIT but insufficient for adenoma (non-invasive neoplasm)
- Exact did not collect blood!

Key Challenges in Colon Cancer Early Detection
- Enhancing Adherence to Current Screening Guidelines
  - 40% of USA population non-adherent
  - Barriers: Cost, discomfort, cultural taboos

Strategy: Circulating Markers
- Tailoring Colonoscopic Screening to Individual Risk
  - Select high risk for endoscopic screening. Rest no screening.
  - Specific demographic risk groups (genetic groups, African Americans)

Strategy: High sensitivity (99%) biomarker panels

Key Challenges in Colon Cancer Early Detection
- Persistently Positive Biomarker Tests, Neg Endoscopy
  - Stool methylated DNA panels report 5% "false positives"
  - Potential Sources: Upper GI; flat adenomas, serrate lesions
  - Non-GI sources

Strategy: Develop longitudinal subsets; specialized endoscopy (molecular probes); upper GI; more extensive screening (pancreas, extra GI source)

Key Challenges We Can Address
- Circulating Markers
  - Can we identify a panel that can equal FIT stool screening?
  - Must a circulating biomarker panel be equal to or beat FIT to be useful?

Sources of Circulating Markers
- Glycoproteins
- Circulating Genetic Material
  - Methylated genes appear to have stronger signals
- Carcinogenesis Associated Genes
  - Rate limiting control of pivotal inflammation mediators
- Antibodies

A Strong Circulating Biomarker
Galectin-3 Ligand--Glycoprotein
- Beta-galactoside-binding protein implicated in tumor progression and metastasis.
- In colon cancer sera, a 40 kDa band distinct from mucin, CEA.
- This band was also detected by Lotus tetragonolobus lectin and Erythrina cristagalli lectin (ECL), indicating that it is a glycoprotein containing fucose and N-acetyllactosamine.
  - R. Bresalier, MD Anderson/GLNE CVC EDRN

Modeling: Galectin-3 Ligand, CYFRA21, CEA
Using 300 EDRN Samples

Circulating Methylated Genes from Clinical Genomics
IKZF1, BCAT1

Carcinogenesis Mechanism Based Gene
Markowitz Laboratory
PGDH as Predictor of Adenoma Recurrence

Autoantibodies
Lampe Laboratory EDRN

Antibodies to Nucleosomes
Volition, Inc

- Family of 39 Different Antibodies, 5 key families
  - Specific DNA Modifications
  - Histone Variants
  - Histone Modifications
  - Nucleosome—Protein Adducts
  - Total Nucleosomes

Panel of 4 Nucleosome Antibodies, Serum Assay
Preliminary Validation by Volition, Inc

- 4800 Symptomatic Patients Undergoing Colonoscopy
  - Sensitivity 81%, Specificity 78% for invasive Colorectal Neoplasms
- Preliminary Screening Detection of Stage I Cancers
  - Sensitivity 74% at Specificity of 90%
- High Risk Adenoma (≥1 cm, dysplasia)
  - Sensitivity 61% @ Specificity of 80%; 530 symptomatic patients
- Tayloring Colonoscopic Screening to Individual Risk
  - Select high risk for endoscopic screening. Rest no screening.

Strategy: High sensitivity (99%) biomarker panels
Will Microbiome Add the Necessary 7% Sensitivity?
Key Challenges We Can Address

- Tayloring Colonoscopic Screening to Individual Risk (goal of 99% sensitive marker panel at specificity of >70%)
  - If we combine circulating markers with FIT will be equal or surpass Exact Sciences panel?
  - Will vimentin beat the Exact panel?
  - Will digital sequencing analytical technology improve stool DNA sensitivity to get us to the 99% target?
  - Will the microbiome be the component that pushes stool panes to the 99% target?

Managing Accrual to Ensure Successful Completion

- Group A--Reopened
  - 50-59 yr
  - First colonoscopy
- Group B--Reopened
  - 60-69 yr
  - Prior colon exam, none within 108 months
- Group C
  - ≥60 yr
  - No prior colon exam
- Group D
  - ≥70 yr
  - No prior OR prior colon exam within 108 months
- Group E
  - ≥65 yr
  - Prior advanced adenomas (>1 cm, sessile)
  - No colon exam within 36 months
- Group F
  - 50-64 yr
  - Prior 3-10 tubular adenomas, no colon exam within 60 months OR

- Prior >10 adenomas, no colon exam within 36 months OR
- Prior advanced adenoma, no colon exam within 36 months OR
- Prior adenoma with high grade dysplasia, no colon exam within 36 months OR
- Sessile serrated polyps, no colon exam within 60 or 36 months depending upon size

Comparison to Equivalent Studies
WHAT HAPPENED???
Age
Community Vs Academic
Screen Vs Surveillance
Registrations on Study

German Screening-Biomarker Trial (BliTz—H Brenner)

- N = 7,950
- Study Duration:
  - 7 yrs

Colonoscopy Screening Rates

- USA: 61%
- Germany : 16%

Why Exact, PreSept, BliTz with Higher Event Rates

- Exact
  - Required 2/3rd enrollments ≥65 yrs.
  - More community sites.
- PreSept
  - Enrolled first colonoscopy screens only.
  - Half enrollment in Germany
  - 65% ≥ 60 yrs
- Blitz
  - Same enrollment as GLNE 010
  - Entirely in Germany (16% colonoscopy screening rate)

Bias

- Screening population—have access to care, including minorities on trial. Minority accrual 17%.
- Pathologist Bias? Fewer academic pathologist make high grade dysplasia diagnosis

Inferences

- Rapid changes in standard of care in USA from 2007 to now.
- Low screening adherence in Germany (16%) results in published cancer event rate of 0.7%. Same as in literature.
- "Downstaging" from invasive carcinoma to advanced adenomas.

Plan

- Cancer Endpoint Only
  - Dysplastic adenomas as endpoints degrade biomarker performance.
  - Reduces the likelihood of successful identification of early detection biomarkers for colorectal neoplastic disease.
  - increases barriers for success to the very biomarkers that the EDRN exists to discover and validate.
  - Degrades the usefulness of the repository samples generated by GLNE 010 for future validation trials.

Plan

- Substantial enrollment in Germany
- USA enrollment restricted to ≥60 yrs, first colonoscopy only

- Incremental industrial funding available (meets Executive Committee requirements)

Analytical Validation

- Galectin-3 Ligand Assay
  - Bresalier Laboratory—Invented Assay
  - BRL—UCLA—Converted to Luminex Assay
  - Going to run at UCLA
- Vimentin Methylation
  - Markowitz Laboratory—Invented Assay
  - BRL—University of Maryland—Converted to qPCR
    - Now extracted from 88 subjects

Team Projects: Aims

- Identify biomarkers that predict the presence of advanced colorectal adenomas
  - Discovery in blood, tissue, stool
  - Promising markers go to validation in blinded reference sets from previous GLNE CVC samples
  - Validated biomarkers to includ in GLNE 010 (cross-sectional colon validation study)

Grady Laboratory
Methylated NTRK3 in Stool DNA-based assay
Microbiome

Mucins and their Associated Serological Panel for Diagnosis of Pancreatic Cancer

Randall Brand, M.D., University of Pittsburgh and Surinder Batra, Ph.D., University of Nebraska Medical Center

Mucins and their Associated Serological Panel for Diagnosis of Pancreatic Cancer
Pancreas CVC
(UPMC and UNMC)
The overall theme of the CVC is to validate the utility of selective mucins and their glycoforms as biomarkers for early detection of PC and effectively identify pancreatic cystic neoplasms with higher lethal potential.

Proposed biomarkers arose from previously funded work in BDLs
Pancreatic Cancer (PC)
Estimated new cases and deaths from PC in the US in 2017:
New cases: 53,070 people (27,670 mean and 25,400 women)
Deaths: 41,780 people (21,450 men and 20,330 women)
PC mortality rates has not changed over the last four decade
Better Diagnostics Tools will lead to better PC treatment
Mucins and Cancer
Mucins and Cancer
Expression Profile of Mucins in Pancreatic Pathologies
MUC4 and MUC5AC are Differentially Expressed in PDAC (
cDNA array, oligonucleotide array and SAGE Analysis
MUC5AC during Progression of PC
MUC5AC appears early and expression is maintained till metastatic stages.
MUC5AC Expression in Different Pancreatic Diseases
Commercial TMA: 101 patients (208 cores)
MUC5AC expression found to be specifically elevated during PC
MUC5AC Diagnostic Significance Assessment
MEASUREMENT OF SERUM MUC5AC
TRAINING SET: UPMC DIAGNOSTIC SIGNIFICANCE (N=241)& UNMC PREDICTIVE SIGNIFICANCE (N=201)
VALIDATION SET I: MAYO CLINIC (N=94)
VALIDATION SET II: UPMC BLINDED SET (N=322)

REFERENCE SET: NCI (N=242)

Training Set

Elevated levels of MUC5AC are observed in both early and late stage pancreatic cancer cases. MUC5AC emerged as highly potential biomarker for differentiating PC

MUC5AC in Combination with CA19.9

The combo (MUC5AC and CA19.9) improves the sensitivity and specificity for differentiating EPC from controls.

Summary MUC5AC+CA19.9

- Elevated levels of MUC5AC are present in early stages of pancreatic cancer patients.
- MUC5AC at its optimal cut-off (20.4 ng/ml) improves the diagnostic efficacy of CA19.9
- The combination of MUC5AC and CA19.9 improve differentiation of
  - EPC cases from benign controls (Sensitivity and Specificity of 85.4% and 71.7%)
  - EPC and chronic pancreatitis cases (Sensitivity and specificity of 86% and 78%) in comparison to CA19.9 alone (66% sensitivity 47.8% specificity for BC; 69.8% sensitivity 31.7 % specificity for CP).

Diagnostic Performance of MUC5AC in Validation Sets

MUC5AC is potential diagnostic marker for identifying early stage pancreatic cancer (Stage 2B)

Reference Set

242 blinded samples collected by NCI under EDRN program

Patients till stage 2B were present in the reference set

CA19.9 and MUC5AC levels

Elevated levels of both MUC5AC and CA19.9 were observed in PC early stages in comparison to various control groups

Similar trend was observed after age, diabetes adjustment

Elevated levels of CA19.9 were observed in acute biliary obstruction cases while MUC5AC showed varying trend in this group

Stage IA/IB/IIA vs Chronic Pancreatitis

MUC5AC performed better than CA19.9 in differentiating chronic pancreatic cases from CA19.9 that was used at its optimal cut-off

Stage IA/IB/IIA vs Acute Biliary Obstructions

MUC5AC has high diagnostic potential in cure biliary obstruction cases and combination performed much better than CA19.9 alone

Summary: CA19.9 and MUC5AC levels

- SIGNIFICANTLY ELEVATED LEVELS OF MUC5AC ARE OBSERVED IN EARLY STAGE PC CASES. (training sets, two centers and blinded EDRN reference sets)
- MUC5AC ALONE DIFFERENTIATE STAGE 1A/1B/IIA FROM VARIOUS CONTROL GROUP WITH AUC BETTER THAN CA19.9
- MUC5AC HAS HIGH DIAGNOSTIC POTENTIAL FOR DIFFERENTIATING CP AND ABO CASES FROM CANCER CASES DURING EARLY STAGES
- MUC5AC EMERGED AS PROMISING MARKER IN COMBINATION WITH CA19.9 FOR STAGE IA/IB/IIA PATIENTS

A Combination of MUC5AC and CA19-9 Improves the Diagnosis of Pancreatic Cancer: A Multicenter Study

Plans for Future Analysis

- Validate the diagnostic potentials of MUC5AC in another blinded set from UPMC (250 EPC; 250 controls)
- PROGNOSTIC EFFICACY OF MUC5AC AND CA19.9 COMBINATION.
- DOES EVLUATING MUC5AC IN LONGITUDINALLY COLLECTED PLCO SAMPLE SET HELP IN IDENTIFYING CANCER PATIENTS WITH IMPROVED DISCRIMINATORY POWER?

- HOW MUC5AC IMPROVES THE PATIENTS STRATIFICATION IN DELENIATING THE RESPONSE TO CHEMOTHERAPY AND SURGERY?

Diagnostic Significance of Circulating MUC4 mucin
MUC4 expression in Pancreatic Cancer
Pancreatic Cancer progression model
Correlation between MUC4 expression and cumulative survival rate in 135 patients with invasive ductal carcinoma, as determined by the Kaplan–Meier method. The survival of patients with high MUC4 expression was worse than those with low MUC4 expression. (Saitou et al. Batra SK. J. Clin Pathology 58;845-52, 2005)

Immunophenotypic expression of various markers in EUS-guided Fine Needle Aspiration (FNAs) in patients with and without adenocarcinomas

EUS-guided FNA samples in patients with and without adenocarcinomas
38 atypical/suspicious cases: 31 (65%) positive for adenocarcinoma, 17 (35%) negative for adenocaroma
Total samples: 114
MUC4 positive (106/114)
93%

MUC4-SERS Sandwich Immunoassay Format
Detection of MUC4 in PC patient
Detection of MUC4 in PC Patient Sera

- Healthy Controls (HC)
- Chronic Pancreatitis (CP)
- Early Stage PC (EPC)
- Late Stage PC (LPC)

MUC4 is 61% sensitive and 95% specific in differentiating
PC from HC.

MUC4 was 76% sensitive and 95% specific in differentiating PC from CP.

- Based on the 95% confidence interval Raman Intensity discriminates:
- PC vs. HC
- EPC vs. HC (p=0.0053)
- LPC vs. HC (p=0.0050)
- PC vs. CP
- EPC vs. CP (p=0.0011)
- LPC vs. CP (p=0.0011)

MUC4, MUC5AC and CA19.9 combination
The combined utility of MUC4, MUC5AC and A19-9 for differentiating EPC from controls. Differences of biomarkers between PDAC compared to control subjects were assessed using logistic regression and ROC curve analysis. Cutoff points were determined for each marker using optimal SN and SP. MUC4, MUC5AC and CA19-9 added up to differentiate PC cases from benign controls with SN of 95% and SP of 100%

Diagnostic Significance of Trefoil Factors (TFFs)
Trefoil Factors
Trefoil factor family (TFF) peptides are mucin-associated molecules co-expressed with MUC mucins and involved in the maintenance of mucosal barrier and the biological behavior of epithelial and carcinoma cells
Trefoil Factors: Potential markers for Classical Subtypes in Pancreatic Cancer
Expression analysis of TFF1-3 in normal, PanIN and PC tissues
All TFF showed significantly high expression in PanIN and EPC
Diagnostic Performance of combination of TFFs, MUC5AC and CA19-9
Combination of TFFs, MUC5AC and CA19-9 can better predict EPC compared to other groups
Diagnostic Performance of combination of TFFs in low and high CA19.9

*TFF1-3 can better predict EPC compared to CP in low CA19.9 group

MUC4 EXPRESSION IN IPMNs
MUC4 EXPRESSION IN IPMNs
MUC4: A Specific-Marker for Malignancy in Early Lesions
Expression pattern of MUC4/8G7 in gastric-type IPMN-H- ONLY IN NEOPLASTIC CELLS EXHIBITING HIGH GRADE DYSPLASIA
MUC4 EXPRESSION AND
IPMN RISK STRATIFICATION

Conclusions

- The results presented here validate the potential of MUC4 as a PC biomarker and the capability of the SERS technology for the detection of MUC4 in PC patients.
- Overall, the diagnostic performance of MUC4, MUC5AC was superior to CA19-9 in distinguishing PC patients from healthy individuals or patients with chronic pancreatitis.
- The combination of markers could provide much needed diagnostic marker set for PC
- MUC4 distinguishes high-risk cystic lesions in pre-surgical EUS FNAs

Biomarkers for Pancreatic Cancer Early Detection

Anirban Maitra, M.B.B.S., The University of Texas MD Anderson Cancer Center

MDACC EDRN CVC Team
Recurrence after Pancreatectomy for Pancreatic Ductal Adenocarcinoma
Early Detection of Pancreatic Cancer & Survival
What would it take to “double” pancreatic cancer survival?

Biomarker Goals for Early Detection of Pancreatic Cancer – Asymptomatic Disease is the Target
High risk families
Cancer syndromes
Pancreatic cysts
“Sporadic” Risk
New onset diabetes

New Onset Diabetes (NOD) as a Biomarker of Asymptoamtic Pancreatic Cancer
Up to ~50% of pancreatic cancer patients may have new-onset diabetes (NOD) as their first symptom
Can pre-date cancer diagnosis by 2 years
~1% of NOD have occult PDAC

Consortium for the Study of Chronic Pancreatitis, Diabetes and Pancreatic Cancer (CPDPC)
NIDDK/NCI Consortium
10,000 NOD patients recruited prospectively

~100 will develop PDAC over follow up with pre-diagnostic blood samples

A Biomarker Continuum That Spans Risk and Early Detection of Asymptomatic Disease

Pancreatic Cancer Early Detection Initiatives at MD Anderson
PanCAN Early Detection Initiative
NCI MCL Consortium
NCI EDRN CVC
NCI BRPC & BPPC Alliance
NCI/NIDDK CPDPC NOD cohort
Extramural

Integrated Sequential Biomarker Discovery and Validation Strategy
MD Anderson EDRN CVC

Enhancing the Anchor Panel with Sequential Autoantibody Discovery

A Multi-Analyte Biomarker Panel [Hanash & Lampe]

Goal: Pre-Diagnostic Cohorts

WHI (Lampe Lab):
47 PDAC (0 to 24 mo. from dx)
35 PDAC (24 to 49 mo. from dx)
87 matched healthy controls

IARC/EPIC (Hanash Lab):
39 early-stage PDAC
82 matched healthy controls
~200 pre-diagnostic PDAC (pending)

PLCO (Hanash Lab)
~175 pre-diagnostic PDAC
~550 matched controls

NOD Cohort
~100 cases
>9,000 controls
Ancillary Committee approval

EDRN Pancreatic Cancer Bakeoff Collaborative

- Directed by JoAnn Rinaudo (NCI)
- Blinded cases and control samples (N = ~180)
- Two CVC sites (UPMC and MDACC, with assist from Mayo Clinic)
- Centralized study design and statistical analysis at DMCC (Ying Huang)
- Participating laboratories:
  - Brian Haab VARI
  - Surinder Batra UNMC
  - Sam Hanash MDACC
  - Anna Lokshin UPMC
- **Goal**: A curated biomarker panel that can make the case for access to pre-diagnostic samples such as NOD cohort.

