EDRN BIOMARKER DEVELOPMENT

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CONTENTS

Early Detection Research Network

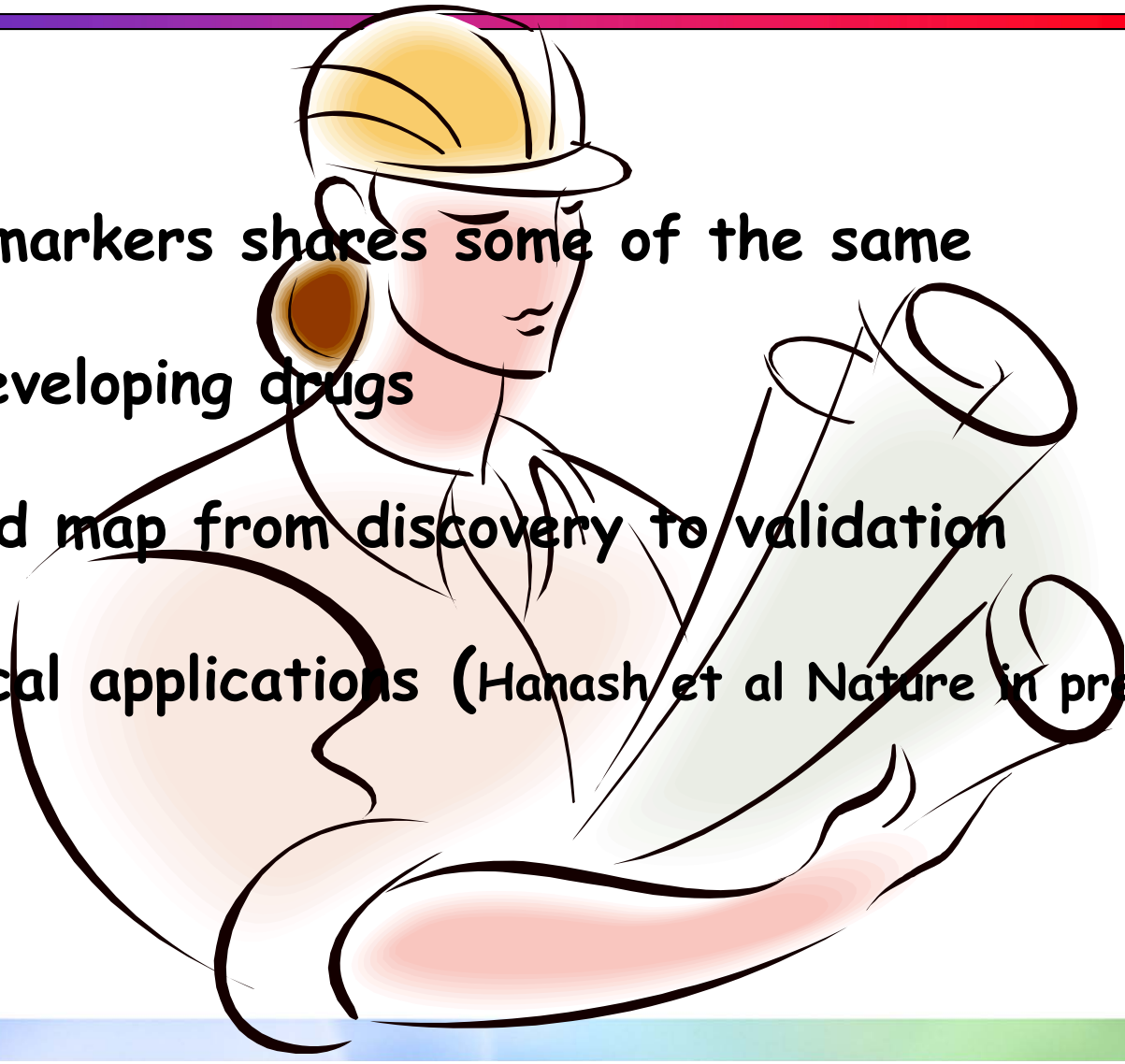
1- Two studies that progressed from discovery to blinded validation in a context of early detection

2- Collaborative work within and outside of EDRN Development laboratories

3- Leveraging resources outside of EDRN

Why so few biomarkers to date?

Early Detection Research Network



- Developing biomarkers shares some of the same challenges as developing drugs
- Requires a road map from discovery to validation for defined clinical applications (Hanash et al Nature in press)

Early Detection Research Network

Autoantibodies as biomarkers for early cancer detection

Immune response to tumor antigens

- **Occurs early during tumor development: may allow early cancer detection**
- **Is not limited to mutated proteins**
- **May involve aberrantly expressed proteins eg oncofetal antigens**
- **Epitopes may result from post-translational modifications eg glycosylation**

ANTI-ANNEXINS I & II ANTIBODIES IN LUNG CANCER

	# subjects	Annexin I Antibody +	Annexin II Antibody +
Lung Cancer	**54**	**16**	**18**
Adenocarcinoma	30	12	11
Squam cell carcinoma	18	3	4
Small cell carcinoma	4	1	2
Large cell carcinoma	2	0	1
Other cancer types	**60**	**6**	**0**
Other controls	**61**	**0**	**0**
Healthy subjects	51	0	0
Chronic lung disease	10	0	0

