Biomarkers for Early Detection and Diagnosis of Lung Cancer: An EDRN Biomarker Discovery Laboratory Experience

From the Thoracic Oncology Laboratory
NYU Langone Medical Center
New York, New York

Disclosures

- **Grants:** NCI EDRN, RO1, TCGA, DOD
- **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
- **Industry:** Fujirebio, 20/20 Gene, MesoScale Diagnostics, Cynvezio, Source MDX, Celera, OPKO, SomaLogic, Genentech, Integrated Diagnostics, Transgenomics, Rosetta Genomics, Calithera, Pinpoint Genomics, Cizzle, Viomics, Myriad, VisionGate
- **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

The Early Detection Research Network

Early Detection Research Network

- Investigator-initiated infrastructure modeled after Cooperative Groups and established in 2000
- Collaborative and team science driven biomarker discovery, development and validation
- Built-in reward mechanisms for collaboration and team science
- Milestone-driven projects with incremental peer-review evaluation
- Inclusive infrastructure that solicits extramural investigators' participation through a unique Associate Membership Program throughout the funding period
- Follows industrial/biotechnology standard practice for biomarker pipeline development

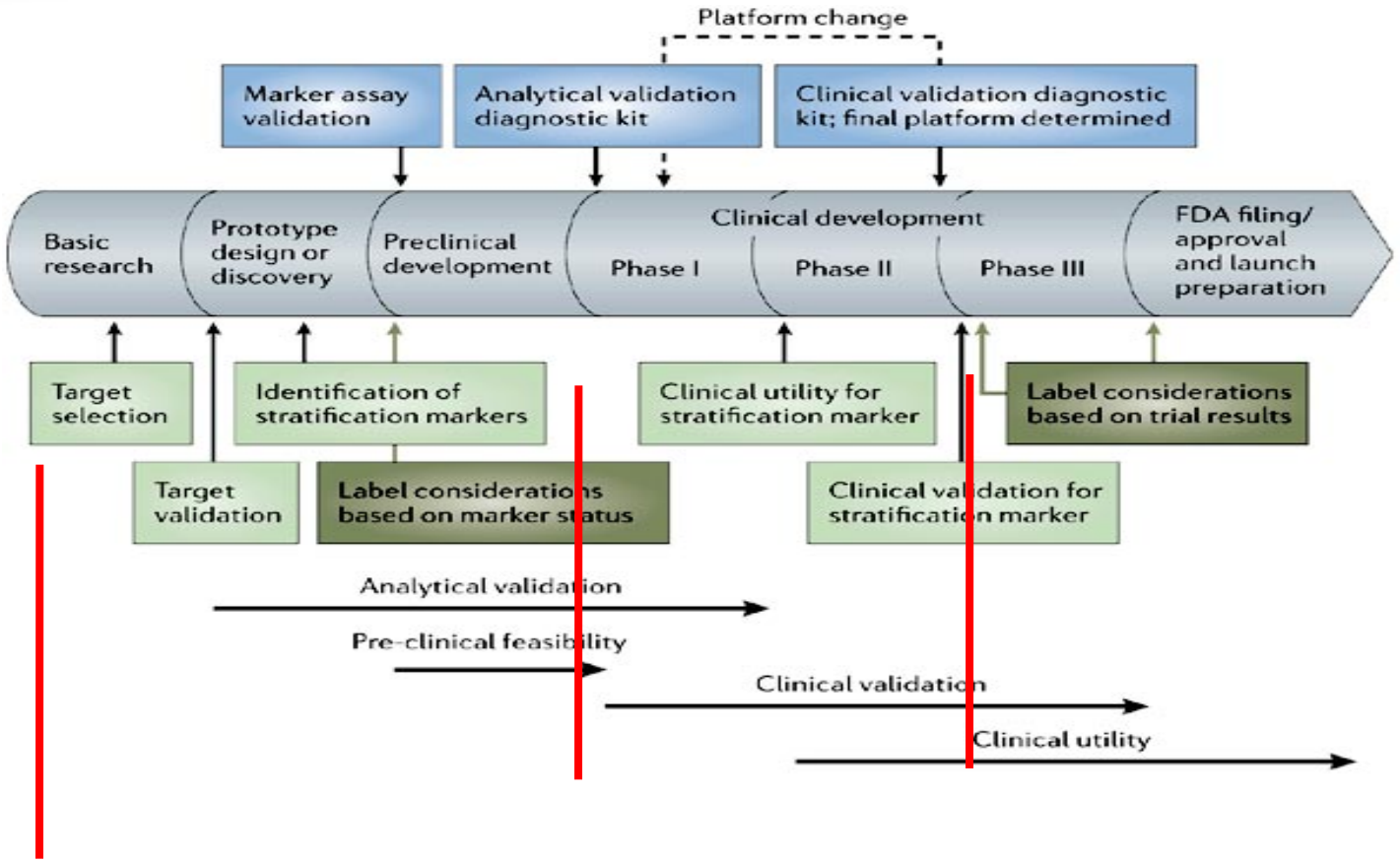
Organized into Collaborative Groups

Early Detection Research Network

https://edrn.nci.nih.gov/collaborative-groups

EDRN Structure is Based on Biomarkers Development Pipeline

Early Detection Research Network



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Nature Reviews | Drug Discovery

BDL → BRL → CVC

EDRN Biomarker Pipeline

Early Detection Research Network

- Hundreds of biomarkers reviewed and evaluated
- More than 500 failed in rigorous testing
- More than 800 prioritized (EDRN Data Base)
- Many of them are in Phase II

https://edrn.nci.nih.gov/biomarkers

Collaborative Activities

Early Detection Research Network

- Extensive interactions; Monthly phone calls including scientific presentations, biomarker prioritization, guest speakers
- Two Steering Committee meetings each year combined with scientific workshop every 18 months; attended by non-EDRN members
- Dedicated Webpages for Each Collaborative Group
- More than 60 network-wide collaborative projects
- Partnerships with other NCI Programs, e.g. CPTAC, SPORES

National Resource

Early Detection Research Network

- Conducts multi-center, multidiscipline trials for biomarker validation
- Informatics and bioinformatic support for data collection, curation,
  storage and queries
- Centralized Statistical Center for data analysis and statistical support (DMCC);
- Fail-safe mechanism for efficient biomarker triaging for large, expensive
  validation studies (use of reference sets)
- Availability of large number of biospecimens (more than 100,000) using
  a uniform protocol
- Shared technologies on genomics, proteomics, and other 'omics' for
  collaborative studies

Collaborative Opportunity for Community and Public

Early Detection Research Network

- More than 200 Associate members, many of which are collaborating with EDRN members;
- Active partnership Foundations
  - Lustgarten Foundation N.Y.
  - Canary Foundation
- Collaboration with China (C-EDRN) and Cancer Research UK;
- EDRN Advocate Forum through quarterly Webinar.

Scientific Excellence

Early Detection Research Network

- ❑Innovative Technologies, Study Designs and Approaches
- ❑Biomarker Discovery
- ❑Preanalytical Validation
- ❑Analytical Validation