Genomic Assays for Early Detection of Pancreatic Cancer

- "*Level 2*" blood based biomarker assay
- Cost limitation - $1,000 range
- Volume limitation – 8mL plasma (ctDNA or exoDNA)
- Highly specific especially with multi-gene NGS panels, *BUT*
- Sensitivity remains an issue

Conclusions: EDRN Pancreatic Cancer Task List

- Cohorts at higher risk for pancreatic cancer have been defined (families, cysts), but "sporadic" risk remains largest subset of patients
  - Need for biochemical risk markers in "sporadic" risk
- New onset diabetes is an easily ascertainable clinical sign for an occult pancreatic cancer but only 1% of NOD have cancer
  - Need for biomarkers that will distinguish T2DM from T3cDM
- <u>Meaningful early detection</u> of pancreatic cancer means diagnosis at asymptomatic stage of the disease, or advanced precursor lesion (PanIN-3)
- Likely biomarker strategy will be a three (four) step strategy:
  - Step 1: Biochemical or clinical risk marker
  - Step 2: Level 1 ED biomarker (proteins/autoantibodies)
  - Step 3: Level 2 ED biomarker (expensive but specific genetic assays)
  - Step 4: IMAGING (did not speak about this but big unmet need)

Acknowledgements

FHCRC CVC Updates in Breast and Colon Cancer Early Detection

Christopher Li, M.D., Ph.D., Paul Lampe, Ph.D., and Charles Drescher, M.D., Fred Hutchinson Cancer Research Center

A*STAR
Mission: We advance science and develop innovative technology to further economic growth and improve lives
>5,400 Staff
>4,500 Researchers, Engineers and Technical Support Staff
>40% of whom come from 60 countries

Diagnostics Innovations ….
Partnerships through
global – local
private – public
academia – industry

Helping our partners create value
Filling the gap in diagnostics value chain

Existing projects within DxD Hub

miRNA qPCR Technologies Comparison

A*STAR

Ovarian Cancer
Ovarian Cancer
CRC
CRC

Background: Identification of 5 bladder cancer (BCa) urinary biomarkers
Bladder cancer all stages versus healthy
Diagnostic performance of 5-urinary biomarkers in discriminating bladder cancer (BCa) all stages patients from healthy subjects:
Three-Biomarker Panel (REG1, TFF1, LYVE1) in Urine for Early Detection of Pancreatic Ductal Adenocarcinoma (PDAC)
Intended Use: The PDAC test is a qualitative urine test that combines the results of three biomarkers (REG1, TFF1, LYVE1) measured individually as three immunoassays into a single numerical score. Using an algorithm and the values of these protein biomarkers, the PDAC software generates a single unit-less numerical score from 0.0 to 10.0. This PDAC test is an aid to assess the likelihood that malignancy is present.

Pancreatic Cancer Protein Biomarkers
Integrated Diagnostics (Dx): Disease Indications
Examples of Singapore NMRC projects with existing prospective clinical samples collection

EDRN Partnership with DxD Hub
Accelerating transformation of diagnostics innovations via collaborations

Fred Hutchinson
Breast, Colorectal, and
Ovary Cancer CVC
Breast: Christopher Li, MD, PhD
Colorectal: Paul Lampe, PhD
Ovary: Charles Drescher, MD
Breast CVC Project:

Phase 3 validation of early detection biomarkers for ER+ breast cancer
Background:

- Mammography reduces mortality by 30% among women 50-69 years of age
- PPV is 60-80% among women 50-69, but only 20% among women <50
- Breast cancer remains the leading cause of cancer mortality in women worldwide

Breast CVC Project:
Phase 3 validation of early detection biomarkers for ER+ breast cancer
Intended clinical applications:

- Inform timing of a subsequent mammogram in women with a negative screening mammogram
- Inform continuation of mammographic screening among older women (beyond recommended age of screening)
- Prioritize women who should be screened with mammography in areas with limited resources

Biomarker discovery
Sample sets evaluated
9 A-list Proteomics Candidates

Next Steps
Colon CVC Project:
Phase 3 validation of early detection biomarkers for advanced adenoma and colon cancer
Background:

- Colonoscopy and FIT reduce mortality.
- Low acceptance rate (65% at best).
  inconvenience
  cost
  insurance
- Proteins in blood might be an alternative

Colon CVC Project:
Phase 3 validation of early detection biomarkers for advanced adenoma and colon cancer
Intended clinical applications:

- Identify people unwilling to undergo or with no access to colonoscopy who should be prioritized for colonoscopy (improve/replace stool tests).
- Among symptomatic patients identify those who have a very low risk of cancer and can avoid colonoscopy.

Transfer to quantitative, high-throughput multiplex technique: modified Luminex

- Bead-based technology
- Suitable for multiplexing
- Antibody-based assay
- •Directly translate from microarray platform

Array vs Modified Luminex – EDRN Samples
Assay Refinement

- Many arrayed antibodies are polyclonal
  Finite resource
  Epitope not defined
- Need to develop pairs for ELISA

CD44
CD44 – defining epitopes
CD44 antibody testing

Luminex Comparison – EDRN Samples
Monoclonal Antibody Development

Colon Cancer Sample Sets

- Early discovery in pre-Dx samples – populate array
- Cardiovascular Health Study (CHS) pre-diagnostic samples (<3 years from Dx)
- EDRN collaborative group project (GLNE7)
- UMN colonoscopy study
- Japanese sera samples
- EDRN sample set 2 from Dean
- Danish plasma samples (Hans J. Nielsen)
- WHI plasma samples
- New collaborative group project (FIT+/-)

Biomarkers for Ovarian Cancer Early Detection

Robert Bast, M.D., The University of Texas MD Anderson Cancer Center

MDACC Clinical Validation Center for
Early Detection of Ovarian Cancer
September 12, 2017

Introduction/Background

- Ovarian Cancer Limited to the Ovaries (Stage I) can be Cured in up to 90% of Patients with Currently Available Therapy
- Disease that has Spread from the Pelvis (Stage III-IV) can be Cured in only 20% or Less
- Only 20% of Ovarian Cancers are Currently Diagnosed in Stage I
- Detection of Preclinical Disease at an Earlier Stage Could Improve Survival by 10-30%

Requirements For Screening

- Postmenopausal Prevalence: 1:2,500
- High Sensitivity: ⍰ 75%
- Very High Specificity: 99.6%
- Positive Predictive Value: 10%

Two Stage Screening Strategies

- Used alone, Neither CA125 nor Transvaginal Sonography (TVS) has Adequate Specificity
- Ovarian Cancer is Associated with rising CA125 and Benign Disease is not
- Very High Specificity and Sensitivity can be attained when rising CA125 is used to trigger TVS in a Two Stage Strategy
- The Risk of Ovarian Cancer (ROC) Algorithm relies on each Woman's own Baseline to determine whether there has been a significant increase

NROSS Screening Protocol

Early Detection of Ovarian Cancer: Background
Two Stage Screening Strategies based on the Risk of Ovarian Cancer Algorithm (ROCA)
The Normal Risk Ovarian Cancer Screening Study (NROSS) conducted by the MD Anderson SPORE in Ovarian Cancer screened 5,729 women over 17 years performing 19 operations to detect 12 ovarian cancers with 9 in stage I or II
The United Kingdom Clinical Trial of Ovarian Cancer Screening (UKCTOCS) enrolled
>200,000 women and showed a decrease in mortality of 20% (P<0.021) in non-prevalent cases of ovarian cancer, albeit with wide confidence limits
Both trials required no more than 3-4 operations for each case of ovarian cancer detected
CA125 is not optimal for an initial stage in that 20% of ovarian cancers do not express the biomarker.
From 110 candidates, HE4 and CA72.4 detected 16% of cases missed by CA125, but did not improve lead time.
Anti-TP53 auto-antibodies detected 16% of cases missed by CA125 and titers were elevated 27 months before clinical detection of disease in CA125 negative cases and 7 months before CA125 in cases detected by the ROCA.
A Multi-Marker ROCA is being developed to achieve greater sensitivity than CA125 alone.
TP53 autoantibodies levels and CA125 values in serum samples from the UKCTOCS screening trial

TP53 autoantibody has lead time over CA125 (longitudinal analysis)
Longitudinal analysis of CA125 and TP53 autoantibodies titers in the UKCTOCS study

MDACC EDRN Specific Aims
Aim 1: To conduct an NROSS2 screening trial to determine the specificity and positive predictive value for a multi-biomarker ROCA potentially including CA125, HE4, CA72.4 and anti-TP53 autoantibodies in a two stage strategy for early detection of ovarian cancer in postmenopausal women at average risk for the disease. We will test the hypothesis that rising values of multiple biomarkers will prompt the referral of no more than 2% of women for TVS and that the overall strategy will achieve a positive predictive value >10%.
Aim 2: To maintain and share a serum and plasma bank to facilitate evaluation of novel biomarkers for early detection. Our goal will be to provide serum, plasma and tissue to multiple sites in the EDRN to identify biomarkers that complement CA125 and provide lead time over CA125.
Aim 3: To collaborate with other Centers in the EDRN Biomarker Development Laboratories to develop a next generation ROCA3 algorithm and an NROSS3 clinical trial for early detection. Our goal will be to develop the ROCA 3 algorithm and initiate an NROSS3 trial before completion of the project period.

ID01-022 Low Risk Ovarian Study
New Participants Accrued March 2016-August 2017
ID01-022 Low Risk Ovarian Study
Number of Specimens Collected 3,908 March 2016-August 2017
Potential Area(s) Of Collaboration With Other Members Of The EDRN

MDACC EDRN Specific Aim 2
Circulating Autoantibody Biomarkers for Early Detection of Ovarian Cancer
This TEAM project proposes a validation study from existing candidate biomarkers by EDRN investigators
Robert Bast, Karen Lu (MDACC)
Karen Anderson (ASU)
Charles Drescher (FHCRC)
Steven Skates (MGH)
Zhen Zhang (JHMI)

- •Biospecimen Sources:
  - Samples obtained at diagnosis and matched controls (Bast, Drescher)
  - Longitudinal preclinical samples from local ovarian cancer screening trials (NROSS, Ovarian Cancer Early Detection Program (OCEDP)
  - Preclinical samples from large cohort studies (WHI, PLCO, UKCTOCS)
- •Biomarker Candidates:
  - Anderson (ASU) – 16 candidates
  - Drescher (FHCRC) – 5 candidates currently with 25 total expected by year 2
  - Bast (MDACC) – 4 candidates currently with 10 total expected by years 2-3.
- Approach:
  - Develop MagPlex Luminex assays for promising autoantibodies
  - Evaluate specificity of candidate biomarkers in the EDRN reference set; Evaluate candidates in the combined MDACC/FHCRC sample set
  - Rank candidates based on their sensitivity at 95% and 98% specificity in the combined MDACC/FHCRC sample set at the time of conventional diagnosis
  - Develop a biomarker panel based on the statistical analysis of the MDACC/FHCRC sample sets
  - Perform subgroup analysis on high- and low-grade serous, clear cell, mucinous and endometrioid cancers and borderline ovarian tumors
  - Evaluate performance of top markers and biomarker panel in pre-clinical samples from participants in local ovarian cancer screening trials as prelude to requesting samples for large population-based cohort studies or ovarian cancer screening trials
  - Develop a standard multi-plex platform assay with Zhen Zhang at JHMI
  - Rank candidates based on assay performance in 100 serum biospecimens from ovarian cancer

cases in the WHI observational study diagnosed within one year of second sample and in samples from controls, or samples obtained at diagnosis from EDRN groups
  - Validate candidates based on assay performance with 1500 serial preclinical samples and 5-fold controls from the UKCTOCS
  - Candidates for Early Detection of Ovarian Cancer
  - Will be Considered Validated If:
  - Clinical sensitivity is achieved (5% or greater) at 98% specificity at least 1 year prior to clinical detection
    - OR
  - There is improved performance of a combined biomarker panel
  - Algorithm
  - Steve Skates will develop a ROCA-like algorithm with the validated antibodies and CA125 and other EDRN biomarkers to be validated

Validation of Lung Cancer Biomarkers: Where the Rubber Meets the Road

Pierre Massion, M.D., Vanderbilt-Ingram Cancer Center

Vaidation of a New Blood-based Biomarker Strategy for the Early Detection of Lung Cancer

Michael Kammer, Amanda Kussrow, Heidi Chen, Darryl Bornhop, Pierre Massion
"where the rubber meets the road"

Lung Collaborative Group
EDRN Lung collaborative group
Proteomic analysis of bronchial epithelium in high risk individuals
FSPG PET imaging for IPNs.

EDRN Lung collaborative group
Mathematical Model Identifies Blood Biomarker–based detectability
CRP and hsCRP

- hsCRP revolutionized the value of the biomarker.
- This happened based on a 30-fold increased sensitivity of the assay.
- CRP is not just a marker of inflammation
- CRP is a marker of Cardiovascular risk
- Can we reconsider existing cancer biomarkers and improve our risk assessment?

CYFRA 21.1
ELISA - enzyme-linked immunosorbent assay

- Measures change in absorbance using capture
  - Direct vs Sandwiched
- Pros: Cost, Throughput, Accessibility
- Cons: Mileage may vary, probes must be developed

Electrochemiluminescence immunoassay (ECLIA)
Compensated Backscatter Light Interferometry (CBSI)
Aim & Significance of the Study

- Aim:

Apply CBSI technology to validate an existing biomarker CYFRA 21.1 to improve the diagnostic accuracy of the test in three clinical contexts: a. in distinction of cancer among indeterminate pulmonary nodules, b. in monitoring of cancer recurrence and c. in preclinical diagnosis.

- Significance: The "so what"

Based on our preliminary data, such biomarker should provide a diagnostic and preclinical tool for early detection of lung cancer, may improve surveillance strategies and save lives.

Patient characteristics
Preliminary Data/Outcomes CBSI for CYFRA 21.1 in 225 blinded serum samples , patients with IPNs

Preliminary cohort: 225 IPNs.
Preliminary Data/Outcomes

Validation plan
HT-CSI vs. Single Molecule Array (SIMOA)
Preliminary Data/Outcomes
AlphaLISA for CYFRA 21.1
Pre Cancer Atlas.

- Histological features of premalignant lesions not adequate for precision risk stratification
- Lack of knowledge of ME and genomic and peigene that dreive cancer
- Longitudinal collect perform molecular profiling.

MIBVAC: Images Aren't Pictures, They Are Data

Robert Gillies, Ph.D., John Heine, Ph.D., Matthew Schabath, Ph.D., and Jae Lee, Ph.D., H. Lee Moffitt Cancer Center

MIBVAC
MIBVAC is an image-analytic centric CVC with four complementary research areas

- Breast (PI: Heine)
- Lung (PI: Schabath)
- Prostate (PI: Gillies)
- Biostatistics (PI: Lee)

Breast Projects
Parent grant project: pre-clinical breast cancer risk prediction using digital breast tomosynthesis (DBT) data and a case-control design for early detection and risk modulated screening strategies

Network collaborative project: improve diagnostic mammography using metrics from DBT, full field digital mammography (FFDM) images and blood biomarkers

- BDL at Duke University (Marks et al and Creatv Microtech)
- CVC at the Moffitt Cancer Center (Heine et al)
- Supported by DMCC and JPL

Parent (core) grant project:
Pre-clinical breast cancer risk prediction

Improve breast cancer risk prediction for early detection and screening strategies

- New dataset: construct 320 pair matched case-control dataset with DBT images as an EDRN resource (HD mode: DBT volume, 2D mammograms and synthetic C view 2D mammograms
- Analytics: translate previously validated breast cancer risk biomarkers from 2D FFDM to DBT and calibrate 2D FFDM images (combine 2D calibrated and 3D metrics)
- Additional EDRN resources: prepare two existing case-control datasets for distribution with images from General Electric FFDM (180 pair) and Hologic FFDM (320 pair)

Progress (year 1)

- 134 cases and 100 controls with DBT images and complete CDEs (below)
- characterize four DBT units for image calibration purposes

Network collaborative project:
Combined Image and blood biomarkers
for breast cancer diagnosis
Improve diagnostic mammography

- Combine image and blood biomarkers with an emphasis on BI-RADS 4 patients. About 80% of these patients result in negative biopsies
- Construct EDRN resource (repository) with FFDM and DBT images, annotated truth images, relevant CDEs, and blood banking

Progress at Moffitt (3 months)

- Establish MTAs

•Establish protocol and IRB approval

- Actively recruiting
- Developing standards for data collection, truth file annotation, and distribution (Duke, Moffitt, DMCC, and JPL)

CDEs
Moffitt Breast Imaging Team
Lung Project
Proposed Aims:

- Specific Aim 1. Establish and curate retrospective lung cancer screening (LCS) and incidental pulmonary nodule (IPN) datasets to develop image-based (radiomic) biomarkers for risk assessment, diagnostic discrimination, and prognostication
- Specific Aim 2. Establish prospective LCS and IPN cohorts, with real-time data curation, feature extraction, and bio specimen collection, for further radiomic development and validation risk assessment, diagnostic discrimination, and prognostication

EDRN Activities:

Supplemental Funding (completed): Curate a retrospective dataset of patients diagnosed with IPNs: Clinical data, risk factor, pathology, radiology reports and CT images

- Collaborative Projects:
- LTP2: Prospective patient recruitment: patient data, biospecimens, CT images
- LTP3: Process pre-surgical CT images for feature extraction; develop radiomic predictors for recurrence and to discriminate indolent vs. aggressive LCs.

New Papers: Risk Assessment

New Papers: Prognostication

New Analyses:
Do Transfer Learning Features and convolutional neural networks (CNN) improve risk prediction in lung cancer screening?

New Analyses: Do Transfer Learning Features and convolutional neural networks (CNN) improve risk prediction in lung cancer screening?
Establishing new (prospective) LCS and IPN cohorts

Lung Cancer Team

Prostate Projects
(PI: John Wei, MD; U Michigan)

- Specific aim 1. To examine the incremental specificity for high grade prostate cancer (ie Gleason 6+) of prostate MRI above and beyond PSA, PCA3 and TMPRSS2:ERG with the sensitivity held constant at 90% in the initial biopsy setting.
- Specific aim 2. Evaluate the potential for novel laboratory biomarkers, (e.g. urine RNA sequencing chip, tissue prints for methylation field effect) to predict findings on MRI
    - To evaluate the relationships between laboratory biomarkers and MRI sequences.
    - To develop an algorithm that optimizes laboratory biomarkers to predict high-grade prostate cancer from a prostate MRI fusion biopsy.
- Specific aim 3. To evaluate the role of radiomics to improve upon standard MRI protocols in the diagnosis of high-grade prostate cancer

Fusion of MP-MRI delineated Prostate ROIs for targeted biopsy
Near-term Focus

- Multi-site validation.
    - –Disseminate Texture/Diffusion/T1 phantom to all accruing sites (Moffitt/Michigan initially)
    - –Use QIBA metrology to qualify sites and generate stats

- Database.
    - –Establish XNat image repository (identical structure to Vandy dbase)
    - –CDEs in NCI Hub (JPL?)

For targeted therapies, benefit is measured in months

Biostatistical Projects
Planned clinical validation studies for lung cancer radiomics biomarkers

- Validation Study 1: to improve the accuracy of the initial LDCT-based diagnosis of lung cancer with a significantly higher positive predictive value (PPV) from current 3.6% to >7~8%, using integrated (Bayesian) logistic regression modeling with radiomics imaging biomarkers and clinical risk factors
- Validation Study 2: future risk assessment of individuals without cancer despite positive LDCT screening by probabilistic logit model with imaging biomarkers

Validation Study 1: Imaging-based Improved Diagnostics

- For general public, LC prevalence 0.074% (CDC 2015)
- Among NLST cohort of high smoking subjects:
    –At T0, LC prevalence 1.0~1.2% (13.5 times higher than public)
    –At T1,
- Among T0 positive without LC, LC prevalence 1.4%
- Among T0 negative, LC prevalence 0.3~0.6%
    –At T2,

•Among T0 positive & T1 positive without LC, LC prevalence 1.9%

- Among T0 negative & T1 negative without LC, LC prevalence ~0.4%
- •LC Prevalence is still low and highly variable among heavy smokers at different screening stages & populations at risk
    –PPV greatly varies due to low and varying prevalence, significantly undermining utility of early detection biomarkers with high false positives
- Bayesian probabilistic diagnostic model by integrating radiomics imaging biomarkers and clinical risk factors

Project performance of integrated Imaging biomarker model for LC diagnostics

Integrated Imaging Model-based Diagnosis of Lung Cancer using nomogram probabilistic score system

Clinical validation study plan for lung cancer imaging biomarkers

- Validation Study 1 based on the NLST cohort (year 2~4): the initial LDCT diagnosis of lung cancer can be improved with a significantly higher PPV by integrating imaging biomarkers and clinical risk factors
- Validation Study 2 based on the NLST cohort (year 3~5): Future risk assessment of individuals can be made for positive LDCT screening subjects without cancer, using integrated imaging-based probabilistic biomarker model

Executive Committee Meeting (closed)
Minutes not provided

Public Private Partnerships
Session Moderator: Lynn Sorbara, Ph.D., National Cancer Institute

Welcome
Lynn Sorbara, Ph.D., National Cancer Institute
Dr. Sorbara welcomed everyone and introduced the first speaker.