CARET Validation strategy

Blinded validation study

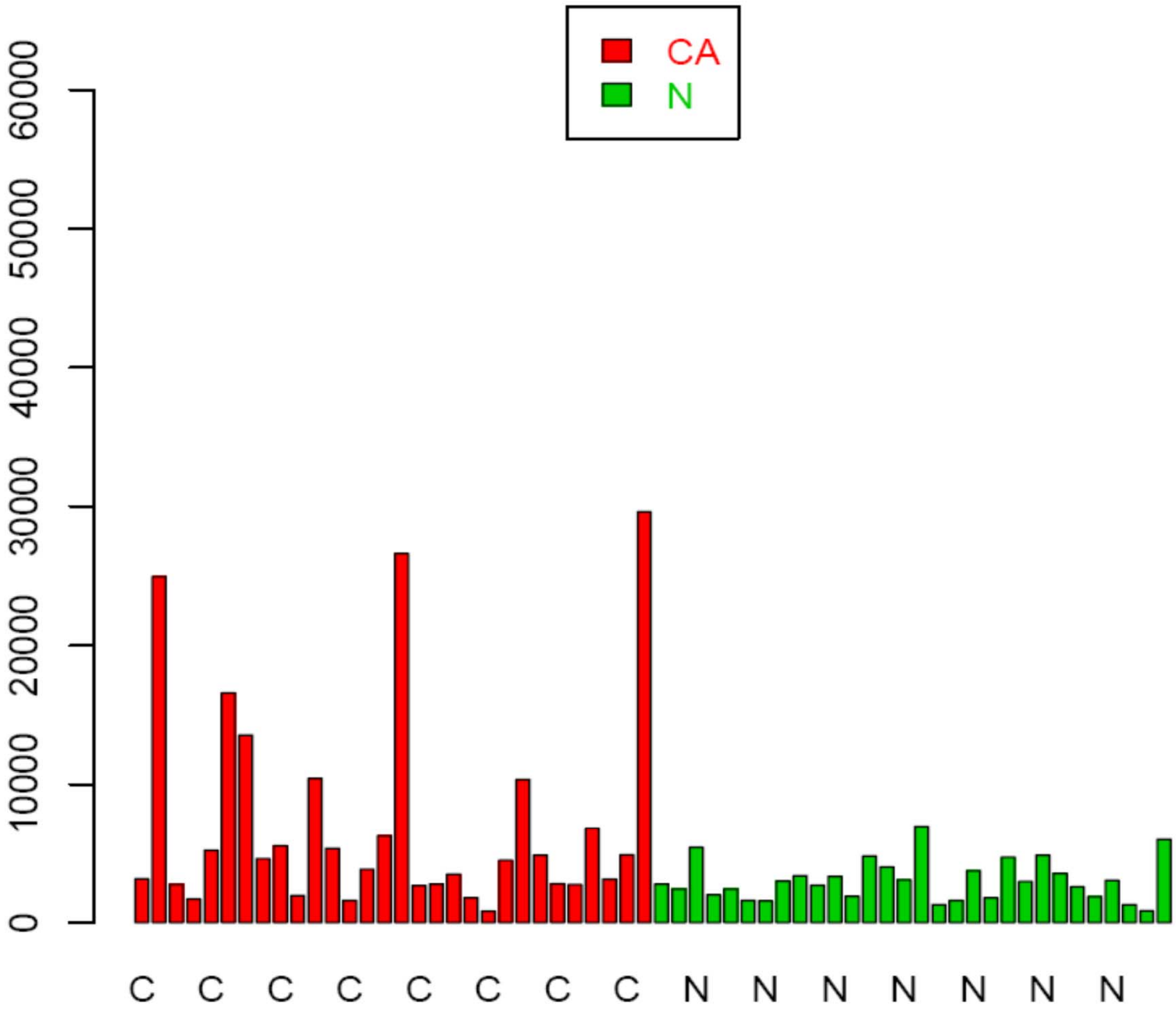
Approach: Protein microarrays

Contents: Natural proteins derived from tumor cell line(s**)**

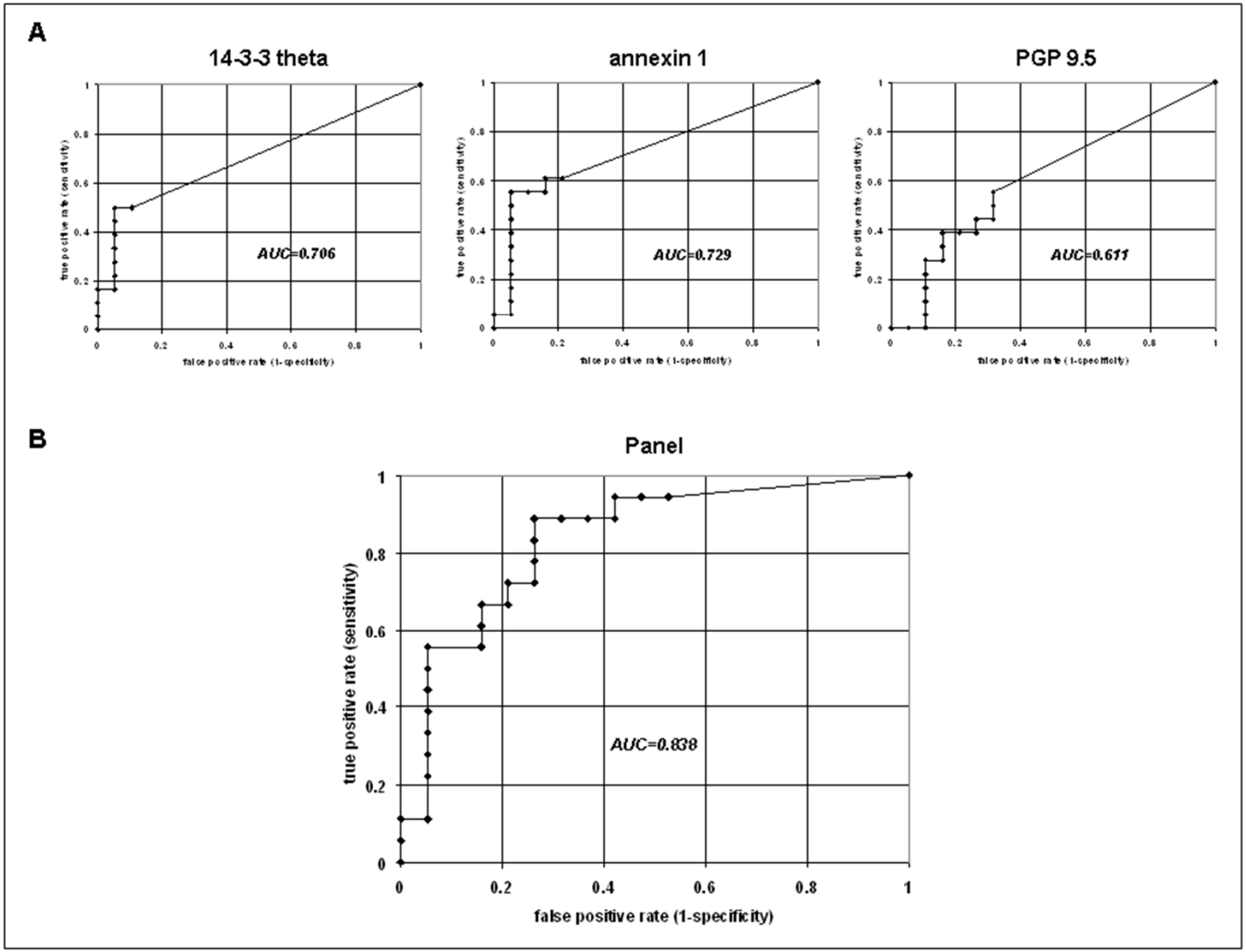
Samples: Collected ~1 yr prior to lung cancer dx from 100 cases and 100 matched controls

Targets: Annexin, PGP9.5, 14-3-3 theta

Data analysis: NCI EDRN Data Management Center


CA
N
0
10000
20000
30000
40000
50000
60000
C C C C C C C C N N N N N N N

Identification of 14-3-3 theta as an antigen that induces a humoral response in lung cancer
Sandra R. Pereira-Faca et al (Cancer Research '07)



Validation Phase 2

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- Validate intended clinical application:
  Blood test in combination with CT scanning

- Demonstrate increased specificity and sensitivity of CT scans when combined with an autoantibody marker panel for high risk subjects

- Retrospective component

- Prospective component

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Pancreatic cancer markers from discovery in the mouse to blinded validation study in pre-diagnostic sera