https://edrn.nci.nih.gov/network-consulting-team

Clinical Assays in Use

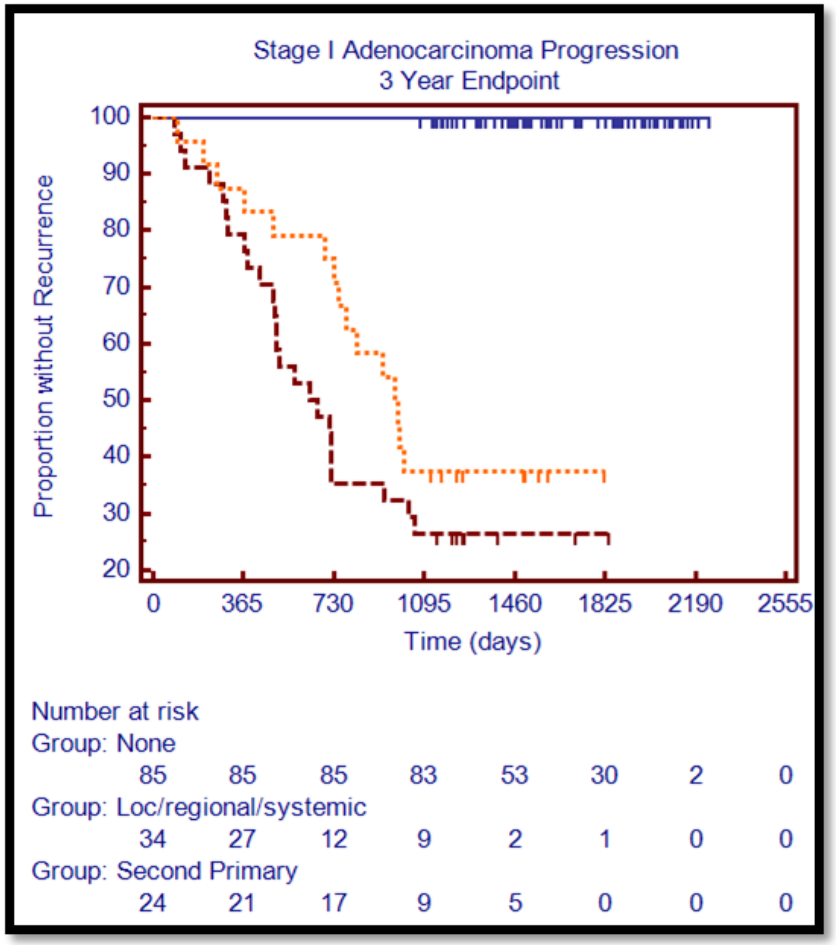
Early Detection Research Network

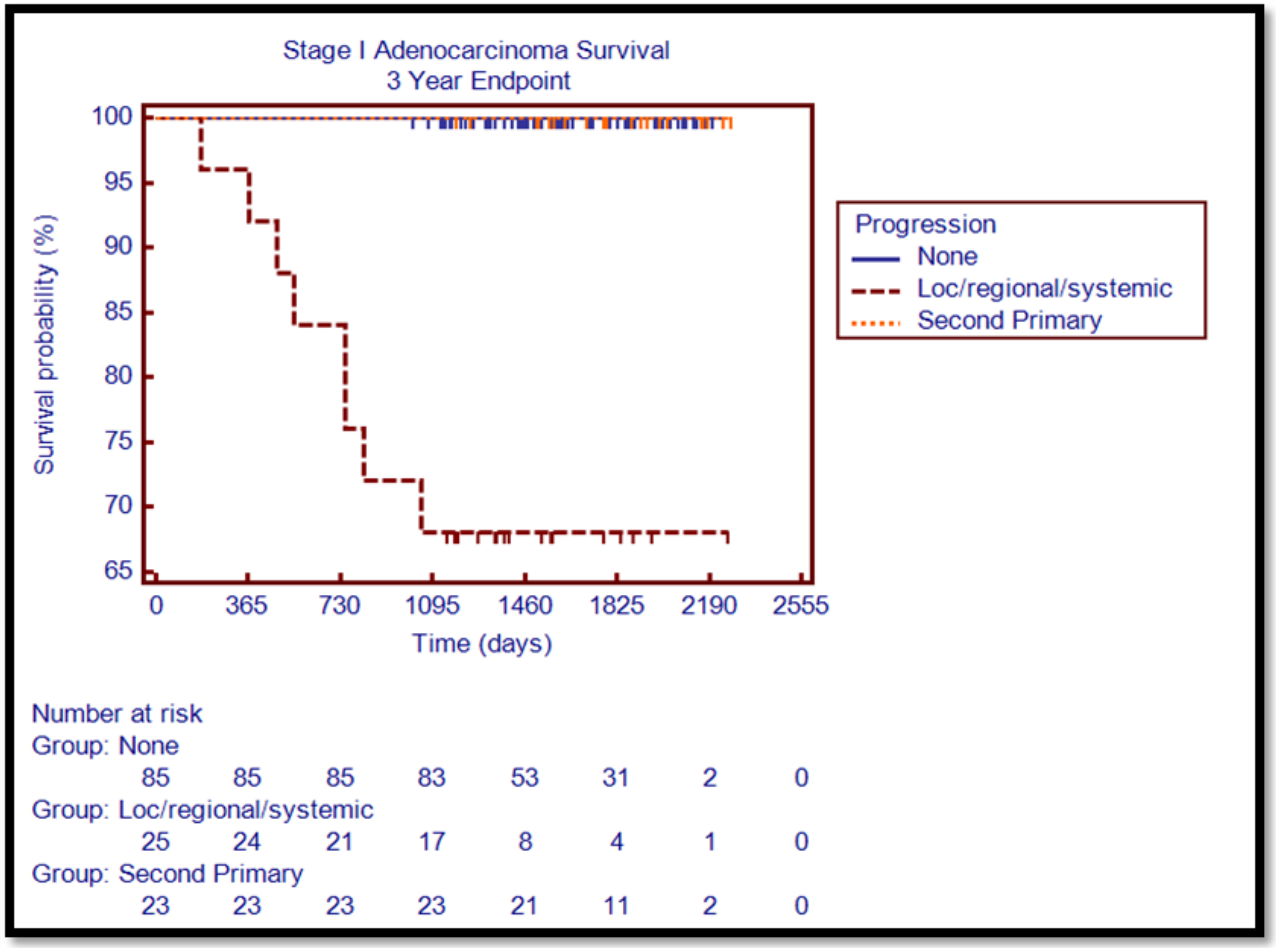
Detection/ Biomarker Assay	Discovery	Refine/ Adapt for Clin Use	Clinical Validation	Clinical Translation
Blood proPSA		√	√	FDA approved
Urine PCA3		√	√	FDA approved
Urine/TMA assay for T2S:Erg fusion for Prostate Cancer	√	√	√	CLIA in process
FISH to detect T2S:Erg fusion for Prostate Cancer	√	√	√	In CLIA Lab
Aptamer-based markers for Lung Cancer		√	√	In CLIA Lab
Proteomic Panel for Lung Cancer		√	√	In CLIA Lab
OVA1™ for Ovarian Cancer		√	√	FDA Approved
SOPs for Blood (Serum, Plasma), Urine, Stool,		√		Frequently used by biomarker research community
Vimentin Methylation Marker for Colon Cancer		√	√	In CLIA Lab
ROMA Algorithm for CA125 and HE4 Tests for Pelvic Mass Malignancies		√	√	FDA Approved
Blood/DCP and AFP-L3 for Hepatocellular Carcinoma		√	√	FDA Approved
Blood GP73	√	√	√	Together with AFP-L3 used in China for monitoring/risk assessment of cirrhotic patients for HCC
Bronchogen		√	√	In CLIA Lab

Is there a need for Early Detection/Diagnostic or Prognostic Biomarkers?

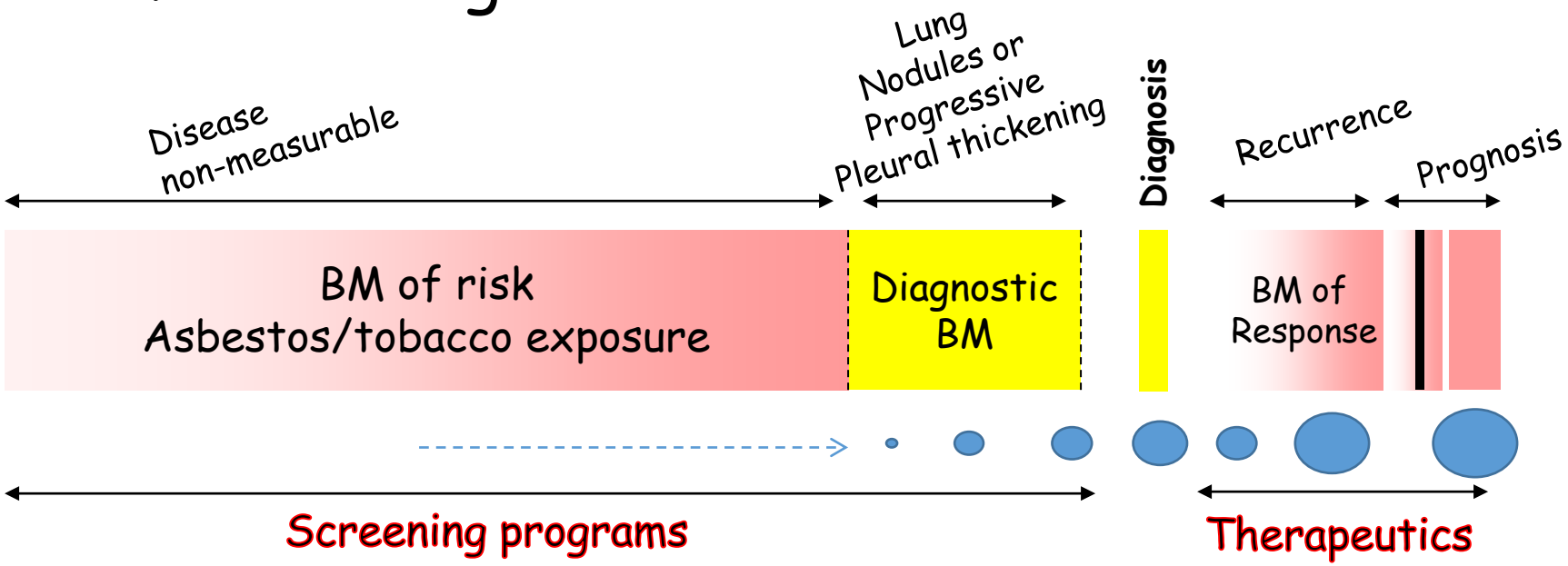
NYU Lung Cancer Progression/Survival Cohort

Patients (2006-2011) with Absolute Follow-up as of February 2013



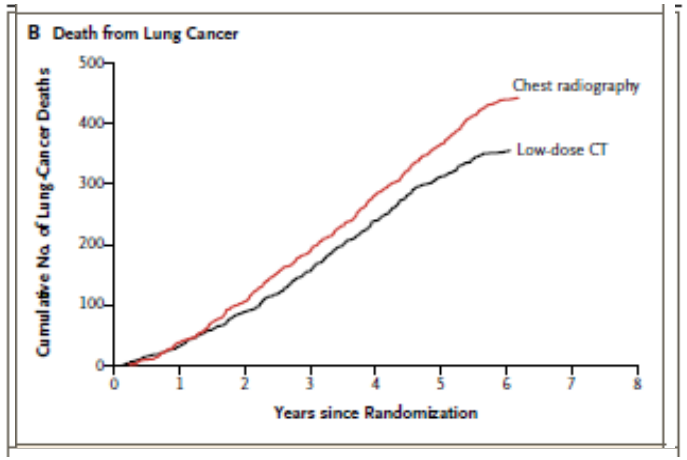


Where do (blood) biomarkers fit in the natural history of mesothelioma and lung cancer?