Experiences and Insights on Partnering in Biomarker Discovery towards Clinical Translation
Gerard J. Davis, Ph.D., Abbott Diagnostics, Project Manager and Research Scientist
Opening comments

Public private partnership perspective
Stakeholders in developing effective tests
Collaboration in "TMUGs" process
Tumor Marker Utility grading system

CRC Collaborations
Pilot panel/algorithm area:
Biomarker panel & algorithm approach
Initial Report: Pilot data for a new marker

EDRN collaborative Study
Individual marker assessments: TIMP-1 & CEA

EDRN collaborative Study: Classification Trees
Sensitivity Estimates for TIMP-1 + CEA

Danish Endoscopy II Trial
Further Panel Development Efforts

Pilot CRC panel: clinical performance
Discrimination of Non-disease/Control vs. Cancer:
CRC +/- High Risk Adenomas vs. All others
4 marker panel/algorithm similar performance as 8 markers (not shown)
Top markers/features: age, gender, CEA, Cyfra 21-1, hs-CRP, ferritin

Pilot Biomarker Panels including Gal3-Ligand

HCC Collaborations
Clinical Discrimination:
advanced liver disease & liver cancer (HCC)

JHMI cohort: Developing a pilot panel

EDRN cohort: assessing the pilot panel

Biologics & assay considerations
Biologics Discovery
Strong capabilities for developing targeted and novel biologic reagents (monoclonal antibodies, recombinant antigens, recombinant Fabs, etc.)

- Broad competencies for hybridoma development, cell culture, antibody purification and fragmentation, animal immunization, and analytical characterization
- Capitalizes on 30+ years of internal development experience including a vast bank of catalogued hybridoma cell lines
- Track record of designing reagents capable of driving best-in-class immunoassay performance (e.g. HIV Ag/Ab 4th Gen, hsTnI)
- Advanced methods and expertise, assuring high performance, efficient/stable cell lines, and scalable/manufacturable processes
- Multiple PMA and BLA approved biologics demonstrating firm compliance with FDA regulations

In vitro diagnostics Systems
IVD analyzers are automated high throughput, random access instruments for the clinical laboratory (e.g. ARCHITECT, Alinity)

The immunoassay format utilizes chemiluminescent microparticle immunoassay (CMIA) technology

Closing comments
Strengths of EDRN:
Common objective/charter
Focus on aiding in early detection of cancer

Help address this clinical need
Open, with inclusion of participants with diverse backgrounds
Reference specimen sets to assess markers

Future considerations:
Continue efforts to engage diverse stakeholders
Explore potential rapid prototyping on IVD platforms earlier
Explore mechanisms for greater openness in analyses of collaboration study data

Elucidating Cancer Mechanisms through Integrated Biology
Carolina Livi, Ph.D., Agilent Technologies, Inc., Academic Segment Manager
Highlights of Agilent Research Biomedical Solutions in Action
Our Support of Universities and Thought Leaders
Biology Is Integrated
Multi-omics Increases Biological Understanding
Omics Measurements
Genomics and Transcriptomics

- Agilent SureSelect capture NGS
- Agilent Microarrays
- Agilent qPCR
- Agilent Microfluidics

Proteomics

- LC/MS

Metabolomics

- LC/MS
- GC/MS
- Seahorse (functional metabolism)

Mass Spectrometry Overview
Time-of-Flight (TOF) MS Overview

Metabolomics Overview:
Discovery, Hypothesis-driven and Targeted Approaches
Batch Qualitative Flux Analysis Workflow

Agilent VistaFlux Workflow

- Metabolomics provides static information on cellular molecular composition
- Qualitative flux analysis reveals in vivo pathway activity
- Qualitative flux analysis tracks the flow of metabolites through a pathway

VistaFlux Stable Isotope Tracing

Isotopologue Tracking
Use of stable isotope labels (13C, 15N, and 2H)
Monitor stable isotope incorporation
Qualitative Flux Analysis of a Cancer Cell Line with Enzyme Mutation (IDH2)

- Goal: Understand impact of IDH2 mutation on glutamate metabolism in chondrosarcoma cell line
- Tracer experiment with U-13C-Glutamine (Gln)
- Monitoring metabolites labeled in green
- Triplicate analysis for each time point (0, 0.5, 1, 3 and 8 hr)

Qualitative Flux Results: Isotopologue Extraction
Oxoglutarate

Qualitative Flux Results: Isotopologue Extraction
2-Hydroxyglutarate

Qualitative Flux Results: Isotopologue Extraction
Succinate

Visualizing Qualitative Flux Results
Quilt Plot Display of Isotopologue Results

- Quilt plot displays all time points and isotopologue results
- Statistically significant (*) label incorporation in M+5 isotopologue of 2-HG at 3 and 8 hours

Qualitative Flux Analysis Experiment
Comprehensive View of Isotopologue Results on Network

Summary

- This experiment confirms glutamine is the major substrate used to produce 2-HG in IDH2-mutant CS-1 cells
- For 2-HG, the presence of the M+5 isotopologue instead of the M+2 or M+4 isotopologue demonstrates the direction of the reaction
- We thank Justin Cross and the many VistaFlux customer collaborator for access to datasets and valuable feedback

Latest Information From Agilent
Omics eSeminar Series
http://www.agilent.com/en-us/training-events/eseminars/emerging-omics
Thank you!
http://www.agilent.com
SurePrint Technology
Foundation of Agilent's Genomics Products

Building on Agilent's Leadership in Oligonucleotide Synthesis

Quality Control Solutions
Microfluidics Portfolio

Bioanalyzer and TapeStation Kits
DNA, RNA and Protein Assays

Custom Design Arrays to Your Experimental Needs
Multiple Microarray Formats:

Wider Dynamic Range for Analytical Sensitivity to Low Expressors

- Agilent gene expression microarray data provides the expected normal distribution of signals, with few probes in the noise
- Competing platforms suffer from compressed signals without a normal distribution

Multiple Techniques for Gene Expression Studies

GLNE-Clinical Genomics Collaboration
Lawrence LaPointe, Ph.D., Clinical Genomics
Clinical Genomics
Headquartered in Bridgewater NJ (Offices/labs in Sydney AU)
Portfolio of >90 patents and patents pending
Distribute 3.5+ million FIT kits in the US and Australia
FDA, TGA approved manufacturer
CLIA approved lab for molecular diagnostics (Nov 2016)
Clinical Genomics: history in CRC
A decade of discovery & valication
UP-regulated panels: mRNA
DOWN-regulated panels: hypermethylataion
Clinical Genomics: decade of discovery & validation

COLVERA
PCR: methylated CpG sites covered by primers and probes
Either target methylated = positive
94.% of CRC tumors hypermethylated for BCAT1/IKZF1
4,000+ prospectively tested patients

Colvera: Generation 1 clinical validation
Specificity:
No association with: history of CRC, age, gender or smoking

Colvera
CLIA registered.
2X sensitivity vs CEA for recurrent CRC

Future directions
Technical improvements to increase sensitivity for methylated biomarker targets

History
What this collaboration means to Clinical Genomics:
Leverage clinical investment
Access to expanded clinical testing sites
Simplified logistics for multiple trials
Exceptional scientific feedback
What private companies can offer the public researchers
* clear, market driven feedback about end users
* understanding of regulatory and reimbursement frameworks

Access to innovative, venture-backed technologies
The keys to success
Transparency
Scientific integrity
Aligned interest
Patient benefit
Corporate imperative to give investors a return
Ultimately cost saving (or accepted benefit)

Illumina's Cancer Research Portfolio and Dedicated Workflows
Jonathan Bibliowicz, Ph.D., Illumina, Inc., Sr. Sales Specialist, Sequencing & Data Analysis
Global cancer
1 in 3 women; 1 in 2 men

Moving From Single Gene to Multi-Gene
The Challenge: how to find more with less?
Challenge
Iterative testing
Next-Generation Sequencing using Illumina technology
Sequencing Costs Declining
Multi-Gene Cancer Panel
TruSight® Tumor 15
TruSight® Tumor 15

Comprehensive process from prepared sample to report
Pre-Defined Report
Automates variant detection
The Value of RNA in Cancer Research
RNA enrichment panel targeting 1,385 oncology genes

Low input, optimized for all sample types (including FFPE)
Fusion discovery - detects both known and novel fusion gene partners
Enables RNA-Seq on a desktop sequencer (~3M reads per sample)
Intuitive BaseSpace App

TruSight ® RNA Pan-Cancer
RNA-seq analysis – FPKM, DE, SNPs, Fusions
TruSight Tumor 170
Multi-Biomarker Cancer Panel
TruSight Tumor 170 Content
Gene List and Variant Classification
TruSight Tumor 170 Library Preparation Method Overview
DNA and RNA workflows processed in parallel and sequenced together

Multi-Omics Approaches
Answering more complex questions
Illumina Benchtop Portfolio Overview

Exosomes Enable Liquid Biopsy Diagnostics with RNA, DNA and Protein
Johan Skog, Ph.D., Exosome Diagnostics, Inc., Chief Scientific Officer
Biomarker Verification and Clinical Grade Assays
Session Moderator: Lynn Sorbara, Ph.D., National Cancer Institute

Biomarker Verification and Clinical Grade Assays
Sanford A. Stass, MD., University of Maryland Baltimore
Biomarker Reference Laboratory

Clinical Laboratory Improvement Amendments (CLIA)

- CLIA-88(final regulations published in 1992): Concerns arose regarding quality of laboratory testing (cholesterol screening, Pap smears).
- Law requires laboratories doing testing for "diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of human beings" be certified (initially by HCFA, now by Center for Medicaid and Medicare Services (CMS).

Clinical Laboratory Improvement Amendments (CLIA) (Cont'd)

- The Clinical Laboratory Improvement Amendments (CLIA) (est. 1988, finalized 1992, and revised 2003).
  –CMS regulates all laboratory testing through CLIA
  –CLIA addresses issues of good laboratory practice, such as:
- Equipment maintenance
  –Proficiency testing
  –Competency testing
- Personnel training and documentation
  –Result reporting
  –CLIA does NOT address clinical utility, test sensitivity, result interpretation

College of American Pathologists (CAP)

- World's largest pathologists association.
- Leader in laboratory quality assurance.
- Federal government recognizes CAP as equal to or more stringent than the government's own inspection program.
- Deemed status with CLIA.
- CAP accreditation awarded (7,585 US and 381 International laboratories) by Commission Laboratory Accreditation based on results of stringent on site inspection every two years including examination of:

- Standard operating procedures/laboratory records
- Quality control procedures/quality assurance programs
- Qualification of directors and staff
- Laboratory equipment
- Facilities
- Safety Program
- Overall laboratory management

What Can Cause Inaccurate Results?
Pre-analytic errors

- Collecting sample from wrong patient
- Collecting the wrong sample
- Mislabeling or failing to label sample
- Storing sample incorrectly prior to testing
- Improper Transportation of samples
- Damaged reagents or test kits

What Can Cause Inaccurate Results?
Analytic errors

- Failing to follow an established algorithm
- Reporting results when QC tests out of range
- Incorrect sample or reagent measuring
- Using improperly stored or expired reagents
- Instrument calibration bias
- Reporting results beyond acceptable linearity range
- Conducting testing in wrong test mode

Laboratory Errors Happen

- Root Causes?
  - Training
  - Competence
  - Environment
  - Policies and Procedures
  - Test system performance
  - Understandable report created with an effective interpretation

CAP'S Accreditation Program helps laboratories achieve these outcomes using:

- 21 discipline specific checklists that are a blueprint for running a high quality laboratory.
  - ~ 40% of requirements exceed federal requirements and reflect the more stringent standards of CAP.
- Peer inspection model focuses on education and information sharing.
  - Specialty inspectors for emerging high complexity areas
- Proficiency testing monitoring beyond regulated analytes because all tests should be done correctly

Standards and Checklists

- Purposes:
  - Standards are the broad principles the laboratory must meet in order to achieve accreditation.
  - Checklists provide detailed requirements that inspectors use to determine whether laboratories meet the Standards

CAP 2-year accreditation cycle covers the entire lab

Standard Operating Procedures (SOPs)
CLIA/CAP Compliance

- Specimen Handling/Specimen Rejection Criteria/Specimen Storage
- Principle/Procedure

- Equipment
- Supplies
- Controls/Reagents/Reagent Preparation
- Quality Control/Criteria for Controls
- Interpretation and Procedure Limitations
- Troubleshooting Guide
- References

Specimen Handling

•Specimen collection and handling:
  - Methods for collection
  - Proper labeling
  - Method for delivery
  - Method for specimen preservation
  - Procedure for safe handling
- Proper recording of specimens received

Specimen Handling (cont'd)

- Criteria for rejection.
- No aliquot is ever returned to the original container.
- Aliquoting procedure prevents any possible cross-contamination.
- Schedule for retaining specimens.
- Verify identity and integrity through all steps.
- Condition documented upon receipt (evidence of tampering, inadequate volume, etc.)
- Specimens limited access, secured area.

Equipment Validation/Quality Control (QC)

- New equipment "clinically validated".
- All equipment regular Q.C.
  - Q.C. records reviewed monthly.
  - Records maintained in Q.C./Troubleshooting Notebooks.
- SOP for set-up/normal operation of equipment.
- Instructions/regular schedule for checking critical operating characteristics.
- Function checks to detect trends/malfunctions.
- Tolerance limits for acceptable function.
- Instructions for troubleshooting/repairs.
- Surveys to include UV/VIS spectrophotometer and analytical balance.

Equipment Validation/QC (cont'd)

- Document repairs/service procedures/maintenance.
- Spectrophotometer wavelength calibration checked regularly.
- All calibration curves rerun/verified after servicing or equipment recalibration.
- Criteria for acceptable background levels taken regularly.

Equipment Validation/QC (Cont'd)

- Temperatures checked/recorded.
  - Water baths,
  - Heating blocks,
  - Incubators and ovens,
  - Refrigerators and freezers
- Individual wells (or a representative sample thereof) of thermocyclers checked for temperature accuracy pre and post placed into operation.

Miscellaneous Supplies

- Volumetric glassware of certified accuracy (Class A, NIST Standard or equivalent).

  - Non-certified volumetric glassware must be checked for accuracy of calibration before initial use and at specified intervals.
  - Reconstitution of lyophilized calibrators, controls or proficiency testing materials, or any other tasks requiring accurate volumetric measurement, performed with measuring devices of Class A accuracy, or those for which accuracy has been defined and deemed acceptable for the intended use.
- Appropriate thermometric standard device (e.g., NIST certified) for calibration checks.
- All non-certified thermometers checked against an appropriate thermometric standard device.

Controls/Reagents/Reagent Preparation

- Document QC of reagent shipments of the same lot number
- New reagent lots checked against old reagent lots or with suitable reference material before or concurrently when placed in service.
- Appropriate validation studies performed/documented for use of any reagents that do not follow manufacturer's recommendations.
- Reagents/solutions properly labeled:
  - Content and quantity, concentration or titer
  - Storage requirements
  - Date prepared or reconstituted by laboratory
  - Used within labeled expiration date.

Quality Control/Quality

- All SOPs set specific criteria for controls.
  - Tolerance/acceptability limits defined for all control procedures, control materials, and standards.
- All assays include external positive, negative, and amplification controls as QC for the specific assay.
  - Distinguish a true negative result from false negative due to PCR failure. False negatives are often due to PCR inhibitors in the specimen; demonstrate that a different DNA/RNA sequence from the one being tested is amplifiable in the same specimen.
- Evidence of corrective action when control results exceed defined tolerance limits.
  - Unacceptable controls documented on worksheet/Troubleshooting Notebook.
- Quantity/quality (intactness) high molecular weight DNA/RNA assessed.
  - Nucleic acids processed promptly/stored adequately to minimize degradation.

Interpretation of Results

- Criteria for assay parameters/data interpretation.
- Clearly define interpretation guidelines resulting in a level of consistency between laboratories and clear delineation of positive, negative, or indeterminant results.

Personnel Training & Competency

- Training new staff.
- Annual training/evaluation includes:
  - Annual competency tests using in-house blind samples, CAP/CDC survey/exchange samples, or written tests.
  - A minimum of 8 continuing education hours.
  - HIPAA, Compliance, and Safety/Hazard training.

Experience with BDL to UMB-BRL Assay Development and Implementation – Lessons Learned

MSA Assay Validation for Detection of Bladder Cancer SOP/Qualification

- This assay consisted of Short Tandem Repeat PCR analysis of 15 previously validated microsatellite markers located within 14 gene loci (D4S243, D21S1245, FGA, D17S695, D16S476, D9S171, IFN-A, D20S48, D17S654, D16S310, TH01, D9S162, D9S747, MBP and MBPa).
- Implementation issues between commercial and UMB-BRL CLIA/CAP laboratories.
  - Difference in Primer pairs used

- Need for new Positive Control
- Refinement of SOP with regard to interpretation and acceptance criteria
- New reagents (PCR master mix and HLPC primers)
- Clear definition of interpretation guidelines that result in level of consistency between laboratories
- Clear definition of all aspects of these assay parameters in the SOP

Assay Development/Validation of GP73 Biomarker for Hepatocellular Carcinoma

- Glycoproteomic studies suggest hepatocellular carcinoma has greatly elevated serum levels of "total" and fucosylated forms of normal Golgi resident glycoprotein (GP73) compared to cirrhosis.
- Collaboration with Dr. Timothy Block (Drexel University) established ELISA for quantitative GP73.
- Confirmed reagents and materials for the quantitative GP73 assay and established SOP with CLIA/CAP standards for assay in the UMB-BRL using LiCor equipment.
- Parallel testing for the quantitative GP73 assay with Dr. Block's research laboratory established that the ELISA assay demonstrated inter-laboratory (100% concordance) using the LiCor equipment, indicating successful transfer of the assay from Dr. Block's laboratory to the UMB-BRL CLIA/CAP laboratory.
- The Licor would not be the most likely equipment used in the clinical laboratory. LiCor optics is not as sensitive resulting in false negatives. The Liaison, or a like system that uses chemoluminescent technology, is a better fit for clinical laboratory since it is more sensitive and would result in fewer false negatives.

Potential Biomarker for Colon Cancer
Transfer of Vimentin Gene Methylation Assay from BDL (Sanford Markowitz, MD, PhD, Case Western Reserve University) to UMB-BRL CLIA/CAP Laboratory

Application of a Vimentin Gene Methylation
Potential Biomarker for Colon Cancer

Potential Biomarker for Colon Cancer
Transfer of Vimentin Gene Methylation Assay
From BDL (Case Western Reserve University) to
UMB-BRL CLIA/CAP Laboratory (Cont'd)

- PCR Amplification Buffer affects sensitivity
  - Roche buffer (Essential) vs. ABI Taqman universal buffer.
- The Roche buffer produced lower Ct values (mid –high 20's to low 30's) which improves assay sensitivity vs Taqman (high 30's to 40's). Therefore, the Roche buffer was used.
- Reagents (e.g. buffers) were verified, optimized and standardized for clinical application.
- The UMB-BRL implemented HPLC purified primers and probes.
- Certain elements of the assay were modified to adapt to CLIA/CAP standards
  - Inclusion of standard curve using CpGemone DNA, etc.
  - The UMB-BRL implemented use of diluted CpGenome DNA (methylated and unmethylated) versus diluted cell line.
- Evaluation of DNA clean up on the stool samples
- Repeat testing of previous pre-validation study samples demonstrated a high degree of concordance between BDL and UMB-BRL.
- Specimen processing SOP for samples received at UMB-BRL developed.