Pancreatic cancer mouse model

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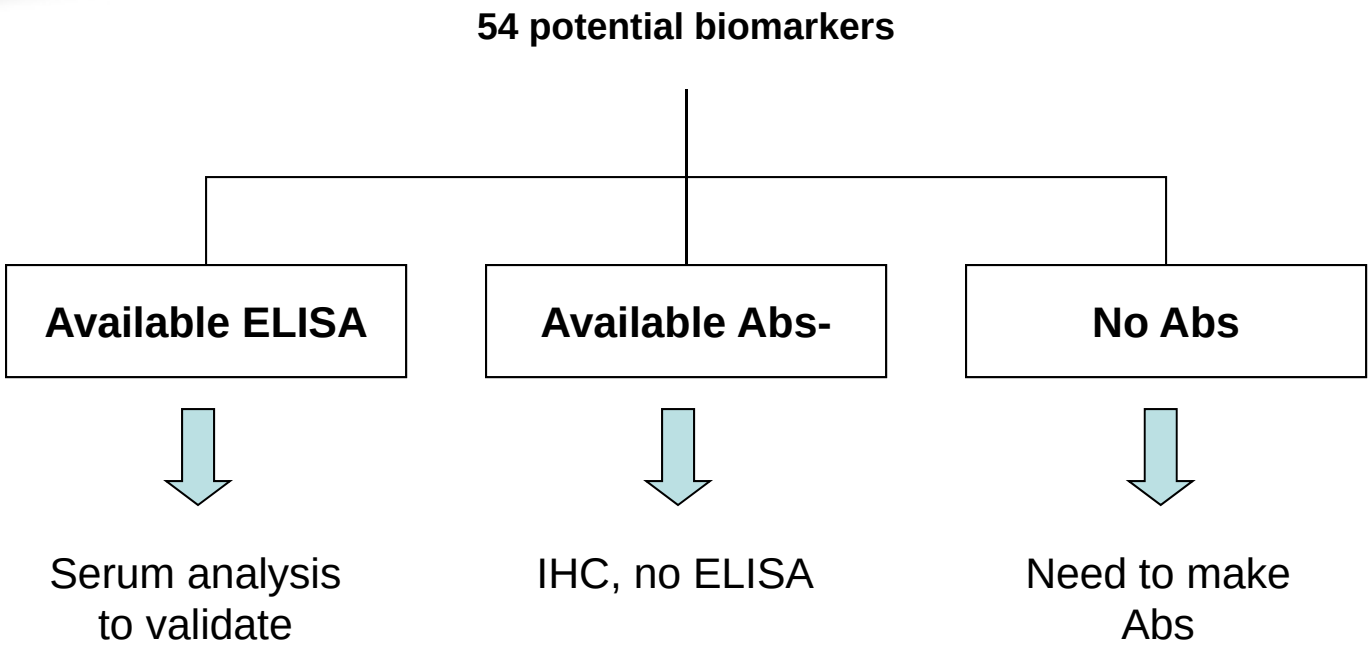
K-ras activation + Ink4a/Arf for pancreatic cancer