Reduced Lung-Cancer Mortality with Low-Dose Computed Tomographic Screening

The National Lung Screening Trial Research Team*



Thoracic cancer diagnostic biomarkers: The Necessary Steps to Believing a Biomarker

Sullivan-Pepe, JNCI 2001- EDRN

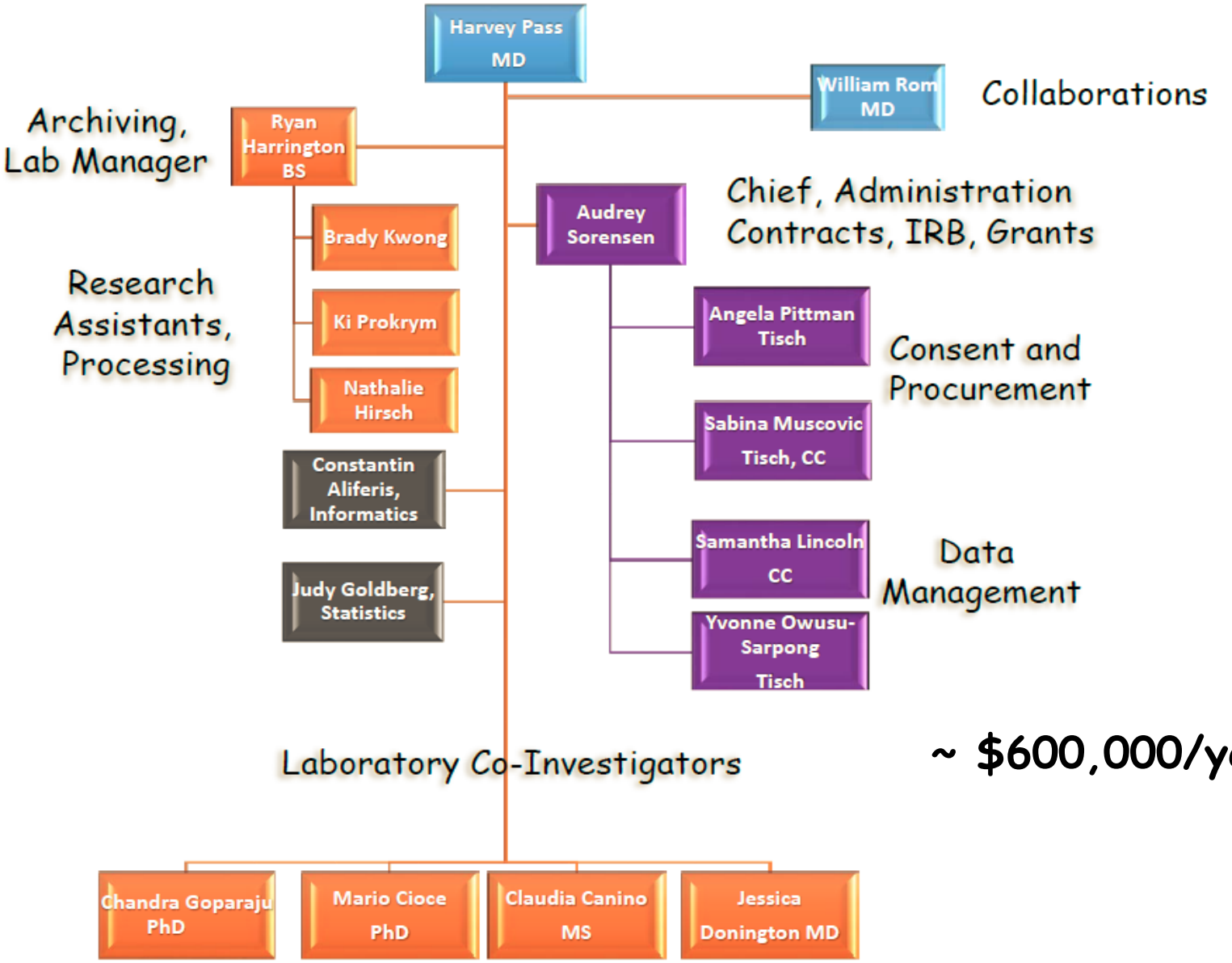
Candidates	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
	Discovery, Prediction	Assay validation	Retro-longitudinal	Prospective screening	Cancer Control
SERUM/PLASMA					
MALDI TOF MS profiling	x	x	x		
SomaMers	x	x	x		
Specific antigens /proteins	x	x	x		
miRNA	x	x	x		

Challenges

Early Detection Research Network

- Who to screen? Risk group?
- How often should chest CT be offered?
- How to handle the high false positive rate (benign lung nodules) ?
- How to streamline the Follow-up?
- Is it Cost effectiveness | implications
- How to implement for public policy
- What is the Impact on surgical | treatment approaches?
- Role(s) of molecular biomarkers?

What does it take to do this ?



~ $600,000/year in salary costs

How do you orchestrate it?

Consenting
Tissue/Blood Procurement
Administrative Core

Consenting
Tissue/Blood Procurement
Data Management

Consenting
Blood Procurement
Data Management
Regulatory

Extramural Scientists, Industry

IRB Contracts

ILO, MTA

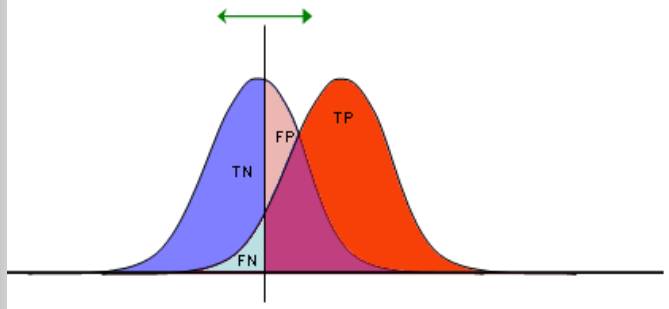
Processing Archiving in Freezer Works Storage

Tube Type	Cat#	Number	Volume (cc)	Yield (cc)
BD serum	366430	3	10	8-10
BD K3 EDTA plasma	366450	3	7	8-10
				Buffy Coat Frozen
BD CPT plasma	362760	2	4	5-6
				Cell Pellet cryopreserved

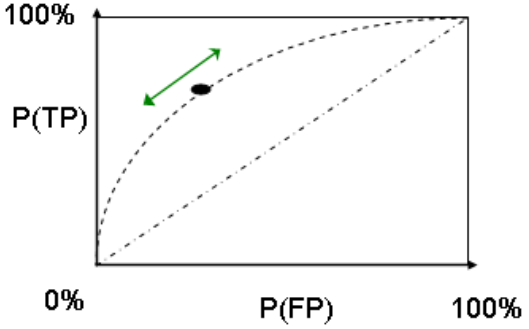
Scientists

How to quantitate how good a biomarker is: the ROC Curve

		Condition (as determined by "Gold standard")		
		Condition positive	Condition negative	
Test outcome	Test outcome positive	**True positive**	**False positive** (Type I error)	Precision = $\frac{\Sigma \text{ True positive}}{\Sigma \text{ Test outcome positive}}$
	Test outcome negative	**False negative** (Type II error)	**True negative**	Negative predictive value = $\frac{\Sigma \text{ True negative}}{\Sigma \text{ Test outcome negative}}$
		Sensitivity = $\frac{\Sigma \text{ True positive}}{\Sigma \text{ Condition positive}}$	Specificity = $\frac{\Sigma \text{ True negative}}{\Sigma \text{ Condition negative}}$	Accuracy

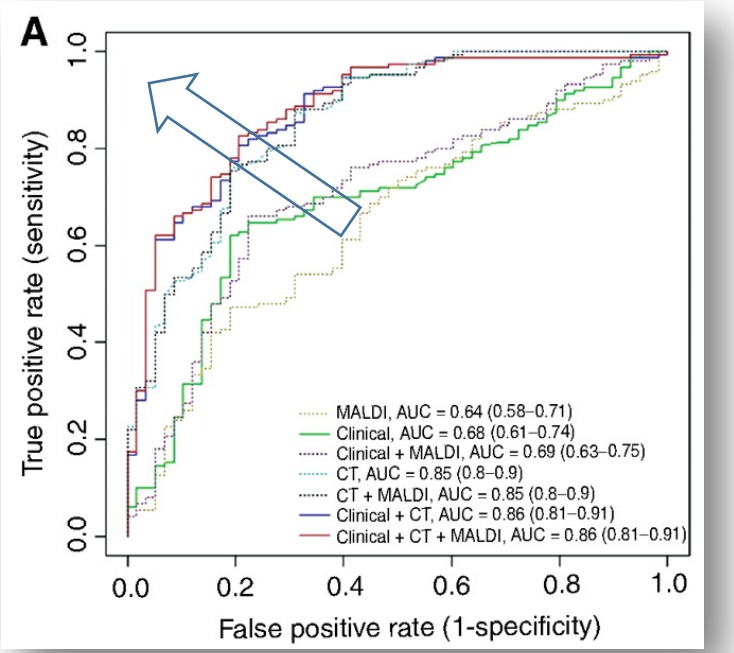


TP	FP
FN	TN
1	1



How good should the biomarker be?