Overview
Implementing BDL Laboratory Assays into CLIA/CAP-Compliant Assays
Issues/Lessons Learned

- "Paper Towel Syndrome"
  - Lack of organized SOP without clear definition of all assay parameters (e.g. fragmented SOP written on paper towels, isolated notes in lab coat pockets, etc.)
- Modification of SOPs

- BDLs may have used assays prior to discovery/development of a biomarker. SOPs may have been modified on an ongoing basis without documentation/rationale making it difficult to implement/evaluate assay efficacy.
- Consistency of assay performance
- Alternate methods/different technologists

- Staff training
  - Appropriate training
  - Potential for inconsistencies in running assay from batch to batch
  - Assure staff complies with procedures as delineated in SOP and assure different staff do not "do it their own way"

Overview
Implementing BDL Laboratory Assays into CLIA/CAP-Compliant Assays
Issues/Lessons Learned (Cont'd)

- Compliance with CLIA/CAP QC Standards
  - Standard controls or presence of controls in assay.
  - Equipment calibration, SOP, records, validation, QC, maintenance and monitoring, including pipet QC
  - Reagent QC including, source, tracking by lot and shipping number, storage conditions; HPLC grade not used
  - Kits used for testing not at clinical level (e.g. kits purchased outside U.S. which may not meet clinical standards) and contents mixed
  - QC of in-house reagents and expiration dates
- The same model of equipment/platform or different equipment may not function the same and produce comparable results between the developmental laboratory and the BRL.
- Alternate methods used in the BDL for the same assay by different technologists or same assay performed differently by technologists in BDL makes it difficult for BRL to develop CLIA/CAP SOP.
- Lack of clarity on how BDL calculates results
- Reagents not stored at proper temperatures; temperatures of refrigerators/freezers not recorded
- BDL assays may use equipment that is not in general use in a clinical laboratory
- Budget comparisons between BDL and BRL may be different due to differences in final BRL assay.
- Interpretation guidelines/results must be developed based on validation and be clearly defined

BRL and BDL CLIA/CAP Assay Recommendations
If you don't know where you are going you might end up some place else
Yogi Berra
BRL and BDL CLIA/CAP Assay Recommendations

- BDL and BRL communicate early in the process of assay development/implementation for testing in a CLIA/CAP laboratory.
- Perform unblinded parallel studies between BDL and BRL during assay development/implementation to assess efficacy of all assay parameters.

BRL and BDL CLIA/CAP Assay Recommendations (Cont'd)

- Clearly validate and define interpretation guidelines that result in consistent agreement between BDL and BRL
- Early identification by the BRL of promising platform(s) used by BDLs so BRLs will be prepared for technology transfer
- Assays used by BDLs may not be in use in BRLs/clinical laboratories or are assay adaptations. BRLs need to learn and optimize reliability of these assays early in the process
- Clearly define all aspects of assay parameters in the SOP
- Continued parallel testing as the biomarker development laboratory develops and the BRL evaluates biomarker(s) for clinical implementation
- Incorporate well defined quality assurance oversight/methods to provide more consistent results throughout the development and implementation process

UMB-BRL Core Facilities and Resources
Potential Areas of Collaboration with Other Members of EDRN

Johns Hopkins University Biomarker Reference Laboratory

Daniel Chan, Ph.D., Johns Hopkins University

Translation of Cancer Biomarkers into
Clinical Diagnostics – EDRN BRL at JHU
Daniel W. Chan, Ph.D., DABCC, FACB
Professor of Pathology, Oncology, Radiology, Urology
Director, Clinical Chemistry Division & Co-Director, Pathology Core Lab
Director, Center for Biomarker Discovery & Translation
Johns Hopkins Medical Institutions, Baltimore, Maryland, USA

Center for Biomarker Discovery and Translation
Johns Hopkins University (www.biomarkercenter.org)

An Integrated Approach for Biomarker Discovery, Validation and Translation – JHU Clinical Chemistry
Johns Hopkins Hospital Clinical Chemistry Division:
250 staff, 5000 patient specimens/day, 12 million test/year
Director: Dr. Daniel W. Chan (CLIA/CAP certified lab)
Clinical Diagnostics – 2 Pathways
1. CLIA certified clinical lab (LDTs)
2. FDA cleared/approved (Medical Devices).

Ref: Translation of proteomic biomarkers into FDA approved cancer diagnostics: issues and challenges. Anna K Füzéry, Joshua Levin, Maria M Chan and Daniel W Chan. Clinical Proteomics (2013)10:13

LDTs vs FDA Devices
According to the FDA, "A laboratory developed test (LDT) is a type of in vitro diagnostic test that is designed, manufactured and used within a single laboratory." – CLIA (Clinical Laboratory Improvement Amendments) certified lab.

Recently, a letter from FDA to Tom Harkin, Chairman, Committee on Health, Education, Labor and Pensions, US Senate stated that

- FDA is providing notification for its intent to issue draft guidance entitled Framework/or Regulatory Oversight of LDTs and an accompanying draft guidance document.
- This Guidance proposes a risk-based, phased-in framework for oversight of LDTs in a manner that is consistent with FDA' s current regulation of in vitro diagnostic devices.

LDT vs FDA – Warning!

LDTs vs FDA Devices

- The FDA's review of analytical validity is done prior to the marketing of the test system, and therefore, prior to the use of the test system on patient specimens in the clinical diagnosis/treatment context.
- The FDA's premarket clearance and approval processes assess the analytical validity of a test system in greater depth and scope. The FDA's processes also assess clinical validity, which is the accuracy with which the test identifies, measures, or predicts the presence or absence of a clinical condition or predisposition in a patient, as part of the review that is focused on the safety and effectiveness of the test system.
- Unlike the FDA regulatory scheme, CMS' CLIA program does not address the clinical validity of any test.

FDA Intended Use and Submission for Review

- FDA 2 types of review:
    1. Premarket Notification 510(k) – "Clearance": "Substantially equivalent (SE) Class II devices
    2. Premarket "Approvals" (PMAs): Class III devices

- Risk classification for cancer diagnostics:

High risk: Screening, detection, diagnosis, prediction, companion diagnostics.
Intermediate risk: Tumor Staging, prognosis with clinical outcomes.
Low risk: Detection of recurrence and monitoring of response to therapy.

Establish Performance goals for clinical diagnostics

- To achieve high analytical accuracy (measurement of biomarker concentration).
- To obtain long term consistent results (within and between labs).
- To achieve interchangeable biomarker results between assays in order to minimize clinical confusion (different assays).
- To achieve high clinical accuracy: diagnose clinical conditions correctly (separate targeted diseases from non-diseases).

Characteristics of Clinical Diagnostics
Analytical Performance

- Consistency: Reproducibility (precision – within run, between run), lot-to-lot variation.
- Accuracy: Recovery, linearity, method comparison (correlation).
- Sensitivity: Limited of detection (LOD), Limited of quantitation (LOQ), functional sensitivity (20% CV).
- Specificity: Interferences (matrix, bilirubin, lipid, drugs etc), HAMA, high dose hook effect etc.
- Standardization: Traceability, calibration curve.
- Instrumentation: Variation.
- Quality management.

Characteristics of Clinical Diagnostics
Clinical Performance

- Define "Intended Use".
- Select a targeted population: Patient inclusion and exclusion criteria.
- Identify pre-analytical and biological variability.
- Determine accuracy: Method comparison (correlation)
- Establish reference values: Healthy population
- Determine sensitivity and specificity using receiver operating characteristic curves (AUC): Separate disease from non-disease.
- Establish test predictive values: positive and negative.
- Multiplex testing: Models, training vs testing (validation), study design to minimize systemic biases from non-disease associated factors.
- Evaluate cost effectiveness and clinical outcomes.

Daniel W. Chan, Ph.D. and Zhen Zhang, Ph.D.
(Translation of cancer biomarkers)

Ovarian Cancer
Wall Street Journal 3-9-2010

Case study: Ovarian Cancer

- A 50 years old woman was presented at the Johns Hopkins Hospital clinic. Physical examination revealed masses in the pelvic area. An ultra-sonography was performed, however, the result was not diagnostic. Her serum CA125 was 105 U/mL. What was the diagnosis?

OVA1 – Wall Street Journal 3-9-2010
The 1st proteomics IVDMIA (In vitro diagnostic multivariate index assays) cleared by the US FDA with Partnership from Vermillion, Inc.

- Intended Use: The likelihood of malignancy in patients with ovarian adnexal mass.
- "The test, which is called OVA1 …was shown to correctly flag 92% of cancers, when used along with radiological imaging and a standard patient work-up, in a study of 27 hospitals, doctors' offices and

clinics. physicians using their usual detection methods but not OVA1 had previously found 72% of the cancers."

Study Design

Use of Data from Multiple Sites

Bioinformatics:
UMSA for Nonlinear Classification

Model Evaluation and Comparison: ROC Curves

Lesson learned: Do you have the right approach for biomarker discovery and validation?

Do you have the right specimens for biomarker discovery?

- To obtain the right specimens from the right targeted population for biomarker discovery.
- For example, if the goal is to discover biomarkers for aggressive prostate cancer, specimens should be obtained from patients with and without aggressive prostate cancer.

Overa (OVA2)
FDA 510(k) clearance – March 18, 2016

- Proteomic IVDMIA: Overa = A panel of CA125, Transferrin, Apolipoprotein A1, FSH and HE4.
- Specificity improved from 54% to 69%.
- Same sensitivity = 91%.
- A single cutoff for all patients.
- Validation of a second-generation multivariate index assay for malignancy risk of adnexal masses. Robert L. Coleman, Thomas J. Herzog, Daniel W. Chan, Donald G. Munroe, Todd C. Pappas, Alan Smith, Zhen Zhang and Judith Wolf. GYNECOLOGY (2016) 215:82, 1-10.

Choosing the right bridge!
Translation of Biomarkers

Bridge to Nowhere!

EDRN SC GU Group meeting 9/21/2005 (Seattle)

EDRN SC GU Group meeting 9/21/2005 (Seattle) (cont)

Prostate cancer biomarkers decision gate
National Cancer Institute (NCI), Early Detection Research Network (EDRN)
Daniel W. Chan (PI-BRL)

In 2005, 14 biomarkers were submitted to EDRN for consideration (Chair of EDRN GU group – Daniel W. Chan).
Based on preliminary data from the investigators, the top 5 biomarkers were selected for pre-validation using the same prostate clinical specimen reference set (blinded): ProPSA, human Kallikreins, EPCA2, PCA3 and TSP1.
Three biomarkers (human kallikreins, TSP1 and EPCA2) failed pre-validation. Pro-PSA and PCA3 were recommended for clinical validation.

EDRN Public-Private Partnership

PCA3 Molecular Urine Test - Non-coding RNA (PROGENSA)
To determine the need for repeat prostate biopsies in men who have had a previous negative biopsy:
Hologic, Inc.
FDA approved 2/15/2012. Cut-off: PCA3 RNA/PSA RNA < 25 = negative.
EDRN study: Initial biopsy >60, repeat biopsy <20.
Can urinary PCA3 supplement PSA in the early detection of prostate cancer? J Clin Oncol (2014) 32:4066-72.
Wei JT, Feng Z, Partin AW, Brown E, Thompson I, Sokoll L, Chan DW, Lotan Y, Kibel AS, Busby JE, Bidair M, Lin DW, Taneja SS, Viterbo R, Joon AY, Dahlgren J, Kagan J, Srivastava S, Sanda MG.

Summary - Translation of cancer biomarkers into clinical diagnostics
1. What new biomarkers are needed clinically?
2. Define the clinical "intended use" (FDA) for unmet needs.
3. What performance criteria should be established for clinical diagnostics?
4. How should biomarkers be validated to meet analytical and clinical requirements?
5. How to develop clinical diagnostics?
6. What are the regulatory requirements for CLIA certified lab or FDA clearance/approval?
7. Are these new clinical diagnostics cost effective and can be paid?

Conclusion: Translation of cancer biomarkers into clinical diagnostics
Translation of cancer biomarkers into clinical diagnostics will require close collaboration and partnership between NCI EDRN researchers and BRL, clinicians, industry and regulatory agencies.

Charge to Collaborative Groups
Sudhir Srivastava, Ph.D., M.P.H., National Cancer Institute
No slides prepared, audio failed.

Wednesday September 13, 2017

Collaborative Group Meetings (closed)
Minutes not provided

Scientific Workshop Planning Committee Meeting (closed)
Minutes not provided

Showcase Presentations from Collaborative Groups:
Session Moderator: Christos Patriotis, Ph.D., National Cancer Institute

The Immuno-biology of Pulmonary Premalignancy
Steve Dubinett, M.D., University of California, Los Angeles
David Geffen School of Medicine at UCLA
Representing the BU/UCLA BDL

Rate limiting step to develop new interception approaches is lack of understanding of the earliest molecular events associated with disease initiation and progression

Loss of immunosurveillance with tumor progression

Loss of immunosurveillance with tumor progression

TCGA Lung Squamous Carcinoma – leukocyte signature genes* appear to be important in Stage I
(A) Heatmap of GSVA-derived scores of 22 immune-related cell profiles in 229 samples. Samples and cellular profiles were ordered based on unsupervised hierarchical clustering.
(B) Survival of Stage I patients in G2 (n = 49) and G3 (n = 37). HR= 2.4 (95% confidence interval 1.2 - 4.7).
(C) Kaplan-Meier curves of Stage II and higher patients in G2 (n = 33) and G3 (n = 39). HR= 0.9 (95% confidence interval 0.4-1.8).

TCGA Lung ADC – Immune-related pathways appear to be important in Stage I
Tumor immune profile of TCGA lung ADC
(A) Heatmap of GSVA-derived scores of 16 immune-related pathways in 502 samples. Samples and pathways were ordered based on unsupervised hierarchical clustering.
(B) Kaplan-Meier curves of Stage I patients in G2 (n = 101) and G3 (n = 91). Hazard ratio = 2.0 (95% confidence interval 0.99-4.25). (C) Kaplan-Meier curves of Stage II and higher patients in G2 (n = 77) and G3 (n = 90). Hazard ratio = 1.2 (95% confidence interval 0.74-1.98).

Rationale
- The complete mutational profiles of atypical adenomatous hyperplasia (AAH) premalignant lesions and adenocarcinoma in situ (AIS) have not been studied
- Molecular mechanisms underlying AAH formation and potential progression are not well understood

- The role of the immune system and microenvironment in premalignancy and tumor progression is not fully investigated

PCA of Premalignant Adenomatous Lesions
1) Identify immune contexture in premalignancy and the associated tumor
2) Identify AD premalignant neoepitopes
3) Derive novel molecular subclasses of AD premalignancy, using genomic and transcriptomic heterogeneity

Extending the PCA to premalignant lesions for adenocarcinoma (AAH):
Immune recognition and suppression in lung premalignancy
The battle between host and tumor starts very early

Work flow for neoantigen discovery and validation

Experimental design
- FFPE tissue blocks from 41 patients with multiple AAH lesions, AIS and ADC obtained from UCLA Lung Cancer Tissue Repository
- For each patient, the following regions were isolated by Laser Capture Microdissection (LCM) and sequenced utilizing whole exome sequencing:
  1-3 regions of normal lung
  2-4 premalignant AAH lesions
  1-3 regions of AIS (where present)
  1-3 regions of invasive ADC
- A total of 50 normal areas, 89 AAH, 15 AIS and 55 ADC were sequenced

Mutational landscape in pulmonary premalignancy
Whole-exome sequencing of at least three non-overlapping regions of adenocarcinoma,

AAH and normal airway epithelial cells (n=41)
Immune infiltration and neoepitopes in lung adenocarcinoma premalignancy

Dysregulated pathways in lung adenocarcinoma premalignancy

Human bronchial epithelial cells after selection for migratory capacity

Ten Years of Mesothelioma Biomarkers: Controversy and Evolution
Harvey Pass, M.D., New York University
Disclosures
- Grants: NCI EDRN,TCGA, DOD, Technion, CDC
- Philanthropy: Rosenwald Foundation, Stephen A. Banner Lung Cancer Foundation, Belluck and Fox, Levi, Phillips and Konigsberg, Simmons Foundation, Baron and Budd, multiple patients who shall remain anonymous
- Royalties: NCI cell lines
- Industry: Fujirebio, 20/20 Gene, MesoScale Diagnostics, Cynvezio, Source MDX, Celera, OPKO, SomaLogic, Genentech, Integrated Diagnostics, Transgenomics, Rosetta Genomics, Calithera, Pinpoint Genomics, Cizzle, emeraldLogic, HTG, Freenome,
- Patents (no money): Osteopontin for diagnosis and prognosis of MPM; fibulin 3 for diagnosis and prognosis of MPM; mir-29c* for prognosis of MPM; mir-31 for diagnosis of MPM, HMGB1 for diagnosis of MPM

Survival of 336 Pleural mesotheliomas

"Fluid Based Screening" for Mesothelioma
- How do patients present in the US?
  - 91% have an exposure to asbestos
  - 90% of pleural patients present with an effusion
  - 77% of abdominal patients present with ascites

- Effusions do NOT OCCUR OVERNIGHT
    - Screening is done in asymptomatic patients with a history of exposure to asbestos
- Therefore, you are attempting to define plasma biomarker changes which could reflect the development of an asymptomatic effusion or some "perturbation of mesothelial physiology" over time.
- A change in marker indices could lead to a CT of the chest/abdomen
    - But the markers must be specific for a given "disease/malignancy"

Not a good marker for diagnosis due to lack of specificity

But what about diagnostic specificity???

FBLN-3 has function in MPM

The development of our MPM assay is illustrated in Figure 21. (A) SOMAmer with 5' biotin and a photo-cleavable linker between the SOMAmer and the 5' biotin are pre-bound to streptavidin coated beads (either magnetic or agarose beads can be used) and beads are added to samples in microwells (B-H) Schematic sequence of assay steps leading to quantitative readout of target proteins. (B) Proteins, shown as different shapes, and beads with SOMAmers are mixed in solution (C) SOMAmers attached to magnetic beads bind to proteins specifically (gold and green) and some non-specifically (blue). Unbound proteins are washed away (Catch 1) (D) Tagging: Proteins bound to SOMAmers are tagged with NHS-biotin. (E) Photocleavage and kinetic challenge: UV light (hy) cleaves the linker (L) between the SOMAmer and the 5' biotin, releasing SOMAmers into solution. Taking advantage of a SOMAmer's slow-off rate from its target protein, further specificity of a SOMAmer to its protein target is derived from a kinetic challenge, by adding excess anionic competitor ("random" SOMAmers) to the SOMAmer-protein complex in solution; cognate complexes (gold and green) dissociate slowly, but non-cognate complexes (blue SOMAmer) dissociate rapidly and competitor prevents re-binding. (F) Catch 2: The SOMAmer-protein complexes that remain after the kinetic challenge are captured onto new streptavidin (SA) coated magnetic beads by the biotin tag on the protein from the NHS-biotin labeling of the protein (D) and unbound SOMAmers are washed away. (G) Elution: SOMAmers are eluted into solution by disrupting complexes (e.g. proteins denatured with sodium perchlorate) (H) Readout: Eluted SOMAmers are hybridized to complimentary probe sequences on coded Luminex beads and quantified by flow cytometry on Luminex.