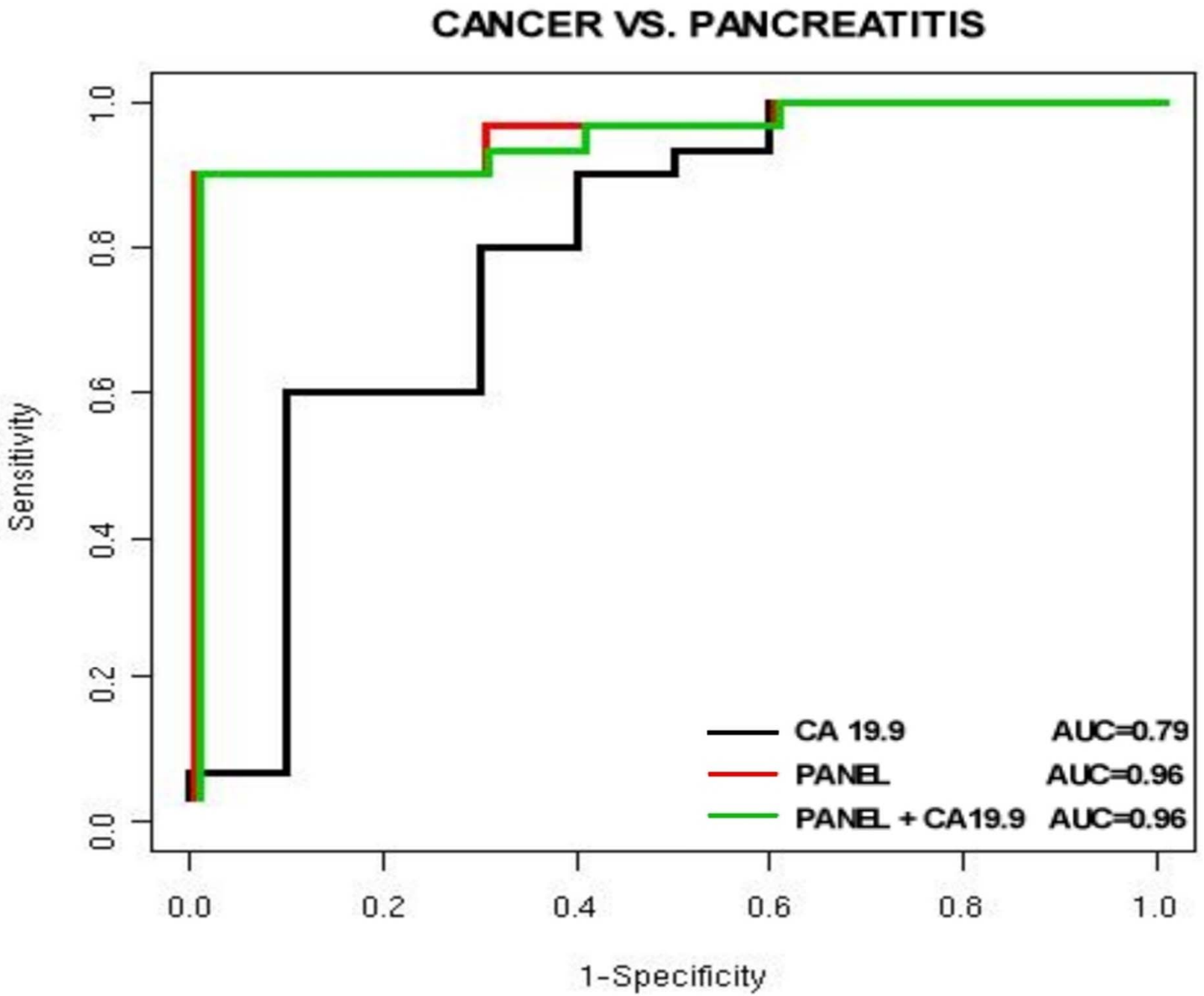
R. DePinho and N. Bardeesy

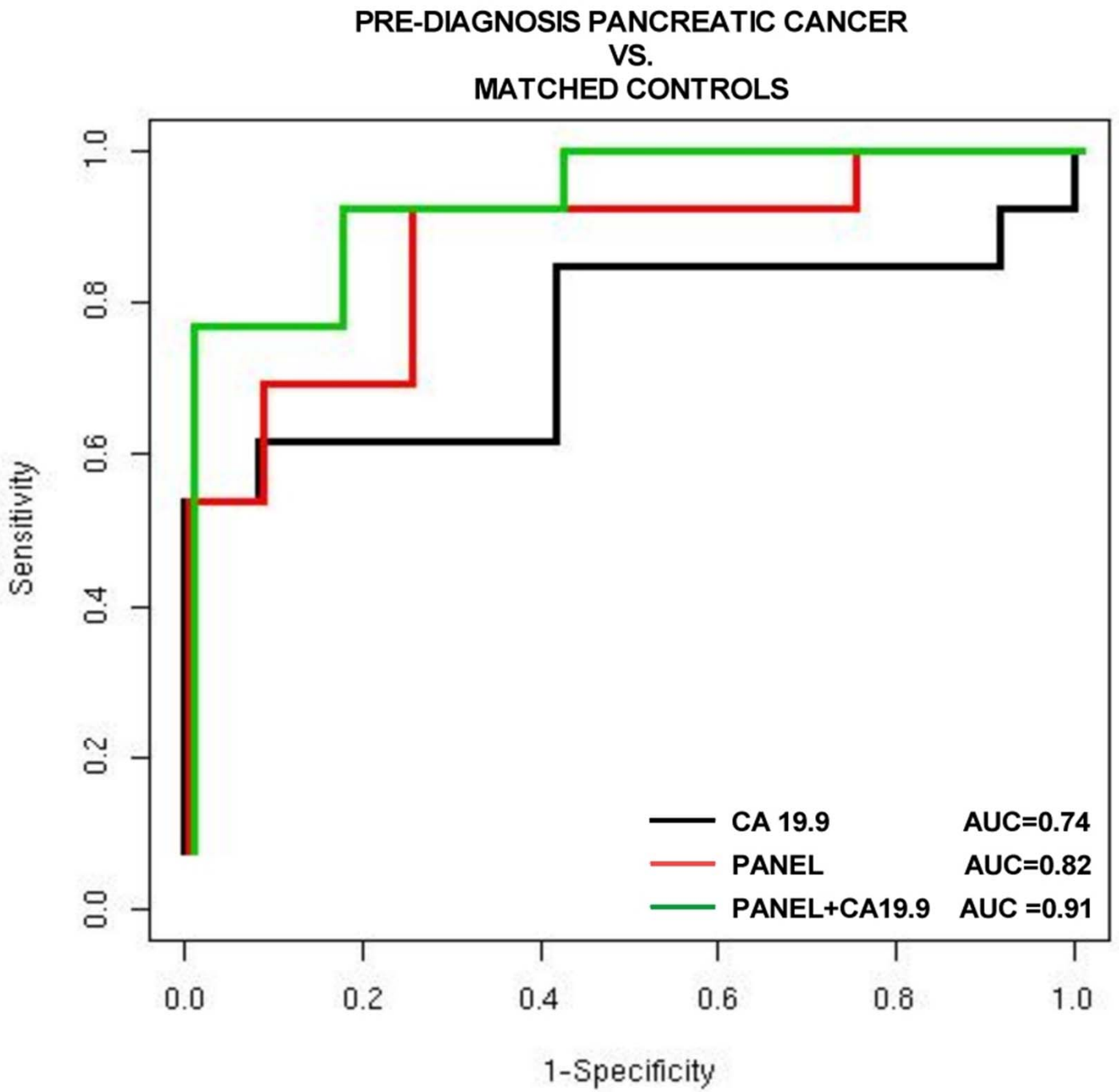
-**Plasma from mice with early stage tumor and matched controls**