- Better than standard of care.
- What are the metrics?
  - Performance of the test: PPV & NPV
  - ROC curves (TPR vs FPR).
  - Net reclassification Improvement (NRI) index
  - Change in decision making.



Pecot, CEBP 2012

What do physicians want?

- **A negative test result (that is, "benign") to be correct with high probability (more than 90%) to ensure that malignant nodules are not accidentally eliminated**
  - a high negative predictive value (NPV)
  - An NPV of 90% reduces the posttest probability of cancer to 10% or lower
    - That is a twofold reduction in cancer risk from the 20% pretest probability of cancer among patients selected for invasive procedures!

- The diagnostic test must be developed and validated on intended-use samples from multiple independent sites without demographic bias on key clinical parameters such as age, nodule size, and gender
  - Ideally in stage IA cancers.
  - The intended-use population should have a high occurrence of current and former smokers

What do patients or people at high risk or people with nodules want????

- They want their doctor to tell them with the least invasive way possible that either
  - You do not have to worry about having lung cancer
  - Your test tells us that something is brewing and we need to repeat the test over the next 3-6 months or longer
  - Your test tells us that the nodule you have on your CT scan is either definitely a cancer or definitely benign

THE LEAST INVASIVE WAY TO DO THIS IS BY LOOKING FOR SOMETHING IN THE BLOOD WITH A SIMPLE BLOOD DRAW!!!!

Who Studies Biomarkers?
EDRN Lung Collaborative Group: 2010-

Investigator	Site(s)	Platform/Expertise
C. Croce	OSU BDL	MicroRNA and ultraconserved non-coding RNA
S.Dubinett **A.Spira** **M. Lenburg** **D. Elashoff**	UCLA-Boston LC BDL	Genomics, microRNA, Central/Peripheral Airways; Role of Inflammation and biomarkers; RNASeq
D. Liebler **D. Taab**	Vanderbilt BDL	Mass spec, LCMRM; shotgun proteomics;plasma/tissue
P. Massion	Vanderbilt CVC	Case – control studies for diagnostic discovery; prospective CT imaging with collection of specimens; archive repository
H. Pass **M. Huflejt**	North American Mesothelioma Consortium	Plasma/Tissue; Genomic, proteomic, glycomic, microRNA diagnosis and prognosis for mesothelioma; lung collaboration with W. Rom and industry
W. Rom	NYU CVC	Ongoing screening (1143) and r/o lung cancer (1047) prospective cohorts; collaborations with industry/H.Pass
D. Sidransky	Hopkins BDL	Epigenetic/methylation markers serum, plasma, sputum, BAL
S. Stass	U. Maryland Biomarker Reference laboratory	Analytic and Clinical Validation of lung biomarkers; microRNA for early detection/standardization of qPCR techniques

BDL **CVC** **BRL**

Early Detection/Diagnostic/Prognostic Blood Based Platforms: Mesothelioma and Lung Cancer

- Proteomic
  - Serum
    - MRM-Mass Spec
      - Indie Diagnostics
    - SomaMers
      - SomaLogic
  - Plasma
    - Osteopontin
    - Luminex
    - Autoantibodies
- MicroRNA
  - Plasma
    - Croce et al
  - Exosomes
    - Cazzoli et al

❖ Multiple Reaction Monitoring Mass Spectroscopy proteomics

❖ Nucleotides contructed to bind to specific proteins and to remain bound during various manipulations

❖ Short "noncoding" ~22 nucleotides in length after processing, double-stranded,
❖ Bind 3'-UTR of target transcripts, leading to:
  ❖ Ribosomal interference
  ❖ Degradation of transcript
❖ May act as oncogenes or TSGs
❖ Very stable in plasma/serum/FFPE

❖ microvesicles specialized in the transport of different types of RNA in particular microRNAs

A Blood-Based Proteomic Classifier for the Molecular Characterization of Pulmonary Nodules: Indie Diagnostics

- Shotgun Proteomic analysis of tumors.
- Selected candidate proteins for testing in the blood

Steps in refining the 388 candidates down to the 13-protein classifier.

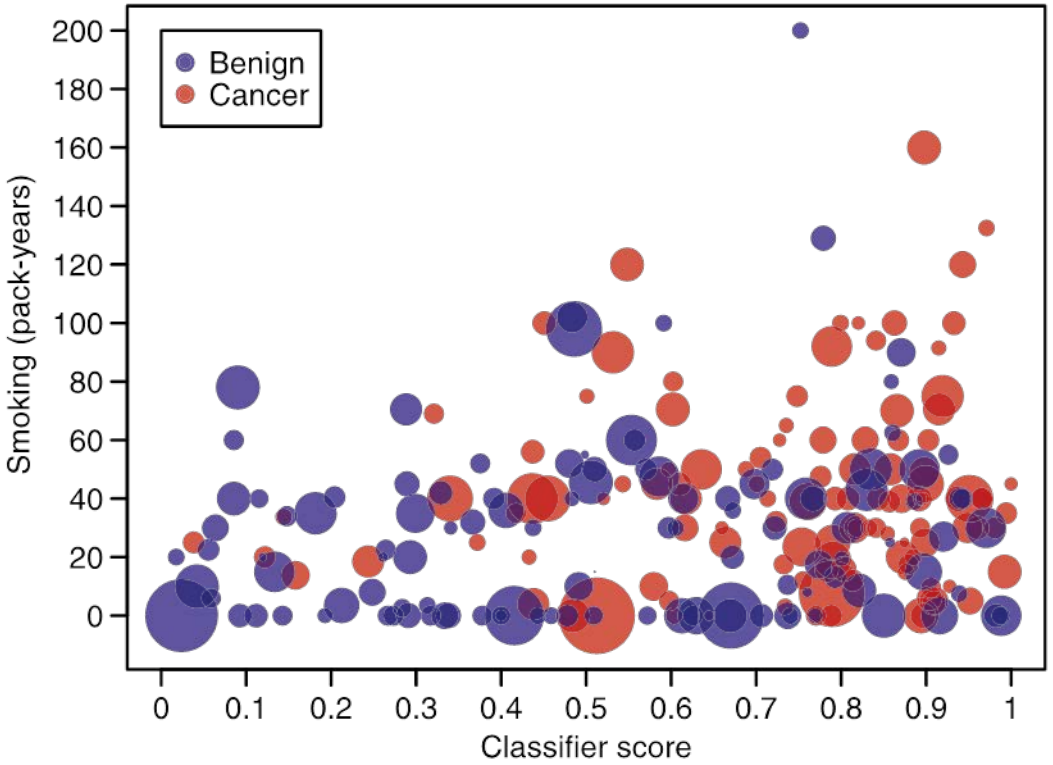
Number of proteins	Refinement
388	Lung cancer–associated protein candidates sourced from tissue and literature
371	Number of the 388 protein candidates successfully developed into an MRM assay
190	Number of the 371 MRM protein assays detected in plasma
125	Number of the 190 MRM protein assays detected in at least 50% of cancer or 50% of benign discovery samples
36	Number of the 125 detected proteins that were cooperative
21	Number of the 36 cooperative proteins with robust MRM assays (that is, no interfering signals, good signal-to-noise ratios, etc.)
13	Number of the 21 robust and cooperative proteins with stable logistic regression coefficients

- Developed 13 multiple reaction monitoring MRM assays. LRP1, BGH3, COIA1, TETN, TSP1, ALDOA,GRP78, ISLR, FRIL, LG3BP, PRDX1, FIBA, GSLG1
- Training and testing algorithm.