So what else is new????
Hypothesis:
Buffy Coat/PBMC RNA Immune RNA expression profiles differ between asbestos exposed, unexposed, and mesothelioma populations

Nanostring Immune-Oncology Panel
- 770 gene panel
    - gene expression of human immune response in all cancer types
- 24 different immune cell types and populations
    - 109 genes to cell surface markers for 24 different immune cell types and populations,
    - 30 common cancer antigens
    - Genes that represent all categories of immune response including key checkpoint blockade genes.

Possibilities and different microenvironments
Plasma
- EDTA
    - Non Cellular
        - Protein

        - cfDNA, microRNAs, exosomes and microvescicles
    - Cellular
        - Buffy coat
        - Granulocytes, platelets, granulocytes, monocytes
- Citrated
    - Non Cellular
        - Protein
        - cfDNA, microRNAs, exosomes and microvescicles
    - PBMC
        - Platelets, monocytes, lymphocytes

Serum
- Non Cellular
    - Protein
    - cfDNA, microRNAs, exosomes and microvescicles

17 High Risk vs 17 AE vs 17 MPMs

Healthy Age and Tobacco Matched Cohort vs Asbestos Exposed

Asbestos Exposed vs Mesothelioma

Validation: BC
Asbestos Exposed vs Healthy Matched

But who the hell cares about mesothelioma???

What about early stage lung cancer (cStage I?
- Can this BC immunooncology method separate patients by
    - histology
        - their chances of recurrence/progression within the first 5 years after complete resection?

Overview of ADS1 (n=48) vs. GR (n=24)

Overview of ADS1 (n=48) vs. GR (n=24)

Overview of ADS1 (n=48) vs. HR (n=18)

ADS1 (n=48) vs. HR (n=18)

ADS1 (n=48) vs. HR (n=18)

Prognostic Comparisons – Overview (102 patients)

DE – Progressed (28) vs. Not Progressed (66)

Immune Cell Profiling – Progressed (28) vs. Not Progressed (66)

Thanks to…
- NYU Thoracic Lab
    - Chandra Goparaju PhD
    - Ryan Harrington BS
    - Amanda Beck MD
    - Joe Levin MD
    - Nathalie Hirsch MD
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    - Haining Yang, PhD
    - University of Toronto

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    - Indie Diagnostics
    - SomaLogic
    - Fujirebio
    - Karmanos Cancer Institute
    - Anil Wali PhD
- Early Detection Research Network, NCI, NIH
    - DMCC
    - Mark Thornquist PhD
- Lung Cancer Foundation of America/IASLC

The Old Standbys……..
Other Players

Tissue

MPM, OPN, and Prognosis

Can Plasma Biomarkers "add value" to already established Clinical Prognostic Indices?

- EORTC and CALGB Prognostic Indices are already an established clinical Prognostic Index for MPM
- Can any of the Biomarkers we have talked about add value to the EORTC Prognostic Index?
    - Take the most relevant biomarkers in the literature and investigate that…..
- SMRP, Osteopontin
    - Discovery Set: 83 MPMs from NYU and Wayne State University
    - Blinded Validation Set: 111 MPMs from Princess Margaret Hospital

Present EDRN Renewal, 2016-2021

Biomarker Discovery with SomaLogic Slow Off Rate Modified Aptamers Platform

Aim 1: Develop a novel SOMAmer based proteomic platform in order to validate plasma and pleural effusion diagnostic and prognostic biomarkers

Subaim 1a: Develop and technically validate a 14 SOMAmer, luminex based assay (SOMA14 NYU-MPM) combining Fibulin-3 with the 13 SOMAmer MPM test.
Subaim 1b: Validate the using new cohorts from the NYU Thoracic Surgery Archives
Subaim 1c: Validate the plasma prognostic accuracy of the Soma 14 NYU MPM assay
Subaim 1d: Determine whether the Soma 14 NYU MPM assay can diagnose MPM pleural effusion
Subaim 1e: Blinded validation of the diagnostic and prognostic capabilities of SOMA14 NYU-MPM using plasma cohorts from the Princess Margaret Cancer Center and pleural effusion cohorts from NYU and University of Glasgow

Aim 2: To determine the accuracy of HMGB1 and its Isoforms for the diagnosis of MPM pleural effusions

Subaim 2a: Compare sensitivity and specificity of total ELISA HMGB1 pleural effusion levels to MRP and Fibulin-3 using pleural effusions from the NYU Thoracic Surgery Archives
Subaim 2b: Measure Total, oxidized, and acetylated forms of HMGB1 in pleural effusions using pleural effusions from the NYU Thoracic Surgery Archives using a novel mass spectroscopy platform
Subaim 2c: Blinded diagnostic validation of HMGB1 and Fibulin-3 ELISAs compared to total, oxidized, and acetylated forms of HMGB1 pleural effusion cohorts from NYU and University of South Glasgow.

Aim 3: To determine whether buffy coat/PBMC immuno-oncologic RNA expression can define asbestos exposure and diagnose MPM

Subaim 3a: Further refine and validate Nanostring Immuno-oncology profiles in the diagnosis of asbestos exposure and MPM

Subaim 3b: Blindly validate locked in Nanostring Immuno-oncology profiles for healthy, non-AE vs AE, AE vs MPM, and MPM vs non-MPM using buffy coat/PBMC from the Princess Margaret Cancer Center

Aim 4: Integrate the best analytes from Aims 1-3 into a diagnostic and prognostic profile for separating plasmas from healthy, non-AE vs AE, AE vs MPM, and MPM vs non-MPM and MPM vs non-MPM pleural effusions

The discovery and validation of the proposed plasma and pleural effusion biomarkers from these studies will have the potential to monitor patients for the development of MPM in the future. The profiles that are promising from these studies could then be subjected to for further validation in ongoing longitudinal prospective/retrospective cohorts for the screening of MPM including the EDRN sponsored MPM Screening Trial in Santiago, Chile.

Future Validations and Pre-Diagnostic Opportunities

EDRN sponsored MPM Screening Trial in Santiago, Chile.

- Help from NYU and NCI sought from Chilean Clinic in the screening of asbestos exposed workers as mandated by the Government
- EDRN approved Trial, December 2013
- Chile approved, July 2014
- NYU IRB, approved October 2014
- Site Visit, May 28, 2015
- Funding Approved, October 2016
- Start Date August 2017

<u>The sTRA Antigen: A New Blood and Tissue Biomarker for Pancreatic Cancer</u>

Daniel Barnett, B.S., Van Andel Research Institute

Pancreatic Cancer – Poor Survival, Late Diagnosis

- Pancreatic cancer is the 9th most common cancer in the US and the 4th deadliest (number of deaths annually) in males and females. 1
  - Overall survival is 7%, 15-25% in resected cancers
  - Difficult and Late Diagnosis
  - Resistant to treatments

Novel glycan antigen sialyl-TRA (sTRA) is related to current best test antigen (CA19-9)

Pancreatic Cancer Heterogeneity and Glycans

Glycans detected on captured proteins and glycans by microarray

Patient Sample Sets

sTRA performs as well or better than CA19-9, but strongest together

Low correlation between CA19-9 and sTRA expression

sTRA consistently performs as well or better than CA19-9

sTRA is complementary to CA19-9

sTRA detects PDAC not detected with CA19-9

MUSC – Drake Set

- 52 cases, 8 controls
- Matched blood and tissue samples (cases)
  - All patients had resectable cancer
- Tumor samples analyzed as a Tissue Microarray

Plasma sTRA and CA19-9

Plasma sTRA and CA19-9

Low serum marker levels show expression in tissues

No significant tissue expression difference between high and low serum CA19-9 expression

CA19-9 and sTRA expressions are correlated, but complementary

Patients with high plasma in both

Patients low in both or high in CA19-9 only

Patients with high plasma sTRA only

Acknowledgements

An Update on ctDNA as a Cancer Biomarker

Robert Schoen, M.D., M.P.H., University of Pittsburgh

ctDNA
As cancer cells turn over, release DNA into circulation, urine or stool
Detecting Mutant DNA – somatic mutations within driver genes that are responsible for clonal growth
Exquisite specificity – normal cells do not clonally expand, rarely harbor these somatic mutations, and not in this concentration

Challenges

- Technical: Can we develop tests of sufficient specificity and sensitivity?
- Biological:

1) Are there detectable amounts of DNA from neoplastic cells present in the biological fluid (some tumors don't release)?
2) Somatic mutations vary - panel
3) Mutations not specific to one cancer

Safe-SeqS
Safe
Sequencing
System
Method for detection and quantification of rare mutations

Safe-SeqS
Assignment of unique ID (UiD) to each template molecule
Amplification of each UiD template molecule to create UiD families
Redundant sequencing to amplify: PCR fragments with same UiD are truly mutant if 95% contain identical mutation

Essential elements of Safe-SeqS

Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers

ctDNA KRAS + Protein for Detection of Pancreatic Ductal AdenoCA
221 pancreatic CA – all M0 (surgically resectable) (Stage I-II)
Tumor size median, 3.0cm IQR (2.4-3.8); 24 ≤ 1.5 cm
Positive nodes N=170 (76.9%)
182 controls

KRAS Detection
Safe-SeqS – can detect 1 mutant/10,000 molecules
Codon 12 and 61 mutations
Detected mutant KRAS 66/221 (30%)
N=62 (codon 12) N=4 (codon 61)

ctDNA positivity correlated with stage, more frequent in large tumors

Mutant Templates
# of mutant templates calculated from mutant allele fraction and DNA concentration
15/66 (23%) <2 mutant templates/ml plasma
Avg 9.4/ml plasma
Only 1/221 normals had mutant KRAS
Concordant mutation found in 50/50 cancers tested

Protein Markers
Evaluated long list proteins – 5 promising
Prolactin – found to be elevated if blood drawn after anesthesia eliminated
CA 19-9 – threshold of 100 u/ml – zero positive for healthy controls
CEA, HGF, OPN – used threshold 10% higher than maximum in 273 normals - 100% specificity

Failed Protein Markers

Combination Assay
Sensitivity: 141/221; (64%, 95 CI 57-70)
Specificity: 1/182; (99.5%)

Combination Assay
Combination assay sensitivity: 141/221 (64%, 95 CI 57-70)
Positive assay associated with ↓'d survival – independent of clinico-path results – HR 1.76
27/40 (60%) patients with no symptoms were detected, of whom 19/27 (70%) w/o recurrence at 12 mo f/u

Summary: Pancreatic Adeno CA
KRAS gene mutation and protein biomarkers N=221 CA's, 182 controls
KRAS mutant ctDNA in plasma – 66/221 (30%) – 100% concordance w/tumor
Add 4 proteins (CEA, CA 19-9, HGF, OPN) sensitivity to 64%
Only 1/182 controls was positive – 99.5% specificity
High thresholds to avoid decreasing specificity

Mega Study
1600 cancers, 800 controls
ctDNA Assay with 61 amplicons, evaluating spectrum of mutations in genes across cancers – agnostic screening assay
Combined with proteins to increase sensitivity and site identification

CRC Cohort
- 409 stage I-IV CRC patients, all with matched tumor
  - 71 stage I
  - 186 stage II
  - 121 stage III
  - 31 stage IV
- Average age 64 (range: 22-93)

ctDNA + protein

ctDNA for Monitoring

Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer
ctDNA in Stage II CRC
N=250, post-op and serial plasma
23% adj chemo Rx at clinician direction

Targeted 15 genes using safe-SeqS
20/231 +ctDNA post op (9 p53, 8 APC, 3 KRAS)

Median f/u 27 mo
34 (14.8%) radiologic recurrence
27/178 (15%) no chemo Rx
7/52 (13%) with chemo Rx

ctDNA in Stage II – Results
No Chemo
+ctDNA ↓↓ Recurrence free survival (RFS): HR=18
ctDNA helpful in both low and high risk by clinical pathologic characteristics
CEA did not predict RFS

Recurrence Free Survival (RFS) in patients not treated with adjuvant chemotherapy

Recurrence Free Survival (RFS) in patients not treated with adjuvant chemotherapy

Recurrence Free Survival (RFS) in patients not treated with adjuvant chemotherapy by CEA level

Serial ctDNA: Patients Treated with Chemotherapy

Conclusions
ctDNA is a marker for recurrence in patients with stage II CRC
ctDNA findings discriminate within clinicopathologic subgroups

"DYNAMIC" Study
Circulating Tumour DNA Analysis Informing Adjuvant Chemotherapy in Stage II Colon Cancer

"DYNAMIC" Study
Study Design
Randomised multi-centre biomarker-driven adjuvant treatment study
Primary end-points
Number of patients treated with adjuvant chemotherapy
Recurrence-free survival

DYNAMIC Study

Aims: EDRN Renewal
- Can ctDNA be used as a primary screening modality?
  - Test panel of mutations (PlasmaSeq)
- Can ctDNA in Stage III CRC be used as predictor of disease free and overall survival?
  - Compare CEA to ctDNA
  - Evaluate ctDNA as prognostic marker

<u>Integrated Genomic and Proteomic Biomarkers for Non-Invasive Identification of Aggressive Prostate Cancer</u>
Paul Boutros, Ph.D., Ontario Institute for Cancer Research
Pathway
* Overview of Clinical Space

Prostate Cancer Epidemiology
Incidence:
~1 in 7 men
(1,515 per million)
Mortality:
~1 in 29 men
(242 per million)
Risk factors:
* Age
* Family history
* High-fat diet

* African ancestry

Prostate Cancer Prognosis
Digital Rectal
Exam Imaging Biopsy Blood test

Clinical stratification of localized cancer
Active Surveillance vs Radiotherapy or Radical prostatectomy vs Radiotherapy or Radical prostatectomy + adjuvant treatment

Pathway
* Genomics

Spatial Sampling of Prostate Cancer

Immense Intra-Prostatic Diversity…

…At All Levels of Investigation

Is This Heterogeneity Clinically Relevant?

Multiple Primaries

Training Cohort

Cluster CNA profiles

Clusters are associated with outcome

Classifier evaluation

This Is the Best Performing Signature

Validation in >500 Patients

What About Other Mutation Types?
* WGS of 200 prostate cancer tumour/normal pairs
* OncoScans of all 200 patients
* Methylomes on 150 patients
* Array-based RNA profiling (HTA) on 150 patients
* Clinical outcome
* Lots of bioinformatics

WGS of 200 Prostate Tumours

WGS of 200 Prostate Tumours

Pathway
* Proteomics

What About the Proteome?

Distribution of Driver Gene Abundance

ETS-Fusion Associated Protein vs. RNA

Protein Has Greater Dynamic Range

Protein Weakly Associated to RNA

All Multi-Omic Correlations Are Weak

Protein-RNA Survival Associations

RNA Complements Protein Biomarkers

Pathway

* Fluids

Experimental Design

Important Control: Age Effects

We independently validate 13 of these as weak biomarkers

Signature Identification using a nested machine-learning model

Final multivariate biomarker: 71% accuracy

Can we provide additional functional evidence for these genes?
Methylation in primary tumours
- n=80 samples
mRNA expression in primary tumours
- n=133 samples

AHRGAP1

Current Directions: improved study design

But is there another easily accessible, prostate-proximal fluid we can assess?

UPS-Urine proteomics
Almost all proteins detectable

Individual peptides are predictive

And together form a predicative model

Pathway
* Summary

Summary
Biomarkers can:
* Be developed using multiple data-types
* Overcome spatial heterogeneity

The germline genome:
* Influences tumour progression and mutations
* Is under-studied

These data call for composite germline, somatic, imaging and fluid biomarkers to diagnose, prognose and monitor progression.

CPC-GENE: The People Involved

Development of Prostate Cancer Early Detection and Prognostic Biomarkers
Scott Tomlins, M.D., Ph.D., University of Michigan
Disclosure Information
I have the following financial relationships to disclose:
- The University of Michigan has been issued a patent on ETS gene fusions on which I am a co-inventor.
  - The diagnostic field of use has been licensed to Hologic/Gen-Probe, Inc., which has sublicensed rights to Roche/Ventana Medical Systems
- Consultant for and honoraria from Roche/Ventana Medical Systems, Jansenn, AbbVie, Almac Diagnostics and Astellas/Medivation
- Sponsored research agreement with and travel support from with Compendia Biosciences/Life Technologies/ThermoFisher Scientific
- Sponsored research agreement with Astellas and GenomeDX
- Co-founder of, equity holder in and Laboratory Director for Strata Oncology

Assess Science

- Established screening biomarker (PSA)
- Numerous clinical models and FDA/LDT tests shown to improve upon performance of serum PSA for predicting aggressive cancer on biopsy
- Prostate cancer is extremely common and usually multifocal
- Multiple LDT tissue tests available to improve prediction of undersampled aggressive cancer on biopsy
- Urology is extremely entrepreneural testing specialty

Project Overview
- Identify and validate novel prostate cancer and aggressive prostate cancer specific transcriptomic biomarkers

The landscape of lncRNAs in the transcriptome

Cancer/Lineage Association of MiTranscriptome lncRNAs

Nomination of ARInc1 as a prostate lineage and ancer-specific lncRNA

ARlnc1 (Androgen Receptor lncRNA1) is a prostate lineage and cancer specific lncRNA

Project Overview
- Determine the utility of prognostic transcriptomic tissue based biomarkers

Multiplex RNAseq
Potential advantages
* <1-10ng FFPE or urine RNA vs. >>10ng qRT-PCR for many targets
* Multiplexing is easier by sequencing than most qRT-PCR
* Digital data (sequence information)
* Combinatorial priming for gene fusions/splice variants

306 transcript targeted RNAsep assay

Expected transcript expression

Derived prognostic signatures

Project Overview
- Determine the utility of diagnostic transcriptomic urinary based biomarkers

Serum PSA + urine T2:ERG, urine PCA3

Urine RNAseq panel

Timeline/Implementation

Outcome Expected

Acknowledgements

Outline
- Determine the utility of diagnostic transcriptomic urinary based biomarkers

Challenges
- Ensuring adequate RNA isolation from post-DRE urine collected in UTM
  - Enable validation through EDRN and banked UM samples
- Updating panel for needed content
  - Remove amplicons that take up too many reads
  - Add PCA3
  - Add additional lncRNAs

Optimization

A Unique Tumor Derived Macrophage is a New Blood-based Biomarker for Cancer Screening and Diagnosis

Jeff Marks, Ph.D., Duke University Medical Center
Cha-Mei Tang, Sc.D., Creatv MicroTech, Inc.