-**Plasma from mice with advanced stage tumor and matched controls**

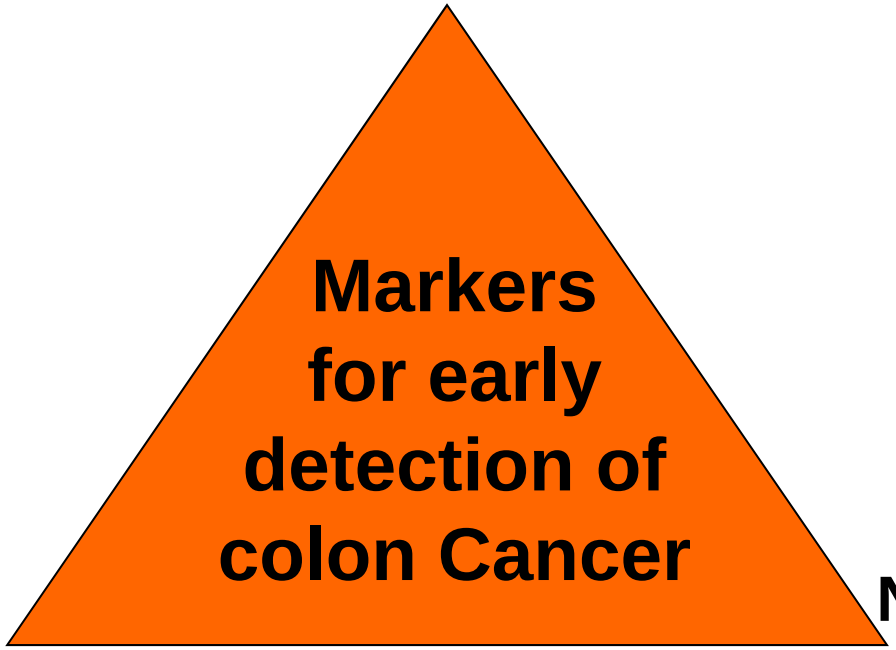
Validation


54 potential biomarkers
Available ELISA
Available Abs-
No Abs
Serum analysis to validate
IHC, no ELISA
Need to make Abs


CANCER VS. PANCREATITIS
Sensitivity
1-Specificity
0.0
0.2
0.4
0.6
0.8
1.0
CA 19.9 AUC=0.79
PANEL AUC=0.96
PANEL + CA19.9 AUC=0.96


PRE-DIAGNOSIS PANCREATIC CANCER
VS.
MATCHED CONTROLS
Sensitivity
1-Specificity
0.0
0.2
0.4
0.6
0.8
1.0
CA 19.9 AUC=0.74
PANEL AUC=0.82
PANEL+CA19.9 AUC =0.91

NHLBI-WHI



10 Academic Institutions

NCI-EDRN

NHLBI-NCI-WHI-EDRN Collaborative study

S. Hanash & R. Prentice
Co-PIs

Early Detection Research Network

100 colon cancer cases that occurred 6 – 18 m following yr 3 blood draw + 100 matched controls

10 teams applied a variety of proteomics approaches to aliquots from the same blood draws

All data compared and integrated

Promising biomarkers to be validated in a second phase

Participating Institutions

Early Detection Research Network

Fred Hutchinson Cancer Research Center	
Harvard Medical School	***R. Kucherlapati***
PNNL	***R. Smith***
Johns Hopkins University	***D, Chan***
Northeastern University	***W. Hancock***
University of Michigan	***A. Chinnaiyan***
Wayne State University	***M. Tainsky***
Eastern Virginia University	***J. Semmes***
Wistar Institute	***D. Speicher***
University of Pittsburgh	***W. Bigbee***

Innovative nature of the study

Early Detection Research Network

Discovery studies at the pre-clinical stage

Reduced bias due to multi-institutional sample collection

Reduced bias due to asymptomatic status of subjects at the time of blood draw

Analysis of aliquots of the same samples by multiple investigators/platforms

Sample blinding at the time of data collection

Centralized integrated analysis of all data collected

Data Analysis

Early Detection Research Network

Data processing: CPAS (M. McIntosh)

Data management: EDRN DMCC (Z. Feng)

Statistical Analysis: WHI (R. Prentice)

Findings

Early Detection Research Network

A total of 2,343 high confidence protein groups were identified which corresponds to up to 2,876 distinct gene symbols (compared to HUPO PPP of 889 proteins identified).