Peptide	Protein	Location
LRP1	Prolow-density lipoprotein receptor-related protein 1	Secreted
BGH3	Transforming Growth factor beta induced protein 3	Secreted
COIA1	Collagen alpha-1(XVIII) chain	Secreted
TETN	Tetranectin	Secreted
TSP1	Thrombospondin-1	Secreted
ALDOA	Fructose-bisphosphate aldolase A	Secreted
GRP78	78 kDa glucose-regulated protein	Endoplasmic reticulum
ISLR	Immunoglobulin superfamily containing leucine-rich repeat protein	Secreted
FRIL	Ferritin light chain	Secreted
LG3BP	Galectin-3-binding protein	Secreted
PRDX1	Peroxiredoxin-1	Cytoplasm
FIBA	Fibrinogen alpha chain	Secreted
GSLG1	Golgi apparatus protein 1	Golgi membrane

Li et al. Sci Transl Med. 2013 Oct

13 MRM predictor of lung cancer among 247 lung nodules 4-30 mm (prevalence 15%)



	N=	Sens	Spec	NPV	PPV
Discovery	143	82	66	95	30
Validation	104	71	44	90	18
Validation 2	37	79	56	94	24

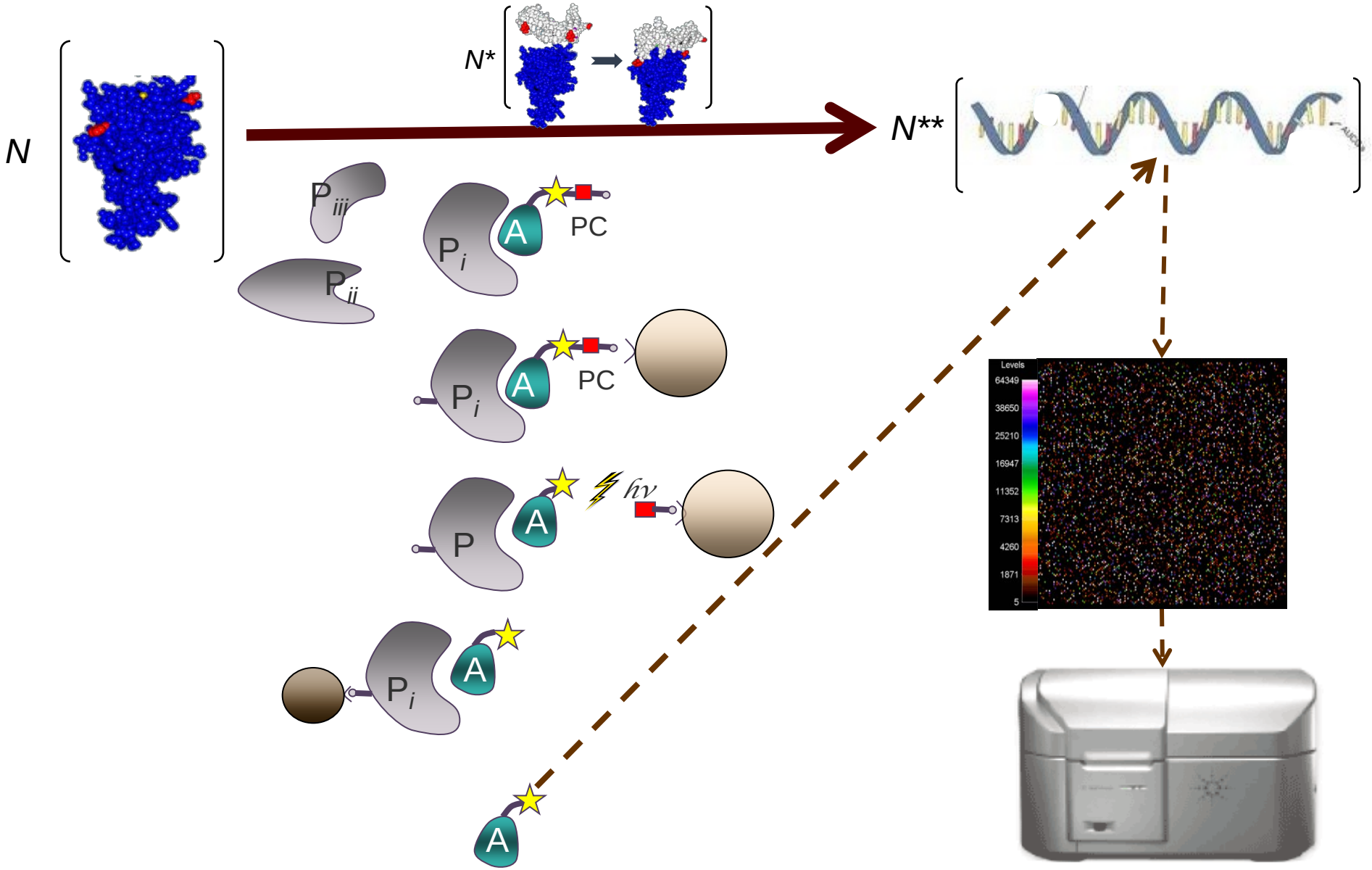
A negative test implies a >2 fold decrease risk for cancer.

High NPV of the test would obviate 1/4 patients with benign nodules from a biopsy

IF THE TEST SCORE IS 0.8 OR HIGHER, THEN THE CHANCE OF THE NODULE BEING BENIGN IS 90% OR MORE!!!!

Li et al. Sci Transl Med. 2013 Oct

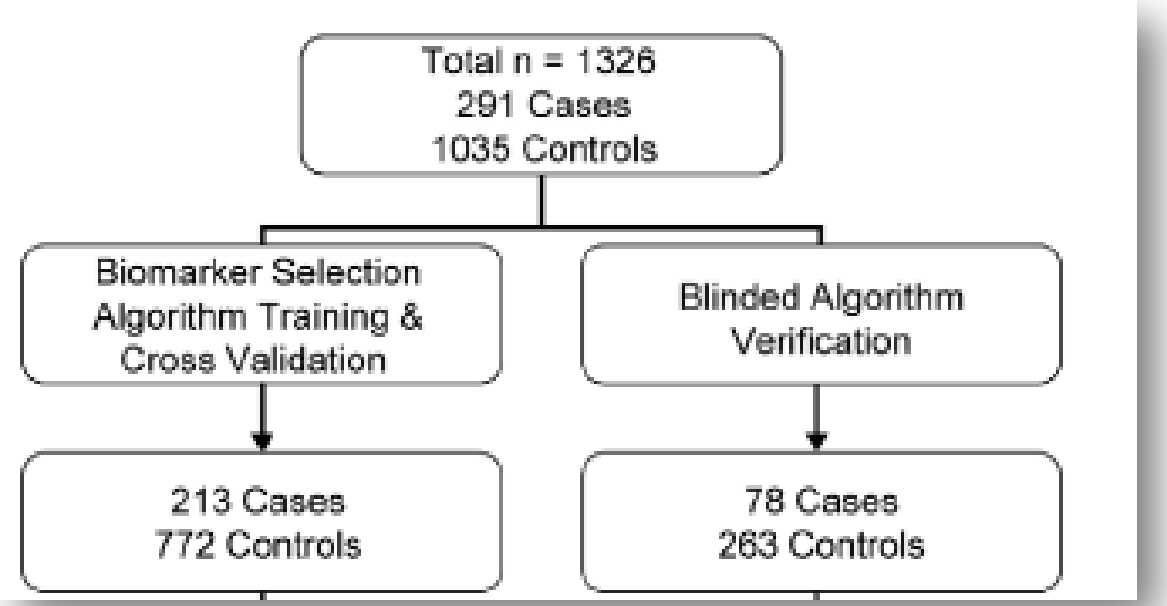
SomaLogic Slow Off Rate Modified Aptamers (SOMA) Platform