Outline
Biomarker
Biomarker isolation method
Assay techniques
Duke pilot study for breast cancer
EDRN collaborative study

Other cancer screening and diagnostic capabilities

Are these Giant Polyploids CTCs?
When we were looking for circulating tumor cells

Biomarker Discovery
Very large cell in cancer patients' blood

Source of Polyploids
Engulfed tumor cells and maybe other cells and debris

Circulating Cancer Associated Macrophage-like Cells (CAMLs)

CAMLs contain ingested tumor markers

CellSieveTM Microfilters
Collect large cells and clusters
Uniform 7 µm pore size and distribution with high porosity
– Rapid, consistent, gentle
– 3 min to filter 7.5 ml of blood
– Eliminates all red blood cells
– Eliminates ~99.99% of white blood cells
Low fluorescence background
Captures both CAMLs and CTCs
CellSave samples good up to 4 days

Assay Techniques
Markers + cell morphology ⇒ accurate cell identification
Can analyze up to 12 markers on the same cell
Cryo-preservation protocol
Bone marrow analysis protocol
Urine sample preservation reagent and protocol
FISH
PCR
Sequencing

Patient Samples
* > 50 healthy controls
– Zero CTCs, zero CAMLs
* Close to 2000 patient samples
– 14 cancer types (peripheral blood)
Breast, Prostate, Pancreatic, NSCLC, renal cell carcinoma, bladder, sarcoma, colorectal, uterine sarcoma, neuroblastoma, esophageal, ovarian, melanoma, liver
– localized, metastatic, remission, recurring
– CAMLs have been found in all 14 cancer types
– CTC range 0 to >900

– CAML range 0 to 105

CAMLs and CTCs by Stage
Importance of CAMLs
Independent, affirmative marker for solid tumors at all stages
High percentage even in Stage I
Applicable for early detection of cancer
Suitable and effective for precision monitoring of therapy
Actionable information

Breast Cancer Screening
Gold Standard Double Blind Study
41 mammography positive patients from Duke University
Median age 52
Double blinded study
CAML blood test versus
Tissue biopsy
Blood test criteria – 1 or more CAML
≥ 30 µm, CD14+
CAMLs differentiated benign vs malignant conditions
(Assuming biopsy is accurate)
Sensitivity (cancer) – 88%
Specificity (no cancer) – 74%

CAMLs for Breast Cancer Screening

EDRN Breast Cancer Collaborative Study
Study goal
1000 mammography BIRAD 4 patients
Predict breast cancer based on presence of CAMLs
Restain to identify CAML is from breast cancer
Study plan
Run 2 tubes of blood per patient separately
Stain for CK 8, 18 & 19, CD31, and CD45
Study Status as of 9/8/2017
Processed 110 patient samples
Developing markers set to identify CAMLs is from breast cancer
Positive markers : mammoglobin, HER2, ER
Negative marker: mamothelin (distinguish from ovarian cancer)

Other Cancer Screening Possibilities
Screening for individual cancers
Screening for group of cancers, such as
screen for the top 4 major cancers and identify the cancer of origin
Women: Breast, lung and colorectal cancer
Men: prostate, lung and colorectal cancer

CAMLs Prognostic of Survival

CAMLs Prognostic of Survival

Summary
CAML biomarker

- Found in all stages of cancer
- In high percentages in Stage I for major cancers

CellSieveTM blood based cell biopsy

- Can always isolate CAMLs

EDRN early detection of breast cancer

- Collected and processed 110 patient samples for BIRAD 4
- Will be combining CAMLs with imaging and immunosignaturing

Many other clinical applications

- Possible for screening for other cancers
- Prognostic for survival

Duke

Acknowledgements

Identifying Candidate Breast Cancer Biomarkers in the Plasma of Xenografted Mice

Amanda Paulovich, M.D., Ph.D., Fred Hutchinson Cancer Research Center

Unique approach combining avatar mice and targeted mass spectrometry to identify blood biomarkers for early detection of breast cancer

Our clinical goal is to identify a blood-based biomarker that could be used in conjunction with mammography to improve sensitivity and specificity.
Limitations of Mammography
Specificity of mammography for distinguishing benign vs. cancerous lesions
Sensitivity of mammography in some patient populations (e.g. dense breasts)
Over-diagnosis (e.g. some DCIS).
This hypothetical biomarker would be impactful by detecting treatable cancers missed by mammography (mortality), and by allowing patients to avoid unnecessary procedures for benign disease (morbidity).

Human proteins are present in the plasma of mice harboring breast cancer xenografts.
We hypothesize that a subset of the human tumor-derived proteins in the plasma of xenografted mice will be elevated in the plasma of women with breast cancer, and that a subset of these will be useful for early detection.

Using the mouse PDX model as a conduit for discovering candidate breast cancer biomarkers offers advantages.

All human proteins in the plasma are, by definition, tumor-derived. This should bias the biomarker candidate pool towards tumor-specific proteins.
The mass spectrometry approach to discovering candidate biomarkers from plasma is greatly simplified, because quantification is not necessary in the discovery phase (i.e. results are binary).
If successful, this approach provides a road map that can be applied to additional solid tumors.

TMT allows relative quantitation of 10 samples at a time.

PDX plasma samples with evidence of hemolysis are contaminated with erythrocyte proteins and were not used for proteomic analysis.
To date, 28 out of 41 samples have passed this initial QC step.

The top ~22 plasma proteins account for >99% of plasma protein mass.
In order to detect low abundance protein biomarkers, we need to deplete the plasma samples of high and medium abundance proteins.

The original plan was to deplete mouse plasma of high and medium abundance proteins with a pair of affinity columns run in series.

However, the IgY-7 column was not available until August 2017, so we used the MARS-3 column, run sequentially.

Replicate MARS depletions were highly reproducible in terms of chromatography, protein yield, and protein content.

A portion of the MARS3-depleted plasma was trypsin-digested, TMT-labeled, and pooled for analysis by glyco-proteomics at PNNL (enrich secreted proteins).

While the SuperMix depletion runs were highly reproducible, protein yield from sample to sample was highly variable.
Due to low protein yields of the doubly-depleted plasma, TMT labeling of the individual samples was not possible.

While there was not sufficient protein mass for TMT labeling, these samples should be enriched for human proteins.
We pooled & analyzed the unlabeled, double-depleted plasma samples from 9 PDX mice for LC-MS/MS analysis.

Data were searched at PNNL and FHCRC using complementary software packages and strategies.
While the peptide numbers varied with the two search engines, the proportion of mouse and human IDs were very similar.

Data were stringently filtered to remove peptides mapping to spectra that gave IDs in both the mouse and human databases.

The human proteins in PDX plasma are enriched for extracellular vesicles and RNA binding proteins.
We hypothesize that extracellular vesicles are the primary origin of human proteins observed in the mouse plasma.

Consistent with this hypothesis, we compared our data with an extracellular vesicle (EV) data set generated from the NCI-60 cell line collection.
83% of the human proteins identified the PDX plasma have also been identified in purified EVs from the NCI-60 cell lines.

The 164 proteins not previously identified in EVs may also be EV proteins.

EDRN Pilot Project: Collaboration with PNNL

Glycocapture enriches for proteins shed or secreted into plasma.
Glycosylation is added as proteins pass though the ER/Golgi
N-glycopeptides can be captured via hydrazide chemistry
The glyco capture approach is complementary to the bRP fractionation of the doubly depleted plasma.
Only 17 glyco-capture candidates were identified from two independent searches of the data.
These findings are consistent with the EV/exosome hypothesis.

Next step: narrowing down the candidate list.
96% of the human proteins identified in the PDX plasma are expressed in human breast cancers.

Although candidate proteins were enriched in breast cancer, none was observed exclusively in breast cancer.
74% of circulating proteins were seen in all CPTAC breast tumor samples, while only 25% were seen in all ovarian tumors, and 17% in all colon tumors.

Using a data set of cultured human mammary epithelial cells (HMECs), we can subtract out proteins seen in normal breast epithelial cells.
170 breast cancer-associated proteins were observed in the plasma of the PDX mice.

Top 5 enrichment classes for the 170 candidates

24 of the 170 breast cancer-associated proteins observed in the mouse plasma have not been observed in human plasma from healthy donors.
The 146 proteins previously observed in plasma could be present at elevated levels in cancer, so remain candidates.

The 24 breast cancer-associated proteins that have not been observed in human plasma from healthy donors.

Summary
The project was delayed due to: delays in obtaining PDX plasmas that were not hemolyzed, delays in obtaining mouse plasma depletion columns.
The need to use an alternative depletion strategy led to technical challenges that reduced protein yield, requiring us to eliminate the TMT label in the first dataset.
Nonetheless, a surprisingly large number of human proteins (951) were detected in the PDX plasma, and these are highly enriched for exosome/EV proteins, suggesting that these candidates may not be generated via shedding, leakage, or secretion.
A high interest set of 170 breast cancer-associated proteins has been identified, including a subset of 24 proteins that have not been observed in healthy donor plasma.

Next Steps
Perform additional due diligence to ensure these are really human proteins; consider analyzing normal mouse plasma.
Attempt to replicate the results with an independent set of PDX mice.
Consider enriching EVs from PDX and human plasmas for proteomic analyses.
AIMS analyses to check for candidates in the plasma of human breast cancer patients.

Data Sharing and Informatics Subcommittee Meeting
Minutes not provided

Thursday September 14, 2017

Contemporary Topic: Interpreting Longitudinal Biomarker Changes for Early Detection
Session Moderator: Jacob Kagan, Ph.D., National Cancer Institute

Overview
Margaret Pepe, Ph.D., Fred Hutchinson Cancer Research Center
An Introduction to Longitudinal Biomarker Evaluations
What is longitudinal biomarker data?
- Biomarker measured in serial samples from each individual.
- CA-125 measured annually for ovarian cancer screening.
- CEA measured monthly for colon cancer recurrence after initial treatment.

Key Idea: Change in biomarker over time may inform about disease onset.

Not talking about markers
- with undetectable levels in controls
- germline gene variants or other lifelong stable measures of risk

Talking about markers
- present in people without cancer (controls)
- controls tend to have their own individual set-point

Talking about
- CA-125 for ovarian cancer screening (Skates).
- PSA for prostate cancer screening (Zheng).
- AFP, DCP for liver cancer screening (Tayob).

Intra-class Correlation Coefficient (ICC)

$$ICC = \frac{\text{variation between subjects}}{\text{var(between)} + \text{var(within)}}$$

Illustration of potential added value from longitudinal biomarker

- Stored annual samples
- Could we have detected cases that occurred after start of year 3 using markers measured up to year 3?
- Single time point marker: M3
- Longitudinal marker: $\Delta 3 = M3 - ave(M1, M2)$

Low within-subject variation in controls (ICC=0.90)

Moderate within-subject variation (ICC=0.70)

High within-subject variation (ICC=0.30)

Recommendations

- Markers that show mild/moderate performance at a single time point
- Consider if there is potential for better performance with longitudinal ascertainment.
- Statistical considerations.
  - Do a preliminary study using serial samples from controls to assess ICC
  - This is cheap compared with larger study to assess biomarker performance for detecting disease in cases.
  - Assay variability.
- Feasibility considerations and complications
  - Will people return to provide serial samples?
  - Algorithms to accommodate missing time points.

Algorithms for using longitudinal biomarker data with real data in real applications

- Ovarian cancer Steven Skates
- Prostate cancer Yingye Zheng
- Liver cancer Mabihah Tayob

Appendix

Ovarian Cancer

Steve Skates, Ph.D., Massachusetts General Hospital

Longitudinal Biomarker Interpretation for Early Detection of Ovarian Cancer

Acknowledgements and Declaration of Interests

Outline

CA125
First trials for early detection of ovarian cancer
Risk of Ovarian Cancer given longitudinal CA125
Ovarian Cancer Screening Trials with ROCA
Longitudinal Test Results
Conclusions

Cancer Death Rates: (US 1930-2006)*

Ovarian Cancer Death Rates - Yellow

Ovarian Cancer – Early Detection Target?

Late stage detection: > 75%
Late stage 5 year survival rate: < 20%
Early stage 5 year survival rate: >85%
Annual Incidence in postmenopausal women: 40-50 per 100,000
(1 in 2,300)

CA 125 Levels in Patients with Epithelial Ovarian Carcinoma and in Various Control Groups

Early Detection with Serum Markers
PPV constraint – 10%
- Low incidence and moderate specificity may result in unacceptably low positive predictive value
- Ovarian Cancer Incidence: 1 in 2,000 women/year
- CA125 Specificity (> 30U/ml) : 98%
- CA125 Sensitivity : 63%
- Positive Predictive Value : 2%

Early Detection with Serum Markers
PPV constraint – 10%
- Low incidence and moderate specificity may result in unacceptably low positive predictive value
- Ovarian Cancer Incidence: 1 in 2,000 women/year
- CA125 Specificity (> 30U/ml) : 98%
- CA125 Sensitivity : 63%
- Positive Predictive Value : 2%

Ovarian Cancer Screening – Previous Studies
Screening for familial ovarian cancer: poor survival of BRCA1/2 related cancers.
J Med Genet. 2009;46:593-7.

Annual surveillance by CA125 and transvaginal ultrasound for ovarian cancer in both high-risk and population risk women is ineffective.
BJOG. 2007;114:1500-9.

Prospectively detected cancer in familial breast/ovarian cancer screening.
Acta Obstet Gynecol Scand. 1999;78:906-11.

CA125 and transvaginal ultrasound monitoring in high-risk women cannot prevent the diagnosis of advanced ovarian cancer.
Gynecol Oncol. 2006;100:20-6.

Biology of epithelial ovarian cancer: implications for screening women at high genetic risk.
J Clin Oncol. 2004;22:1315-27.

[NO] Effect of Screening on Ovarian Cancer Mortality: The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial
JAMA 2011; 305(22):2295-2303.

CA 125 II Assay levels in Ovarian Cancer vs Other Malignancies

CA125 in 3 women with occult ovarian cancer & 3 women without ovarian cancer

Probability or Risk of having Ovarian Cancer
The probability of having ovarian cancer given a CA125 profile accounts systematically for all sources of "signal" and "noise"
The initial baseline level
The variability of the baseline level between women
The slope over time following the change-point
The variability of the slope between women
The amount of rise above the baseline level
Assay and biological variability over time within a woman

Probability or Risk of having Ovarian Cancer
To calculate the probability of having ovarian cancer given a CA125 profile:

Develop longitudinal CA125 model for non-cases

Transform CA125 (log)
Individual constant mean (baseline)
Individual variation about baseline
Y_{ij} = log(CA125), ith patient, time tj
$Y_{ij} = \mu_i + \varepsilon_{ij}$ $\varepsilon_{ij} \sim N(0, \sigma_i^2)$ $\mu_i \sim N(\mu, \omega^2)$
$\omega \approx 2\sigma$. between woman SD = twice within woman SD

Probability or Risk of having Ovarian Cancer
To calculate the probability of having ovarian cancer given a CA125 profile:

Develop longitudinal CA125 model for cases
Baseline, change-point, linear increase in CA125 (fraction p)
Baseline (small proportion not producing CA125: 1-p)

$Y_{ij} = \mu_i + \gamma_i(t_{ij} - \tau_i) + + \varepsilon_{ij}$ $\varepsilon_{ij} \sim N(0, \sigma_i^2)$

$\mu_i \sim N(\mu, \omega^2)$; $\log(\gamma_i) \sim N(\gamma, \psi^2)$; $\delta_i \sim N(2, 0.752)\ T(0,5)$

$\delta_i = d_i - \tau_i$ preclinical duration

$(\mu_i, \log(\gamma_i), \delta_i) \sim MVN$

Probability of Having Ovarian Cancer
Red Dots: CA125 Values
Green Line: Flat Pattern
Yellow Line: Elbow Pattern

Initial odds: From woman's age

Z-values: Distance from Pattern to CA125 values. Smaller distance implies pattern is more likely.

Final Distance:
Sum of squared Z values
Divided by CA125 variability σ^*

Odds: Ratio of average "yellow" to average "green" distance

Final odds: (Initial odds) * (odds ratio)

Probability: Odds/(Odds+1)

ROCA: Risk of Ovarian Cancer Algorithm

ROCA Screening Trials
UK Pilot Trial (Barts III) 1995 – 2001
14,000 normal risk women randomized to screen/ no screen

ROCA study 2001 – 2015
2,400 high risk women, age > 30, CGN/SPORE/EDRN

UKCTOCS 2001 - 2015
200,000 normal risk, postmenopausal women randomized to screen/ no screen

MDACC study in normal 2001-2011
4,000 normal risk postmenopausal women

GOG0199 - screening arm 2003-2011
1,600 high risk women, age > 30

UKFOCSS 2003 – 2013
4,000 high risk women, age > 35

ROCA Screening Trials Results

All 6 trials significantly increased early stage cancer
No other ovarian cancer screening trials had an increase
All other ovarian cancer screening trials: single CA125 cutoff
Individualizing test with ROCA contributed to early stage increase
UKCTOCS had a non-significant Cox regression result for mortality for all cases
UKCTOCS had a pre-specified subgroup analysis for incident cases (80% of cases): a significant mortality reduction

Conclusions
Longitudinal test relies on each patient having their own baseline levels of a test (sigma-within << sigma-between)
Longitudinal interpretation of test for each patient led to significant increase in detection of early stage disease
Longitudinal test most effective when baseline has been measured (incident cases)
Longitudinal test reduced mortality for incident cases
Longitudinal method applied to existing or novel biomarkers may substantially increase impact

Prostate Cancer
Yingye Zheng, Ph.D., Fred Hutchinson Cancer Research Center
Modeling Prostate Specific Antigen (PSA) Kinetics in Prostate Cancer Active Surveillance
September 14, 2017

On behalf of PASS study

PSA in Prostate Cancer Active Surveillance
- AS: a preferred strategy for low-risk disease
- Variations in implementation
- Enrollment, followup intervals and trigger for intervention
- AS protocols are based on re-biopsy and repeated PSA
- Question: How to collect and interpret serial PSA data optimally in the AS setting?
- Implication in biomarker studies

Canary Prostate Cancer Surveillance Study (PASS)
Changes of PSA Over Time in PASS

Research Questions
- Does change in PSA predict time to outcome?
- Reclassification: an increase in Gleason grade, or tumor volume.
- Determine the extent to which PSA kinetics might facilitate improved decision-making for men on surveillance
- Is there a lower threshold for PSAv, where most participants can move to watchful waiting?
- Is there an upper threshold, where most participants will need treatment?

Statistical Models
- To study longitudinal PSA measurements as predictors of reclassification, a two-stage procedure was used in the statistical modeling
- I: Derived features from longitudinal data (e.g., PSA kinetic parameters), which we treated like a putative biomarker
- II: Partly conditional Regression models with longitudinal features (e.g., PSAk) as predictors, predicting the residual time at a landmark time.

Zheng & Heagerty 2004, Marziaz et al., 2017

I. Deriving PSA Kinetics
PSA in Pca Active Surveillance
- Mixed messages:
- Many studies have not looked at PSA kinetics in Active Surveillance (AS)

- PSA screening
- Biochemical Recurrence
- Some studies have found PSA velocity and PSA doubling time to be significant in AS*, while others have not^
- Significant predictor vs. improving model accuracy

* Al Otaibi (2008); Bul (2012); Khatami (2006); Klotz (2010); Kotb (2011); Ng (2008); Zhang (2006)
^ Iremashvili—weak association (2012); Ross (2010); Whitson (2011)

PSA kinetics in the PCa Literature

- PSA velocity (PSAv): rate of rise in PSA
- Simple calculations: slope between two points, or slope from linear regression
- Robust calculations: linear mixed models, joint modeling
- Thompson (2006): 20 definitions for evaluating PSAv
- PSA Doubling Time:
- months it would take for PSA to increase two-fold.
- Calculated from PSA velocity
- If negative PSAv, will need to consider categorizing PSAdt
- There are many different ways to measure PSA kinetics—give quite divergent results

PSA velocity calculation in PASS

- Simple PSAv (PSAvS):
- Used all available PSAs prior to time of interest. At least 2 PSAs required.
- If 2 PSA measurements: change in PSA / change in time
- If 3+ PSA measurements: slope from linear reg. model
- Restricted Simple PSAv (PSAvRS):
- Used 2 PSAs closest to and prior time of interest
- Change in PSA / change in time
- Short term changes, independent of enrollment length

PSA velocity calculation

- BLUP PSAv (PSAvBLUP):
-
- For a new patient with repeated PSA measured up to sij, Best Linear Unbiased Predictors (BLUPs), with

at each time sij was calculated using only past and current PSA measurements PSA(s0, …, sij)

- BLUP PSAv Restricted (PSAvBLUP_R):
- Similar to PSAvBLUP, limited to PSA measurements within 2 years prior of time point

II. Predicting Outcome with longitudinal PSA Kinetics

Modeling Longitudinal Data

Statistical Models

- For time-varying PSA within subject: Cox PH model
- Outcome was time from each PSA measurement to grade and/or tumor volume reclassification, or censor
- Logged measurement time s as predictor
- with robust variance estimates
- Covariates:
- Dx PSA, measurement time s, PSAv at s
- Dx age, prostate size, prior biopsy (most recent percent of biopsy cores involved, history of any negative biopsy)

Subject Inclusions

- Data were from the 2016 PASS data freeze, and the following exclusions were applied:

PSAvS vs PSAvBLUP

Results from Cox models

PSAvBLUP extremes

ROC Curve Analysis
ROC Curves from Cox PH model with diagnostic PSA plus PSAk using 3 or 6 month PSAs, prediction of reclassification event at 3 years since diagnosis. Prediction was made from 1 year after diagnosis.