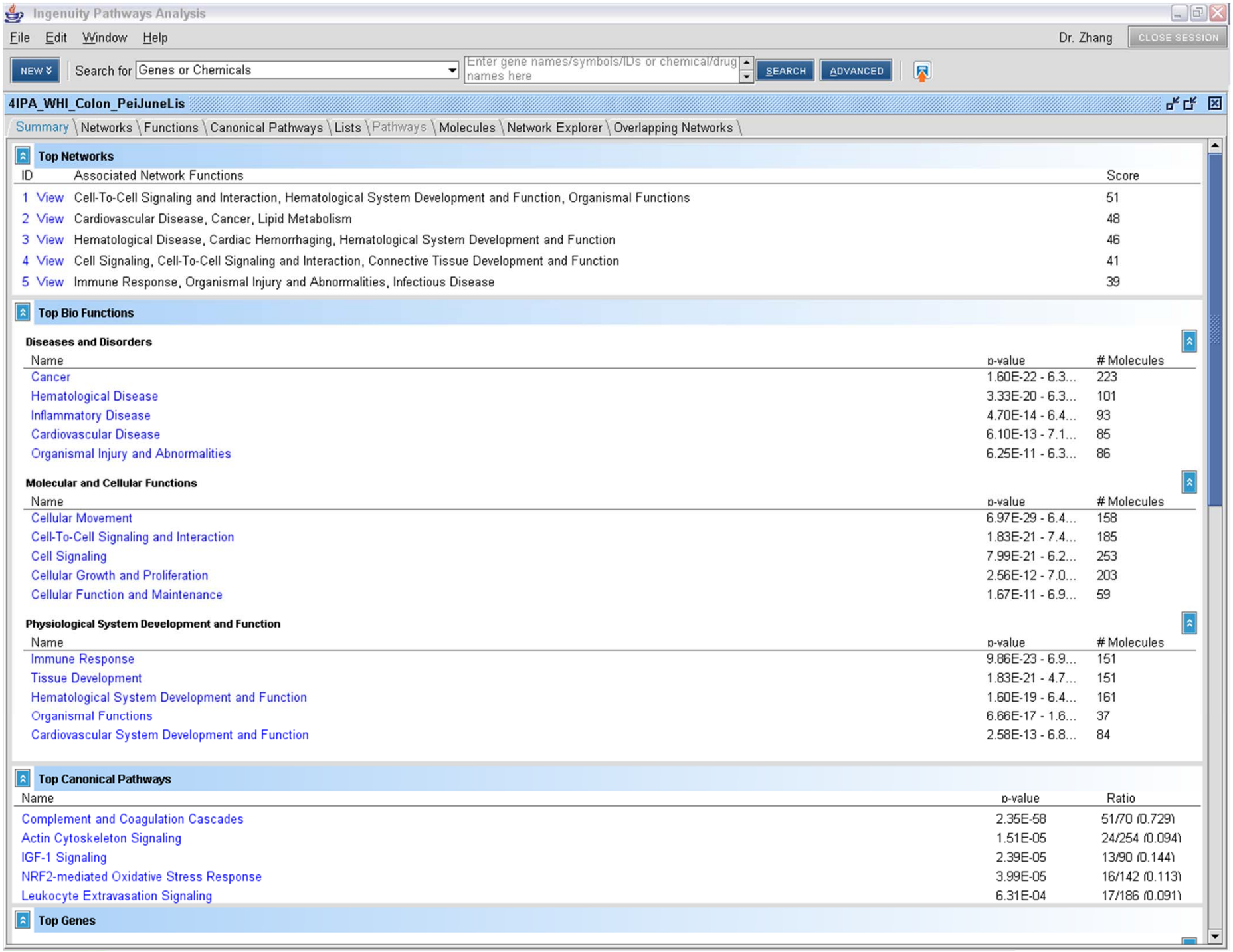
A total of 1,846 of these proteins were identified in at least two separate laboratories.