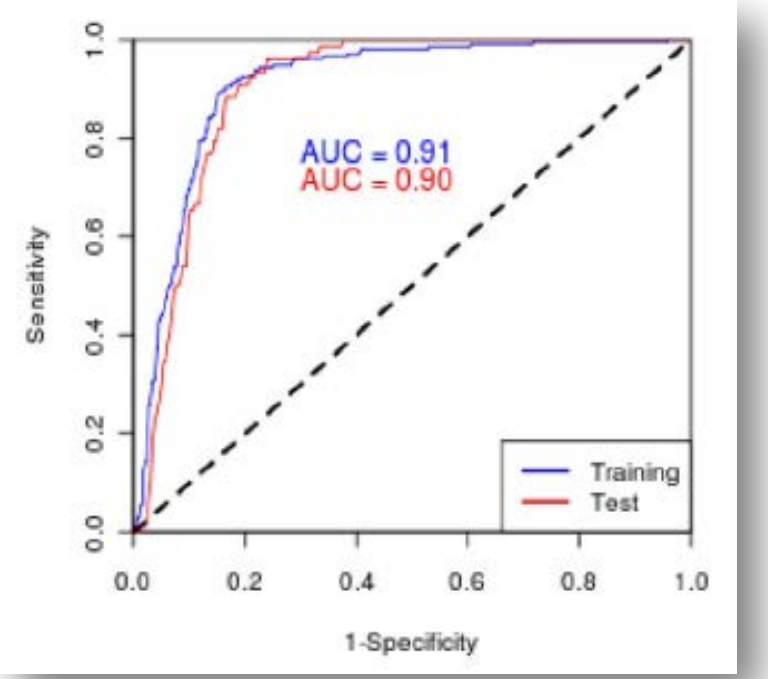


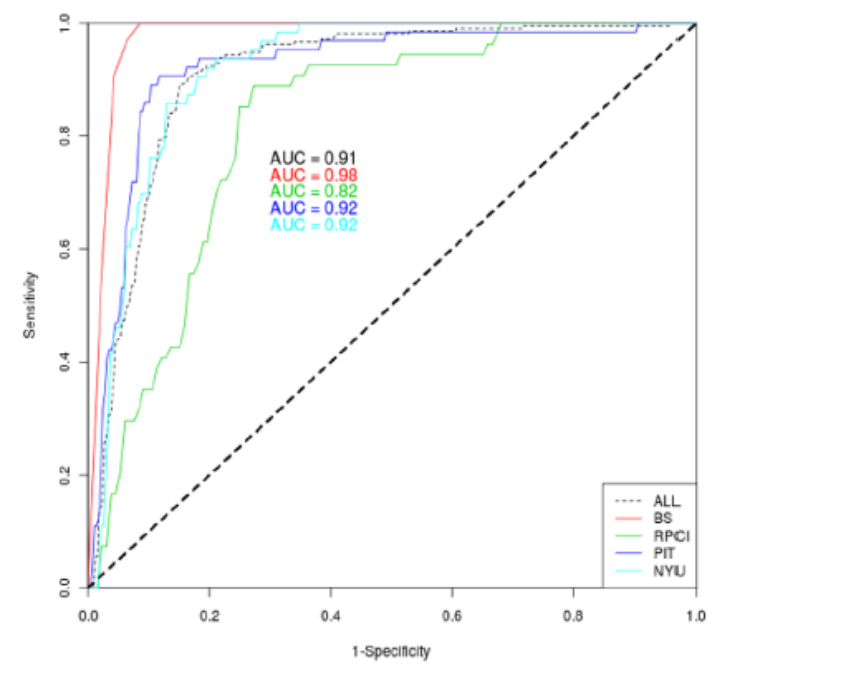
The SomaLogic Proteomic Platform: Modified Aptamer Technology

Metric	Conditions	Result
Sensitivity	Median LLOQ	~ 0.3 pM
Dynamic Range	Over all proteins in plasma or serum Each aptamer at one dilution	10^7 ~10^4
Precision	Median inter-run CV	5%
Sample Volume	Per sample, for >800 proteins and ~25 controls	8 uL
Multiplex Size	Current # unique human proteins per sample	1034
Time to result	Automated assay & hybridization detection	~30 hours
Throughput	One robot, 2.5 FTEs	168 samples/day >1M analytes/week

PLoS One. 2010 Dec 7;5(12):e15003

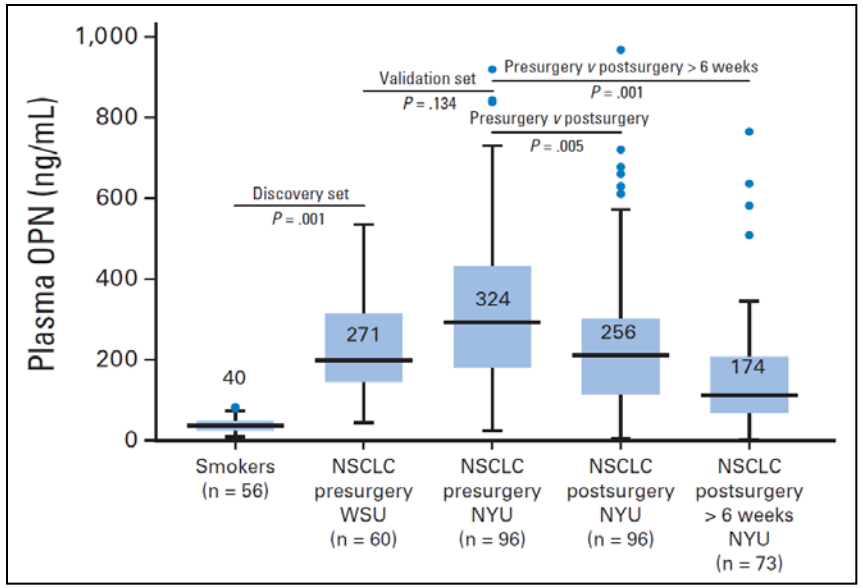


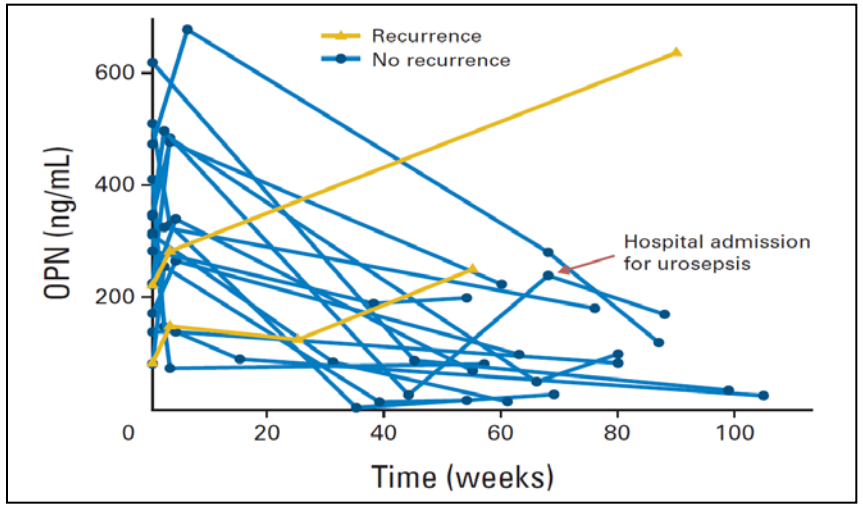




Biomarker	UniProt ID	Direction*	Description
Cadherin-1	P12830	down	cell adhesion, transcription regulation
CD30 Ligand	P32971	up	cytokine
Endostatin	P39060	up	inhibition of angiogenesis
HSP 90α	P07900	up	chaperone
LRIG3	Q6UXM1	down	protein binding, tumor suppressor
MIP-4	P55774	up	monokine
Pleiotrophin	P21246	up	growth factor
PRKCI	P41743	up	serine/threonine protein kinase, oncogene
RGM-C	Q6ZVN8	down	iron metabolism
SCF sR	P10721	down	decoy receptor
sL-Selectin	P14151	down	cell adhesion
YES	P07947	up	tyrosine kinase, oncogene

Osteopontin Plasma Levels in Early Stage NSCLC



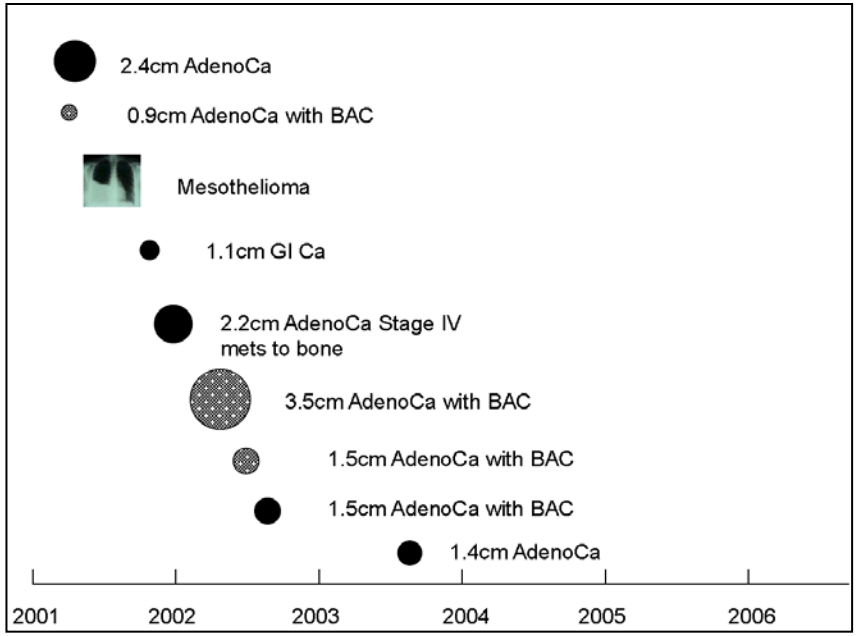


- Plasma OPN elevated in early stage NCSLC compared to smokers without cancer
- Plasma OPN levels decrease in response to resection
- Preliminary evidence for rise in plasma OPN with recurrence
- But…..what about longitudinal studies????????

Blasberg, J. JCO, Feb 2010.