Summary
- Does PSAv predict time to reclassification (grade/tumor volume) in PASS?
- Yes, with adequate measurement
- Issues with compliance
- Determine the extent to which PSA kinetics might facilitate improved decision-making for men on surveillance for low-risk prostate cancer
- need to combine with other clinical variables (and novel biomarkers) for decision making

Acknowledgment
- Anna Faino
- Lisa Newcomb
- Dan Lin
- Matt Cooperberg
- Jim Brooks
- Other PASS investigators

Hepatocellular Carcinoma

Nabihah Tayob, Ph.D., The University of Texas MD Anderson Cancer Center

Improved early detection of hepatocellular carcinoma with longitudinal biomarker screening algorithms

Early Detection of Hepatocellular Carcinoma?
Hepatocellular carcinoma (HCC) has five-year survival
<12%.
Early (or very early) stage HCC:
→ Surgical resection, liver transplantation and loco-regional therapies.
→ 5-year survival is 47-84%
Later stage HCC (symptomatic):
→ 5-year survival is <10%
Key to reducing HCC mortality: early detection.
Key risk factor is cirrhosis: accounts for 80-90% of HCC cases.
→ Hepatitis B and C infection
→ Alcoholic liver disease
→ Non-alcoholic fatty liver disease

AASLD guidelines: screening every 6 months via
Ultrasonography ← mandatory
α-Fetoprotein (AFP)← optional but common
Positive screen → CT or MRI

Current Surveillance Practice
Problems with ultrasonography:
Operator dependent
Difficult to perform in obese patients
→ Sensitivity for early stage HCC in clinical practice: 32%

Serum AFP is a diagnostic biomarker that is widely used in screening.

Sensitivity 41-100%
Specificity 70-90%

Incorporating longitudinal AFP
Lee et al (2013): standard deviation and rate of increase of AFP vs current AFP.
White et al (2015): current AFP and rate of increase in AFP within the last year vs current AFP.
McIntosh and Urban (2003): parametric empirical Bayes (PEB) approach.
Skates, Pauler and Jacobs (2001): posterior risk from Bayesian hierarchical changepoint model.

HALT-C Trial
HALT-C trial design
Enrollment criteria:
Chronic hepatitis C.
Stage 3 fibrosis (Advanced fibrosis or cirrhosis). Radiological imaging to exclude HCC.
History of non-response to interferon-based therapy.
Randomization: long-term low-dose pegylated interferon therapy v.s. no treatment
Follow-up schedule:

HALT-C Trial

Parametric Empirical Bayes (PEB) Method:
Has a deviation in the biomarker trajectory for a particular patient occurred?
Specify a model for the biomarker in control parients.
Parameters are estimated using control patients only.

PEB threshold:
Suppose a patient has completed n screenings.
Compare current AFP to a individually tailored threshold for that patient.
Estimate mean AFP level for patient:

PEB estimate = Weight 1 × population mean
+ Weight 2 × sample mean

PEB Rule: Y_{n+1} > PEB estimate+c1×SD of PEB estimate
Standard Threshold (ST) Rule: Y_{n+1} > c2
Select c1 and c2 to control the false positive rate.

PEB threshold:
Suppose a patient has completed n screenings.
Compare current AFP to a individually tailored threshold for that patient.
Estimate mean AFP level for patient:

PEB estimate = Weight 1 × population mean
+ Weight 2 × sample mean

PEB Rule: Y_{n+1} > PEB estimate+c1×SD of PEB estimate
Standard Threshold (ST) Rule: Y_{n+1} > c2
Select c1 and c2 to control the false positive rate.

Evaluating the screening algorithms
False positive rate (FPR): proportion of positive screens among all the screenings conducted in the control group.

Specificity=1-FPR.

True positive rate (TPR)/Sensitivity: proportion of HCC cases with at least one positive screening during the pre-diagnostic period.

ROC results: cirrhosis at baseline

ROC results: cirrhosis at baseline

Summary: PEB method
Incorporating AFP screening history
→ patient specific thresholds
→ higher sensitivity

AFP does not increase in all HCC cases.
→ Incorporate additional biomarkers into a longitudinal screening algorithm to identify all the disease subtypes.

Incorporating longitudinal DCP
Des-gamma carboxy-prothrombin (DCP) is a blood-based biomarker that is part of the FDA approved triplicate.
Previous generation assay for lectin-bound AFP (AFP-L3%) only.
In the HALT-C Trial:

Bayesian screening algorithm
Our goals:
→ Exploit all the available information in multiple longitudinal biomarkers.
→ Robust and computationally efficient.
→ Unevenly spaced biomarker measures.
→ Missing data.

Decision rule: posterior risk
→ Specify a joint model for biomarkers in cases and controls.
→ Prior probability of disease: target population for surveillance.

Decision rule: posterior risk
Decision rule: posterior risk

Hierarchical model for biomarker level in controls

Hierarchical model for biomarker level in cases
We define an unobserved Iik to distinguish between two possible models for the kth marker in cases.
If Iik = 0

Hierarchical model for biomarker level in cases

Connecting the biomarker models
The indicator variables follow a Markov Random Field
distribution.
What does this mean?
→ The probability that we have observed a changepoint in one biomarker depends on whether we have observed changepoints in other biomarkers.
→ This allows us to borrow information across the biomarkers to detect changepoints.

Calculating the posterior risk
A Markov chain Monte Carlo (MCMC) procedure is constructed to sample from the joint posterior distribution for all the model parameters.
→ Gibbs sampler when full conditionals are easily computed.
→ Metropolis-Hasting algorithm when full conditionals are not straightforward.
→ Reversible jump procedure (Green, 1995) when the dimension of the parameter space depends on Iik .
Monte Carlo integration is used to get estimates of posterior predictive probabilities.
Calculate posterior risk at each screening visit

Results from HALT-C Trial
Apply screening algorithm with log(AFP) and log(DCP + 1) in cirrhosis patients.
Ideal study:

→ Training dataset: obtain samples from joint posterior distribution for all the model parameters.
→ Testing dataset: Estimate the posterior risk in each patient at each screening visit and evaluate performance of the proposed screening algorithm.
Use 10-fold cross validation to evaluate the proposed screening algorithm.

Screening algorithms:
mFB: multivariate fully Bayesian screening algorithm
uFB: univariate fully Bayesian screening algorithm (Skates et al, 2001)
uEB: univariate parametric empirical Bayesian screening algorithm (McIntosh and Urban, 2003)
ROC results

Early detection of HCC

Entire screening period

One year prior to clinical diagnosis

Applying the PEB algorithm in a VA HCV cohort
HALT-C is a randomized clinical trial with 3 month surveillance windows.
AFP measured at local laboratories for each University hospital.
How does PEB algorithm perform in a regular clinical setting?
Department of Veterans Affairs (VA) is the largest integrated health-care provider in the United States.
Important setting to study the PEB algorithm for AFP.

VA HCV cohort:
12,124 patients with cirrhosis diagnosed between 1997 and 2005 and at least one AFP test.
11,222 with no HCC ever 902 with HCC eventually
Use IDC-9 diagnostic codes to identify patients with cirrhosis.
HCC diagnosis first identified with IDC-9 codes and confirmed with manual review.
AFP pulled from the laboratory records at the VA.

Results: HALT-C vs VA

Results: HALT-C vs VA

Results: HALT-C vs VA

PEB Model Parameters

PEB Model Parameters

When to use longitudinal biomarkers?
Factors to consider:
Variability of biomarkers affects the performance of PEB algorithm.
Slow increases in the biomarker may affect performance of PEB algorithm.
→ Small increases below the threshold are not detected.
→ At next screen the PEB threshold is adjusted upwards.

Current and Future work
Multiple ongoing studies to further examine longitudinal biomarker screening algorithms for early detection of HCC.
→ 2010-2015 VA cohort with multiple etiologies of cirrhosis
→ Hepatocellular carcinoma Early Detection Strategy Study
→ Texas Hepatocellular Carcinoma Consortium
Methodological challenges → practical implications for clinical practice.
Parametric empirical Bayes screening v.s. fully Bayesian screening.
Incorporate covariates that can explain variability/trajectory of AFP in the absence of HCC.
Other etiologies of liver disease?
Implementation in clinical practice → develop software.

Acknowledgments

Thank you!

Reports from Collaborative Groups
Session Moderator: Guillermo Marquez, Ph.D., National Cancer Institute

Colon and Esophageal Cancers
Robert Schoen, M.D., M.P.H., University of Pittsburgh
GI Collaborative Group
Colorectal Cancer Projects
Ken Kinzler
Rocky Schoen
Sandy Markowitz
Bill Grady
Kishore Guda
Paul Lampe
Bob Bresalier

PROJECT 1: Plasma Protein AA or CRC Detection Markers
Study 1 - Determine whether 6 specified plasma proteomic biomarkers developed by the GI collaborative and markers contributed by companies predict AA and/or CRC and compare the panel's performance to FIT.

Aim 1. To define sensitivity and specificity of specified plasma biomarkers for the detection of AA and/or CRC with samples that had positive and negative FIT values.
Aim 2. To develop a combination rule for the markers comparing it with FIT and testing whether it makes the 75% sensitivity and ≥70% specificity for CRCs indicated for GLNE10 usage. We will use the minimum number of markers necessary and test combinations with and without FIT to determine the most viable panel. If the performance is not sufficient, we will add/subtract other markers from the GI collaborative group to enhance performance.

Future: If successful. we will test the fixed, combination rule using samples from GLNE10.

Samples
Collected in Denmark from individuals undergoing CRC screening; age 50 - 74 years, with either a positive FIT* test (8,415 individuals) or a negative FIT test (5,112 individuals).

100 FIT-negative with no CRC diagnosed within 2 years post draw
100 FIT-positive no CRC by colonoscopy
100 FIT-positive advanced adenoma
100 FIT-positive CRC (25 stage I, 25 stage II, 25 stage III, 25 stage IV)
100 FIT-negative CRC (25 stage I, 25 stage II, 25 stage III, 25 stage IV)

*SENSA FIT

Biomarker Candidates
Galectin-3 ligand
BAG4
IL6ST
VWF
EGFR
CD44
The sialyl Lewis-A and –X content for the latter two proteins
Abbott assayed proteins
Clinical Genomics methylated genes
Volition America Nu.Q test

PROJECT 2: CRC Diagnostic and Recurrence Early Detection Markers
GOAL:

- To evaluate established panels of ctDNA and aberrantly methylated DNA markers in a common, plasma-based reference set, under a uniform, structured protocol of collection and processing.

STUDY AIMS

- Aim 1. Test the hypothesis that established panels of ctDNA and aberrantly methylated genes used together as a primary screening modality for detection of colorectal cancer will be more sensitive and specific than either panel of markers alone. We will test this hypothesis first in Stage III and IV CRC, then in Stage I and II CRC.
- Aim 2. Test the hypothesis that established panels of ctDNA levels and aberrantly methylated genes will be better for monitoring of CRC patients with regional metastatic disease (stage III) than either panel of markers alone.

PROJECT 2: CRC Diagnostic and Recurrence Early Detection Markers

- A. Samples: Plasma samples collected by UPMC (Rocky Schoen) and Case (S. Markowitz) in stage II-IV (R0) CRC patients and screening colonoscopy control subjects
    - Study 1: Diagnostic test: Case vs. Control design
    - Study 2: Stage III CRC recurrence detection marker:
- B. Markers:
- 1) Circulating mutant DNA (Kinzler)
- 2) Circulating methylated genes:
    - Markowitz, Grady (CWRU, FHCRC): mVIM, mITGA4
    - Claus Anderson (Arhus)
    - Larry Lapointe (Clinical Genomics]
- 3) Protein based markers (Lampe, Bresalier)

NEXT STEPS for PROJECTS 1 and 2

- PROJECT 1: Serum/Plasma Diagnostic markers
    - Validation/Verification in Danish Cohort
- PROJECT 2: Circulating DNA markers
    - Assess candidate methylated DNA markers
- Clinical Genomics and Arhaus markers
- Methylated VIM and ITGA4
- Cross-lab validation
    - Obtain samples from cases and controls for diagnostic markers study
    - Accrue serial plasma samples from stage III CRC patients

<u>Pancreatic Cancer</u>

Aniban Maitra, M.B.B.S., The University of Texas MD Anderson Cancer Center

A Assess the science
C Critique progress
T Timeline for project
I Implementation of the project
O Outcome expected
N New or continuing

A Assess the science

Natural history of cystic neoplasia in the pancreas
Caveat: Vast majority (>90%) of mucinous cysts are not going to progress to pancreatic cancer

Challenge: Identify the minority with aggressive biology using biomarkers that will predict which patients need surgery

New Onset Diabetes (NOD) as a Biomarker of Asymptoamtic Pancreatic Cancer

Up to ~50% of pancreatic cancer patients may have new-onset diabetes (NOD) as their first symptom

Can pre-date cancer diagnosis by 2 years

~1% of NOD have occult PDAC
Chari et al, Mayo

It's a good time to be a pancreatic cancer researcher!
EDRN
1st time has two Clinical Validation Centers (CVCs) in PDAC
UPMC and MD Anderson Cancer Center
Biomarkers of Preclinical Pancreatic Cancer (BPPC) ("Cyst Bakeoff")

Molecular Characterization Laboratory (MCL) ("Overdiagnosis consortium")
Pancreatic cancer MCL (MD Anderson Cancer Center + partners)

Consortium for Chronic Pancreatitis, Diabetes and Pancreatic Cancer (CPDPC)
10 consortium sites, MD Anderson Coordinating Center
"NOD" Cohort (JoAnn Rinaudo)

… but that's not all
Pancreatic Cancer Detection Consortium (PCDC)
Goel / Von Hoff (Baylor/Translational Genomics Research Institute)
Goggins (Johns Hopkins)
Hollingsworth (U Nebraska Medical Center)
Petersen/Zaret (Mayo Clinic/U Penn)
Sutcliffe (UC Davis)
Willmann/Park (Stanford)
Wolpin (Dana Farber)
RFA is still open!

Why do we need a biomarker bake off?

EDRN Pancreas Collaborative Group Bakeoff
"Biomarkers of Resectable Pancreatic Cancer" (BRPC)
Directed by JoAnn Rinaudo (NCI)
Blinded cases and control samples (N = ~180)
Two CVC sites (UPMC and MDACC, with assist from Mayo Clinic)
Centralized study design and statistical analysis at DMCC (Ying Huang)
Participating laboratories:

Brian Haab	VARI
Surinder Batra	UNMC
Sam Hanash	MDACC
Anna Lokshin	UPMC

Goal: A curated biomarker panel that can make the case for access to pre-diagnostic samples such as NOD cohort.

EDRN Pancreatic Cyst Bakeoff
"Biomarkers of Preclinical Pancreatic Cancer" (BPPC)
Led by Aatur Singhi and Randy Brand (UPMC)

Blinded cases and control samples
Intent 1: Distinguish IPMN from other cysts (N= 276)
Intent 2: Distinguish high grade from low grade dysplasia (N = 209)
Centralized study design and statistical analysis at DMCC (Ying Huang)
Participating laboratories:

Aatur Singhi	UPMC	Genomic markers
Brian Haab	VARI	Mucins (MUC5AC, endorepellin)

Walter Park	Stanford	Amphiregulin
Charles Craik	UCSF	Gastricsin/Cathepsin
Michael Goggins	JHU	Methylation markers, telomerase
Koushik Das	WUSTL	Das-1

Goal: A curated biomarker panel that can be tested in prospective cyst cohorts (CVC and MCL)

C Critique progress

BRPC

Samples have been distributed to participating sites

Many thanks to DMCC team for blinding!

BPPC

Cyst fluid samples are being sent to UPMC for blinding and distribution

T Timeline for project

BRPC

Samples will be analyzed by January 31, 2018

Ying to complete statistical analysis in time for March 2018 meeting

Phase 2 will include access to EDRN Reference set (including new prospective samples being collected by CVC and PCDC sites)

BPPC

Two year timeline

I Implementation of the project

DMCC Blinding has been crucial

Implementation being monitored closely by JoAnn Rinaudo (BRPC) and Matt Young (BPPPC)

Currently on course for stated timelines

O Outcome expected

BRPC = access to new "reference set" samples being collected by CVCs and PCDCs, as well as support for access to pre-diagnostic PLCO, WHI, EPIC samples.

BPPC = access to longitudinal cyst fluid samples collected by CVCs and PCDCs

N New or continuing

New EDRN reference set being built by the CVC and PCDC sites

Samples will be tied to imaging data (assistance from JPL)

Potentially pathology data as well for resected cases

Eventual goal: Integrate multi-omics tissue based parameters with biomarkers and imaging

Breast Cancer

Christopher Li, M.D., Ph.D., Fred Hutchinson Cancer Research Center

Breast Cancer Collaborative Group Update on Team Projects

- Identification of biomarkers for ER+ breast cancer (Lead: Christopher Li)
- Breast cancer imaging and blood biomarkers (Leads: Jeffrey Marks and John Heine)
- Avatar mice-to-human breast cancer biomarkers (Leads: Amanda Paulovich and Karin Rodland)

Breast Cancer Collaborative Group Update on Team Projects

- Identification of biomarkers for ER+ breast cancer (Lead: Christopher Li)
- Breast cancer imaging and blood biomarkers (Leads: Jeffrey Marks and John Heine)
- Avatar mice-to-human breast cancer biomarkers (Leads: Amanda Paulovich and Karin Rodland)

Potential Clinical Applications

- Biomarkers to inform timing of subsequent screening in women who have had a negative mammogram:

Goal: Identify markers that inform whether subsequent mammogram should occur in 6 months (early recall), 1 year, or 2 years

- Biomarkers to inform continuation of mammographic screening among women 75-79 years of age:

Goal: Identify women who have a 2-fold higher risk of developing breast cancer who may benefit from opting in for screening.

- Identify high risk women who should be prioritized for screening in areas with limited resources/access to mammography

Goal: Identify a subset of women 50-69 with a 2-fold higher risk of breast cancer who would particularly benefit from screening.