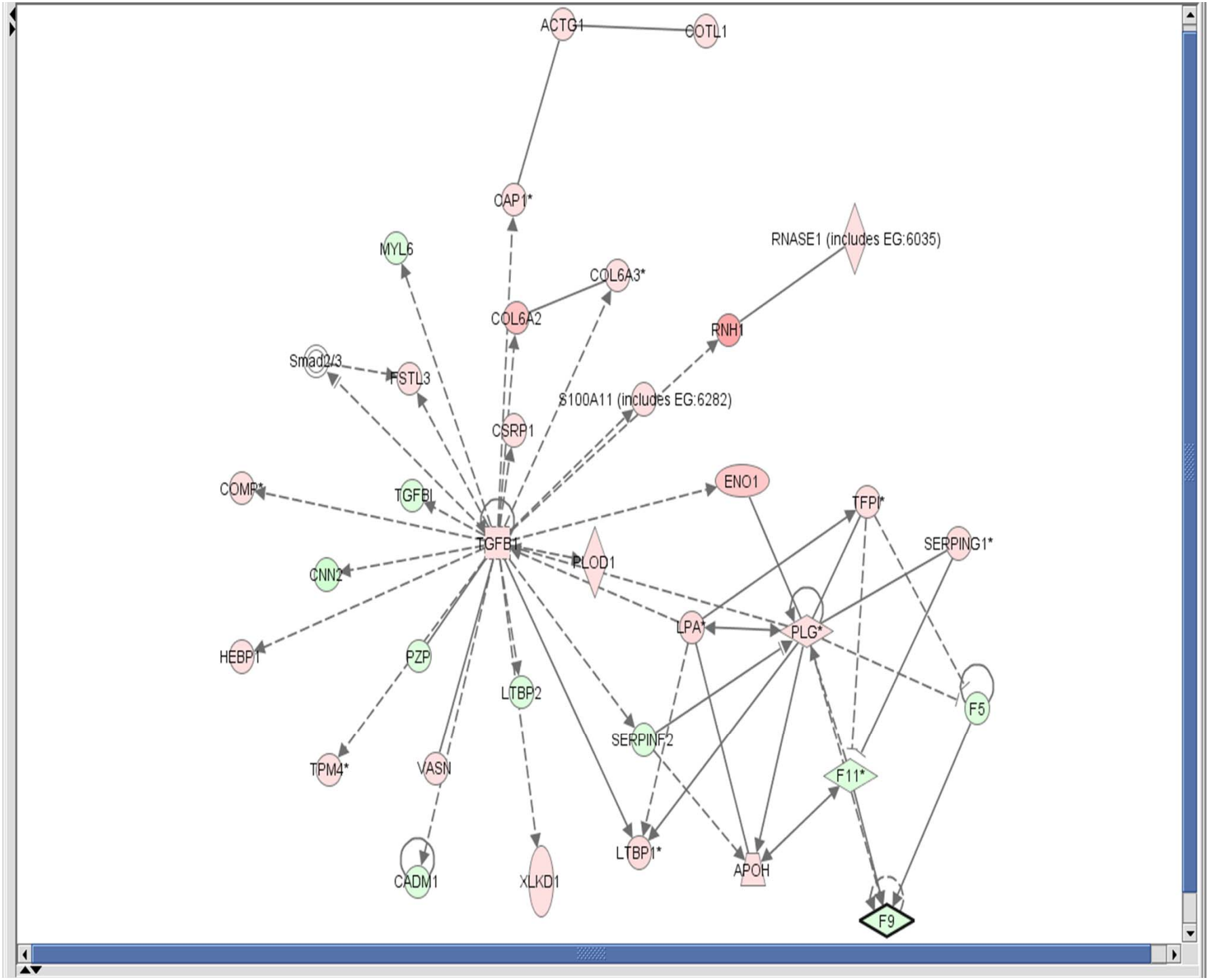
Summary of significance

Early Detection Research Network

65 proteins identified with quantitative values and P<.05 in one ore more labs (41 up, 24 down in pre-diagnostic specimen relative to matches controls).

11/65 proteins showed significance in more than one lab.


Ingenuity Pathways Analysis
File Edit Window Help
Dr. Zhang
CLOSE SESSION
NEW
Search for Genes or Chemicals
Enter gene names/symbols/IDs or chemical/drug names here
SEARCH
ADVANCED
4IPA_WHI_Colon_PeiJuneLis
Summary Networks Functions Canonical Pathways Lists Pathways Molecules Network Explorer Overlapping Networks
Top Networks
ID Associated Network Functions Score
1 View Cell-To-Cell Signaling and Interaction, Hematological System Development and Function, Organismal Functions 51
2 View Cardiovascular Disease, Cancer, Lipid Metabolism 48
3 View Hematological Disease, Cardiac Hemorrhaging, Hematological System Development and Function 46
4 View Cell Signaling, Cell-To-Cell Signaling and Interaction, Connective Tissue Development and Function 41
5 View Immune Response, Organismal Injury and Abnormalities, Infectious Disease 39
Top Bio Functions
Diseases and Disorders
Name p-value # Molecules
Cancer 1.60E-22 - 6.3... 223
Hematological Disease 3.33E-20 - 6.3... 101
Inflammatory Disease 4.70E-14 - 6.4... 93
Cardiovascular Disease 6.10E-13 - 7.1... 85
Organismal Injury and Abnormalities 6.25E-11 - 6.3... 86
Molecular and Cellular Functions
Name p-value # Molecules
Cellular Movement 6.97E-29 - 6.4... 158
Cell-To-Cell Signaling and Interaction 1.83E-21 - 7.4... 185
Cell Signaling 7.99E-21 - 6.2... 253
Cellular Growth and Proliferation 2.56E-12 - 7.0... 203
Cellular Function and Maintenance 1.67E-11 - 6.9... 59
Physiological System Development and Function
Name p-value # Molecules
Immune Response 9.86E-23 - 6.9... 151
Tissue Development 1.83E-21 - 4.7... 151
Hematological System Development and Function 1.60E-19 - 6.4... 161
Organismal Functions 6.66E-17 - 1.6... 37
Cardiovascular System Development and Function 2.58E-13 - 6.8... 84
Top Canonical Pathways
Name p-value Ratio
Complement and Coagulation Cascades 2.35E-58 51/70 (0.729)
Actin Cytoskeleton Signaling 1.51E-05 24/254 (0.094)
IGF-1 Signaling 2.39E-05 13/90 (0.144)
NRF2-mediated Oxidative Stress Response 3.99E-05 16/142 (0.113)
Leukocyte Extravasation Signaling 6.31E-04 17/186 (0.091)
Top Genes


ACTG1
COTL1
CAP1*
MYL6
RNASE1 (includes EG:6035)
COL6A3*
COL6A2
RNH1
Smad2/3
FSTL3
S100A11 (includes EG:6282)
CSRP1
COMP*
TGFBI
ENO1
TFPI*
TGFB1
SERPING1*
PLOD1
CNN2
LPA*
PLG*
HEBP1
PZP
LTBP2
F5
SERPINF2
TPM4*
VASN
F11*
LTBP1*
APOH
CADM1
XLKD1
F9

Phase 2

Early Detection Research Network

Validation of candidate markers using a second set of WHI subjects

Further mining of the data for PTMs, Glycan modifications..

Leveraging non-EDRN resources

Early Detection Research Network

NCI: Mouse Models, Glycomics Alliance, Nanotechnology

Other NIH Institutes: HUPO PPP

Cohorts: CARET, WHI, PLCO

Foundations

- **Lustgarten: Pancreas**
- **Labrecque: Lung**
- **Avon: Breast**
- **Canary: Lung, pancreas, ovary, prostate**

CANARY FOUNDATION

Stopping cancer early...the best possible investment

Canary Lung Project

MISSION: Early detection of lung cancer through a combination of imaging, sputum and/or blood based testing applicable to lung cancer among smokers as well as never smokers.