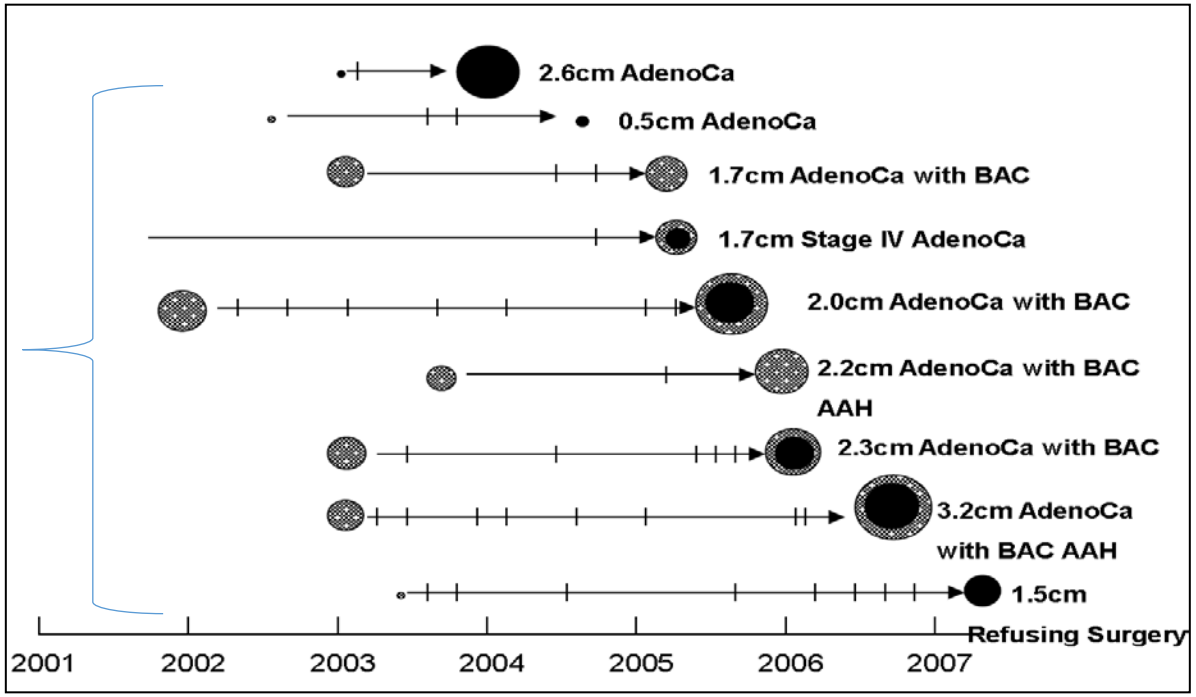
- 1182 subjects; 3771 CT scans with 2174 matched plasma samples.
- Twenty-six NSCLC have been detected since 2001
  - 10 prevalence cancers
  - 16 incident cancers.

Prevalent Cancers



Never Developed Cancer

Incident Cancers



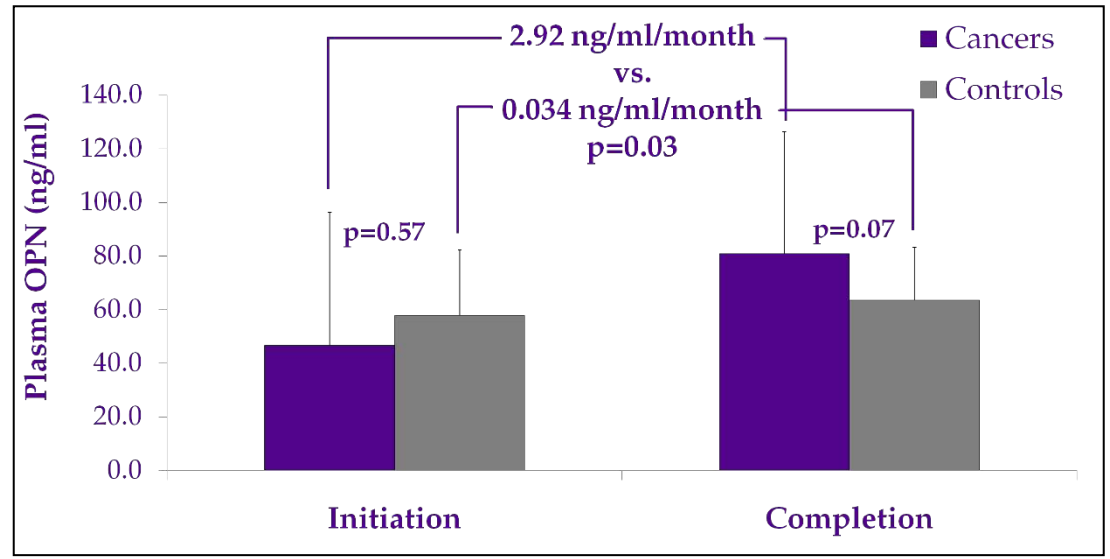
Courtesy NYU Biomarker Center

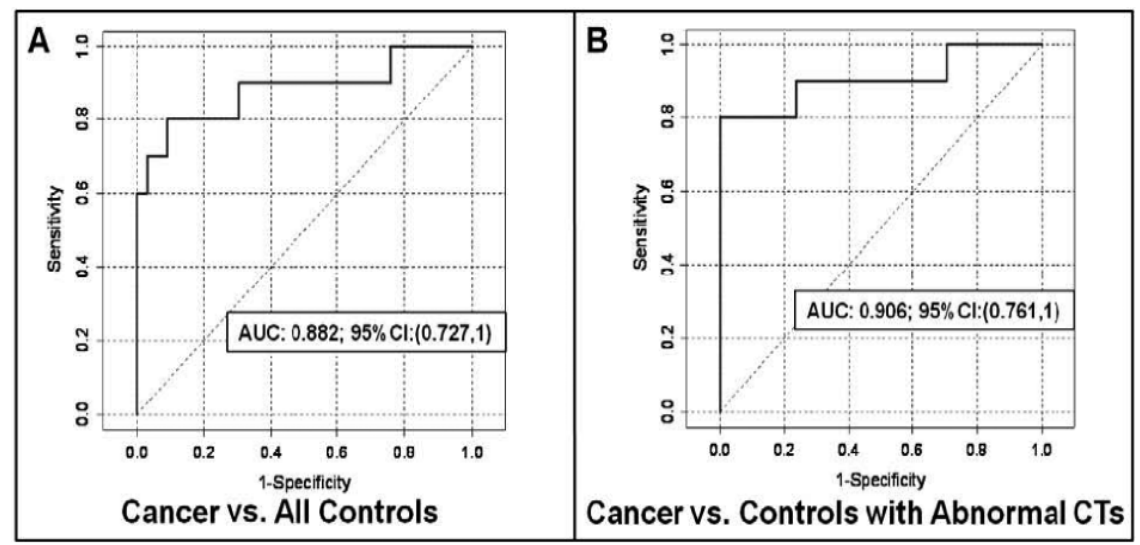
Plasma osteopontin velocity differentiates lung cancers from controls in a CT screening population

Sasha Joseph[a,b], Ryan Harrington[a], Dawn Walter[c], Judith D. Goldberg[d], Xiaochun Li[d], Amanda Beck[a], Tyler Litton[a], Nathalie Hirsch[a], Justin Blasberg[e], Mark Slomiany PhD[a], William Rom[c], Harvey Pass[a] and Jessica Donington[a,*]

Nested case-control study to evaluate plasma OPN as a diagnostic biomarker for NSCLC within this screening cohort.

- Prevalence cancers were excluded
- Incident cancers with serially banked plasma and 1–4 matched controls for each cancer were identified.
- Matched for age, sex, pack years, and the duration of the surveillance interval over which plasma samples were collected.
- Controls were considered free of cancer due to lack of clinical progression on CT screening for an interval of at least 24 months following evaluated surveillance interval.





Cancer Biomarkers. 2012;12(4):177-84.

MicroRNA signatures in tissues and plasma predict development and prognosis of computed tomography detected lung cancer: Boeri and C. Croce, OSU

- Objective:
  - Measure plasma mirs which can pre-diagnose CT detected lung cancer, as well as diagnose lung cancer at the time of CT detection.
- Methods:
  - Two large Italian lung cancer screening programs (28 cancers and 53 cancers)
    - One for discovery and the other for validation.
  - Use microfluidic cards to determine top 100 microRNA profiles of pre-diagnostic (>1y) and diagnostic plasmas
  - For normalization, mirs were expressed as ratios to each other and normalized to plasmas from individuals not developing lung cancer.