Work Flow

Timeline and Milestones (approved 3/2017)

Breast Cancer Collaborative Group Update on Team Projects

- Identification of biomarkers for ER+ breast cancer (Lead: Christopher Li)
- Breast cancer imaging and blood biomarkers (Leads: Jeffrey Marks and John Heine)
- Avatar mice-to-human breast cancer biomarkers (Leads: Amanda Paulovich and Karin Rodland)

Purpose and Goal

- Breast cancer biopsy is performed routinely on a large number of women who have a low probability of cancer
- Goal is to improve predictive accuracy of mammography towards reducing the rate of biopsy by combining image analysis and blood based biomarkers
- Trying to achieve 98% NPV
- In conjunction with this project, the intent is to create a repository of images and matched blood for future studies

Cohorts

- Retrospective cohort of ~1000 images/blood (partially linked to existing breast cancer reference set at NCI-Frederick)
    - Mostly Duke subjects given the blood repository
    - Exclusively full field digital mammography (FFDM)
    - Among other purposes, training of image analysis
- Prospective cohort of ~1000 images/blood
    - Moffitt and Duke – both FFDM and tomosynthesis
    - CAML detection (Creatv MicroTech) can only be performed on freshly collected blood

Progress to Date
Began Prospective Accrual at Duke in February
Almost exclusively BI-RADS 4A and 4B
110 enrolled and analyzed for CAMLs
Immunosignaturing performed on Stage I and benign conditions
330K peptide array
Training and validation paradigm
Multi-institutional training cohorts
Independent analysis by ASU and IBM
Blinded validation underway
Moffitt IRB approved and began prospective accrual recently
Four-way discussion for image storage and annotation
Test images will be delivered to JPL this week

Breast Cancer Collaborative Group Update on Team Projects

- Identification of biomarkers for ER+ breast cancer (Lead: Christopher Li)
- Breast cancer imaging and blood biomarkers (Leads: Jeffrey Marks and John Heine)
- Avatar mice-to-human breast cancer biomarkers (Leads: Amanda Paulovich and Karin Rodland)

Our clinical goal is to identify a blood-based biomarker that could be used in conjunction with mammography to improve sensitivity and specificity
Limitations of Mammography
Specificity of mammography for distinguishing benign vs. cancerous lesions
Sensitivity of mammography in some patient populations (e.g. dense breasts)
Over-diagnosis (e.g. some DCIS).
This hypothetical biomarker would be impactful by detecting treatable cancers missed by mammography (mortality), and by allowing patients to avoid unnecessary procedures for benign disease (morbidity).

Human proteins are present in the plasma of mice harboring breast cancer xenografts.
We hypothesize that a subset of the human tumor-derived proteins in the plasma of xenografted mice will be elevated in the plasma of women with breast cancer, and that a subset of these will be useful for early detection.

We pooled & analyzed the unlabeled, double-depleted plasma samples from 9 PDX mice for LC-MS/MS analysis.
Data were stringently filtered to remove peptides mapping to spectra that gave IDs in both the mouse and human databases.

Although candidate proteins were enriched in breast cancer, none was observed exclusively in breast cancer.
74% of circulating proteins were seen in all CPTAC breast tumor samples, while only 25% were seen in all ovarian tumors, and 17% in all colon tumors.

Next steps
Perform additional due diligence to ensure these are really human proteins; consider analyzing normal mouse plasma.
Attempt to replicate the results with an independent set of PDX mice.
Consider enriching EVs from PDX and human plasmas for proteomic analyses.
AIMS analyses to check for candidates in the plasma of human breast cancer patients.
Further work on clarifying potential clinical applications and design of subsequent human validation studies.

Ovarian Cancer
Steven Skates, Ph.D., Massachusetts General Hospital
Ovarian Cancer Team Projects Current and Previous
Previous
Validation study for serum protein biomarkers for early detection of OC
(PI: Skates)
Current Team Projects
Serum Autoantibodies as early detection for OC (PI: Bast)
tDNA in Uterine Lavage and Serum Protein Biomarkers in early detection of ovarian cancer (PI: Skates)

Ovarian Validation Study – Early Detection Modified design
150+ ovarian cancer candidates discovered in previous round of funding

Circulating Autoantibody Biomarkers for Early Detection of Ovarian Cancer
This TEAM project proposes a study from autoantibody candidate biomarkers by EDRN investigators
Robert Bast, Karen Lu (MDACC)
Karen Anderson (ASU)
Charles Drescher (FHCRC)
Steven Skates (MGH)
Zhen Zhang (JHMI)

A study on the early detection of Ovarian Cancer (OC) with candidate autoantibody markers identified in previous and ongoing EDRN research that demonstrate signal in at least early phase II studies.

Clinical Aim: Early detection of OC using a regular blood test
Target Population(s): Normal risk postmenopausal women

Biospecimen Sources:
- Samples obtained at diagnosis and matched controls (Bast, Drescher)
- Longitudinal preclinical samples from local ovarian cancer screening trials (NROSS, Ovarian Cancer Early Detection Program (OCEDP)
- Preclinical samples from large cohort studies (WHI, PLCO, UKCTOCS)

Biomarker Candidates:
- Anderson (ASU) – 16 candidates
- Drescher (FHCRC) – 5 candidates currently with 25 total expected by year 2
- Bast (MDACC) – 4 candidates currently with 10 total expected by years 2-3.

Milestones:
1) Assemble the necessary serum specimens.
2) Complete a panel of potential AAb biomarkers.
3) Estimate the markers' potential for early detection, as measured by sensitivity up to one year prior to diagnosis at a given high specificity, individually and in combination.
4) Place promising assays on a standard platform.
5) Develop a new algorithm that includes CA125 and multiple AAb biomarkers.

tDNA in Uterine Lavage and Serum Protein Biomarkers in Early Detection of Ovarian Cancer

Specific Aims
- Establish a biorepository of pre-operative uterine lavage biospecimens and serum, plasma, and leukocyte DNA from 200 women scheduled for surgery for potential ovarian cancer
- Determine sensitivity of TP53 mutation detection at high specificity with Duplex Sequencing in uterine lavage biospecimens
- Determine sensitivity of mutation detection in an 18 gene panel at high specificity with Haloplex Sequencing in uterine lavage biospecimens
- Identify optimal classifiers and panels of gene mutations in uterine lavage and serum proteins (close to maximum sensitivity at high specificity) and in the process reveal any complementarity of the two types of biomarkers.

Design
N= 200 – Four accrual sites, 50 patients from each site
Eligibility:
- Age > 50, postmenopausal
- Suspicious for ovarian cancer
- rising CA125 (e.g. ROCA)
- Image abnormalities (U Kentucky or KP TVS criteria)
- Presence of ascites
- Investigator judgment: clinical characteristics - prob(OVCA) > 25%
- Known BRCA carriers with suspicious mass (age > 30)

Samples
Blood – obtained prior to anesthesia, ideally at clinic visit prior to day of surgery
- Serum
- Plasma (or cfDNA via Qiagen tubes)
- Leukocytes (buffy coat - gel separator tube)

Uterine Lavage – after general anesthesia, prior to surgery
TAO endometrial brush – optional, but at clinic visit with 7 days minimum prior to day of surgery (repair endometrial epithelium)

Sequencing
Duplex Sequencing: TP53 Twinstrands (lavage & leukocyte DNA)

- can detect one cancer mutation amongst 24,000 normal genomes

Haloplex Sequencing: McGill University (lavage & cfDNA & tumor)
- can detect cancer DNA down to a frequency of 0.1%
- 4651 amplicons to cover all exons in: ARID1, AKT1, APC, CDKN2A, CTNNB1, FBXW7, FGFR2, KRAS, NRAS, PIK3CA, PIK3R1, PPP2R1A, PTEN, RNF43, BRCA1, BRCA2, MLH1, MSH2, TP53, 9 microsatellites, and mutational hotspots of EGFR, BRAF, POLE and MAPK1 (65.kb total).

Serum Assays
JHU: Best 4 analytes on MSD platform
MDACC: CA125 & HE4 – clinical grade assay (Roche)

Central repository: NCI Frederick
Distribution for:
- serum aliquot to MDACC and JHU
- Uterine Lavage DNA to Twinstrands and McGill
- cfDNA and tumor to McGill
- Leukocyte DNA to Twinstrands

Processing, aliquoting, labelling for long term biobank for reference set

Lung Cancer
Pierre Massion, M.D., Vanderbilt - Ingram Cancer Center
Lung Collaborative Group

EDRN Lung collaborative group

Collaborative group project LTP2
- Assess the science
- Critique the progress
- Timeline for progress
- Implementation for the project
- Outcome expected
- New or continuing

LTP2: Molecular Biomarkers for the Early Detection of Lung Cancer in the setting of Indeterminate Pulmonary Nodules
GOAL: Collaboration among 7 EDRN funded sites across country to improve the diagnostic evaluation of patients with indeterminate pulmonary nodules by determining whether molecular biomarkers for lung cancer diagnosis that are measured in minimally invasive and non-invasive biospecimens are able to distinguish between patients with malignant or benign indeterminate pulmonary nodules that are incidentally detected on CT scan in high risk smokers.

AIM 1: Establish Cohort (n=300)
- Establish a cohort of current and former smokers with indeterminate pulmonary nodules (6-25mm) on whom clinical, radiographic and biospecimen repositories are developed and who are followed prospectively (up to 2 yrs) until final diagnosis.

Clinical, Imaging and Biospecimen Collections

AIM 2: Validate Molecular Biomarkers
- Validate the diagnostic accuracy of existing lung cancer biomarkers in this indeterminate pulmonary nodule cohort (100 cancer vs. 100 no cancer).

Non-invasive biomarkers that discriminate between patients with and without lung cancer could facilitate early treatment and thereby improve lung cancer outcomes, while minimizing complications from diagnostic procedures in patients without lung cancer.

Vanderbilt Lung Imaging Repository
Medical image Analysis and Statistical Interpretation lab

Bennett Landman, PhD

Plans For The EDRN Lung Team Project 3 (LTP3)….and something new

Harvey I. Pass MD

EDRN Meeting, Seattle, September 2017

Disclosure

- All of these specimens were collected at NYU with funding solely from the EDRN

Plans for the LTP3

The specimens for the LTP3 project have been collected and archived under an IRB approved protocol (8896) from September 2006 to June 2015.

The specimens represent 361 patients suspected of having Clinical Stage I-IB lung cancer by the Stage Grouping for the 8th Edition of the TNM classification for Lung Cancer. Essentially, preoperative workup in all patients included CT scanning and integrated PETCT.

Any patient having endobronchial ultrasound or mediastinoscopy prior to resection was confirmed to have non-involved lymph nodes that were sampled.

Of the 361 patients, 27 were found to have N1 disease. No patient in this series had N2 (mediastinal) nodal involvement.

Available Specimens

All of the patients in the series have matching EDTA plasma and Serum.

24 lack CPT plasma, 10 lack buffy coat, and 21 lack PBMC.

Of the 244 with at least one vial of snap frozen tumor, 238 have matching snap frozen lung tissue.

Total RNA from the above 238 matching specimens will be extracted in the NYU Biomarker Discovery Laboratory

As part of the materials which will be available to the investigators, a TMA of all 361 patients, both tumor and normal, will be available (Spring 2018).

150 of the 361 patients apparently have matching high resolution CT scans performed at NYU Radiology Department.

Available Demographics

- Pleural Invasion
- LVI
- Size
- Number of Lymph nodes resected
- Number of lymph nodes positive
- TNM stage
- Stage Grouping
- Adjuvant therapy
- Date Follow-up
- Date of Recurrence
- Type of Recurrence
- Date Death
- Intervals
  - Time to progression, Time to Death, Time to Followup
- Record_ID
- Extent_of_Resection
- IASLC Adenocarcinoma Classification
- Mayo_classification
- Smoking_Status
- Pack years
- Date of Surgery
- Gender
- Ethnicity

- Age
- Number of Tumors resected
- Histology
- VATS vs Open

Demographics of Non Progessors and Progressors

Progression: Clinical Stage I by pStage IASLC 8

Survival : Clinical Stage I

Survival: Clinical Stage I by pStage IASLC 8

Growth pattern-based grading of pulmonary adenocarcinoma – Analysis of 534 cases with comparison between observers and survival analysis

Adenocarcinoma Mayo Classification: Clinical Stage I Can it be beat????

Nanostring Immune-Oncology Panel
770 gene panel which measures gene expression of human immune response in
all cancer types
Combines markers for 24 different immune cell types and populations including 109 genes to cell surface markers for 24 different immune cell types and populations, 30 common cancer antigens and genes that represent all categories of immune response including key checkpoint blockade genes.

Nanostring ImmunoOncology Panel

Prognostic Comparisons – Overview (102 patients)

Differential Expression – Type of Progression

Immune Cell Profiling – Type of Progression

DE – Progressed (28) vs. Not Progressed (66)

Immune Cell Profiling – Progressed (28) vs. Not Progressed (66)

Batch Effect -- Unsupervised Clustering of Normalized Data (12 replicates)
Samples cluster by RLF (i.e. CodeSet lot) rather than by patient ID (i.e same RNA), suggesting a lot-to-lot batch effect
CodeSet lot-to-lot variation (i.e. probe specific efficiency difference) is expected due to the manufacturing process
A calibration process can mitigate the lot-to-lot variation if data from different CodeSet lots need to be combined
Technical replicates run on both lots of CodeSet can be used for calibration

Thanks
- Alan Vaynblatt
- Ki Prokrym
- Nathalie Hirsch
- Ryan Harrington
- Brady Kwong
- Weiming Pai MD
- Chandra Goparaju PhD

Questions?

Prostate Cancer
Martin Sanda, M.D., Emory University
Prostate Collaborative Group - Summary
Assess science:

Critique progress:
Timeline:
Implementation plan:
Outcome expected:
New or ongoing project:
…and…
Synergy:

Michigan (S Tomlins, A Chinnayian):
lncRNA in tissue & urine - Status

- Identified novel lncRNAs associated with aggressive prostate cancer
  - Near saturated knowledge
- Validated performance of a tissue RNAseq assay capable of assessing all classes of transcriptomic biomarkers
  - Preparing for assessment of prognostic utility in context of use
- Standardized performance of a urine RNAseq assay capable of multiplex transcriptomic biomarker quantification
  - Need to develop algorithm to predict high grade cancer on biopsy
  - Assess diagnostic utility in context of use

Emory: Urine Extracellular Vesicle RNA Assay
Assess science:
Targeted RNAseq assay (PreCise) measuring 260 genes
Critique progress:
Pilot experiments to set up the assay have been completed, increased sample eval to enable Comparison of low and high risk prostate cancer is pending
Timeline:
Interim analysis of training set expected by the end of 2017
Implementation plan:
~200 samples ready to run to establish candidate signature in CLIA;
finalize collaborative agreement with industry partner
Outcome expected:
Development of transcriptome signature to discern GG1 vs aggressive prostate ca
New or ongoing project:
Ongoing project, new technique and targets

S. Liu (Toronto) Collaborations in EDRN

- Paul Boutros – urinary microRNA signature
  - Ready for validation in active surveillance cohort
- Thomas Kislinger – urinary proteomic signature
  - Discovery phase
- Tao Liu – urinary proteomic signature
  - Early validation phase

PNNL Team project (T Liu): Selection of candidate biomarkers for aggressive prostate cancer based on targeted proteomics

* Clinical Need: Ability to identify, at the initial biopsy, low risk prostate cancer patients who are candidates for active surveillance versus aggressive treatment
* Using curated literature, PNNL & CPDR selected 52 candidate biomarkers from genomic studies
- Proof of principle pilot study compared prostate cancer (GS 7-9) to benign prostatic hypertrophy (n=10), using OCT-embedded tissue samples from UTHSCSA: completed
- PRISM-SRM analysis of >200 FFPE samples from CPDR with sufficient follow-up after initial biopsy to identify aggressive disease; 105 samples completed thus far:
  - no further progression after 10+ years follow-up – indolent disease
  - biochemical recurrence (BCR) – intermediate response

  - metastatic disease – highly aggressive prostate cancer
- Proteomics discovery using the FFPE samples and inclusion of potential promising candidates (in progress)
- Expansion of panel to non-invasive biofluids, particularly urine (soluble and exosome) – 30 DRE urine (supernatant) received from Emory; PRISM-SRM analysis in progress

Initial results and next steps (PNNL)

- Primary Goal: To identify a panel of protein biomarkers which can be applied to initial prostate biopsies to identify patients suitable for active surveillance (indolent disease)
  - Completed PRISM-SRM analysis of 105 samples; identified 6 possible candidates that associate with either BCR or metastasis ($p < 0.05$)
- TGFB1, SPRC, CCND1, PSA, FOLH1, NCOA2
  - Ongoing proteomics discovery using the FFPE samples for additional promising candidates

Secondary Goal: Expansion of panel to non-invasive biofluids, particularly urine, to possibly avoid need for biopsies
13 candidate biomarkers distinguish high-GS CaP from BPH
6 candidate biomarkers potentially associated with either BCR or metastasis
25 candidate biomarkers initially detected in DRE urine (supernatant)

EDRN Biomarker Reference Laboratory (BRL) at Johns Hopkins Update - Daniel W. Chan, Ph.D. (PI)

- Collaborative studies
- Emory CVC (Martin Sanda) and U. Wash, Canary foundation (Dan Lin) for the PASS (Prospective Active Surveillance Study) study
- PCA3 longitudinal collection and testing of validated biomarkers (PI John Wei and Daniel W. Chan)
- Industrial Collaboration with Minomic Inc. Validation of MiCheck® - a Multianalyte Index Assay (MIA) for prostate cancer.
- Ovarian cancer: Development of an 8 biomarker multiplexed immunoassay on the BioPlex/MSD Sector for the analysis of 200 clinical samples.
- Hepatocellular cancer (HCC): Analysis of a panel of biomarkers (AFP, PIVKA-II), GP73, HGF, DPP4, DPP4/Seprase) for HCC on the Architect Immunoassay Analyzer with Industrial Partner Abbott Labs
- Product Development: An in vitro diagnostic multivariate index assay (IVDMIA) for aggressive prostate cancer. We started with 2 biomarkers previously discovered from our EDRN BRL - fucosylated PSA and sTIE-2 (soluble angiopoietin-2) plus additional promising biomarkers (e.g. AZGP1 & PTK7 by Mass spectrometry MRM assays).

Prostate Collaborative Group - Summary
Assess science:
Critique progress:
Timeline:
Implementation plan:
Outcome expected:
New or ongoing project:
…and…
Synergy:

Prostate Collaborative Group - Summary
Assess science:
multiplex RNA/transcriptome (CPDR, Emory, Michigan)
proteome (EVMS-Toronto, PNNL)
glycome (Hopkins)
Critique progress:
Accomplished: High density multiplex biomarker measurement (eg RNAseq for transcriptome, M-spec for proteome and glycome)
Pending:

Transcriptome: Higher sample throughput to enable training signatures to discern aggressive prostate cancer (Grade Group 2+) from indolent (Grade Group 1);
Proteome/glycome: continue transition from tissue analysis to post-DRE urine EV's
Timeline:
Initial multiplex urine+serum biomarker signatures in 1 year
(Enable subsequent validation and/or combination with imaging data)

Prostate Collaborative Group - Summary
Implementation plan:
Access existing CVC/BDL tissue/urine resources to train next generation proteome/transcriptome/glycome signatures
Explore role for BRL's in export of measures
Outcome expected:
Improve prediction of aggressive prostate ca in two clinical endpoints:
New or ongoing project:
BDL projects: most new, some ongoing
Collaborative projects: 1) Upgrading Study Expansion (ongoing); 2) PCA3 Trial urine interrogation by RNAseq (ongoing/new hybrid); 3) MRI Imaging (new)
Synergy:
Consensus post-DRE urine protocol
Clinical endpoints (initial biopsy; treat or watch)
Resources: PCA3 Trial cohort specimens, URS Study, PASS Trial

Final Remarks

Joshua LaBaer, M.D., Ph.D., Arizona State University/The Biodesign Institute
Sudhir Srivastava, Ph.D., M.P.H., National Cancer Institute

Drs. LaBaer and Srivastava thanked everyone for their attendance and participation.

The meeting was adjourned.