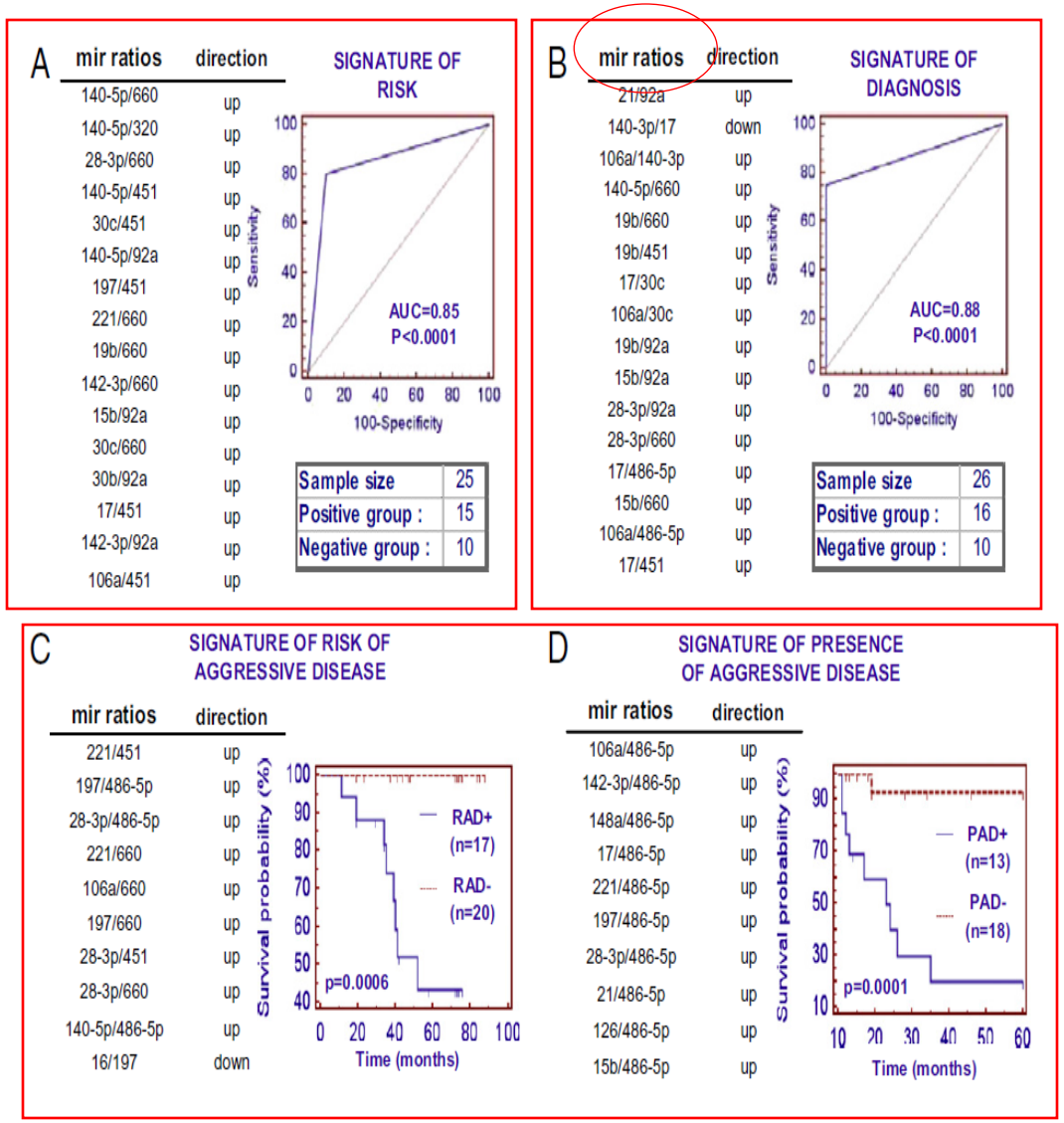
Proc Natl Acad Sci U S A. 2011 Mar 1;108(9):3713-8.

RESULTS

PreDiagnosis: 16 ratios/15 miRNAs discriminated developing lung cancer (80% sp, 90% se); *Validation had a sensitivity of 80%and specificity of 90% (AUC-ROC = 0.85, P < 0.0001*

Diagnosis: 16 ratios/13 miRNAs classified lung cancer (80%sp, 84% se); *Validation had a sensitivity of 75% and a specificity of 100% (AUC-ROC = 0.88, P < 0.0001*

Prognosis: 10 ratios/ 9 miRNAs, identified poor prognosis (dead or with progressive disease) with 100% sensitivity and 100% specificity, and in the validation set, the sensitivity of this signature was 80% with 100% specificity.



Proc Natl Acad Sci U S A. 2011 Mar 1;108(9):3713-8.

Plasma Exosomes for Screening and Diagnosis

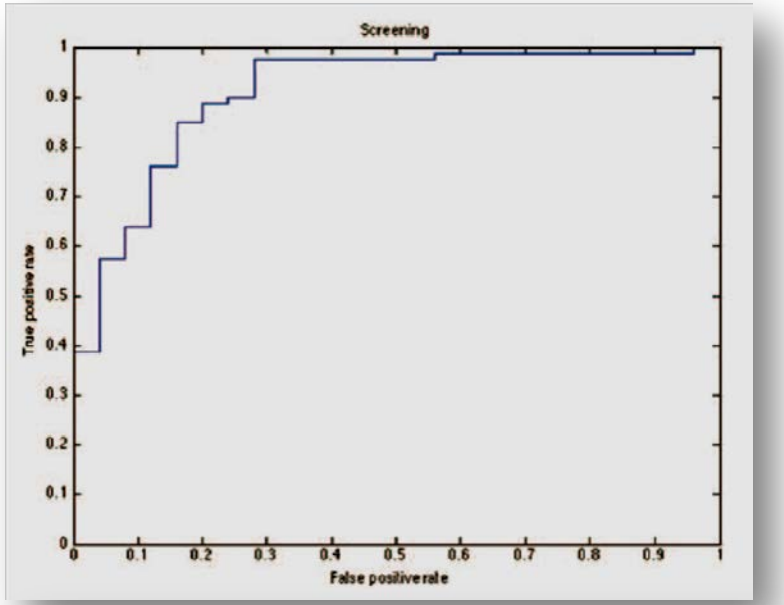
J. Thorac. Oncol. 2013;8: 1156-1162

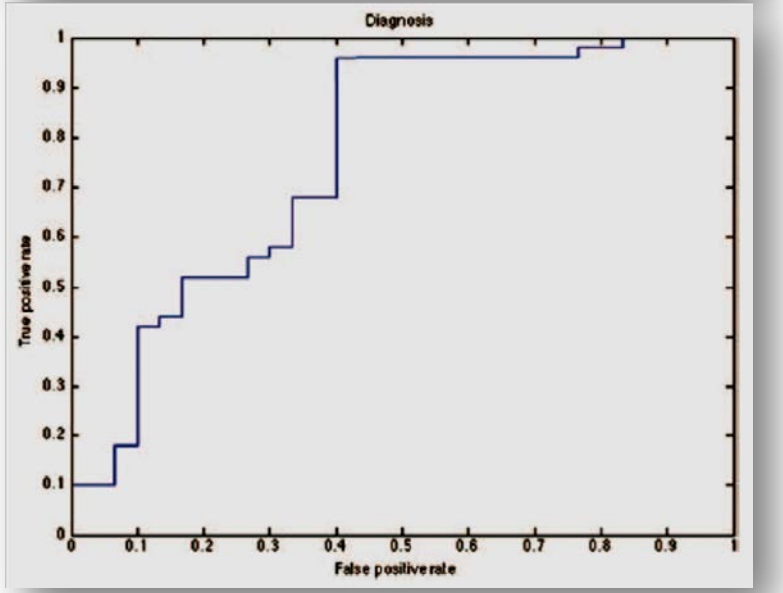
Plasma Exosomes: Population and Results

Variable	Adenocarcinomas (n = 50)	Granulomas (n = 30)	Healthy (n = 25)	p
Study set	(n = 10)	(n = 10)	(n = 10)	
Age, mean ± SD	66.1±14.0	64.8±13.7	65.6±7.4	0.971[a]
Sex, n (%)				0.861[b]
Male	3 (30.0)	3 (30.0)	4 (40.0)	
Female	7 (70.0)	7 (70.0)	6 (60.0)	
Smoking habit, n (%)				0.024[b]
Yes	5 (50.0)	5 (50.0)	10 (100.0)	
No	5 (50.0)	5 (50.0)	—	
Nodule size, mean ± SD	1.42±0.24	1.34±0.56	—	0.683[c]
Validation set				
Age, mean ± SD	70.5±9.2	65.6±9.5	66.7±8.8	0.050[a]
Sex, n (%)				0.126[b]
Male	25 (50.0)	11 (36.7)	16 (64.0)	
Female	25 (50.0)	19 (63.3)	9 (36.0)	
Smoke habits, n (%)				0.151[b]
Yes	41 (82.0)	16 (53.3)	3 (12.0)	
No	9 (18.0)	11 (36.7)	22 (88.0)	
Unknown	—	3 (10.0)	—	
Nodule size, mean ± SD	1.49±0.45	1.34±0.56	—	0.192[c]

[a]One-way ANOVA test.
[b]χ^2 test.
[c]Student's *t* test for unpaired data.
ANOVA, analysis of variance.

Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	AUC ROC
Screening test, %	97.5	72	92	90	90.8
Screening algorithm	α = 0.86 + (miR378a) (−0.3) + (miR379) (0.19) + (miR-139-5p) (0.37) + (miR-200b-5p) (−0.17)				
Diagnostic test, %	96	60	80	90	76
Diagnostic algorithm	α = −0.89 + (miR-151a-5p) (−0.15) + (miR-200b-5p) (0.07) + (miR-30a-3p) (0.05) + +(miR629) (−0.17) + (miR100) (0.04) + (miR-154-3p) (−0.05)				





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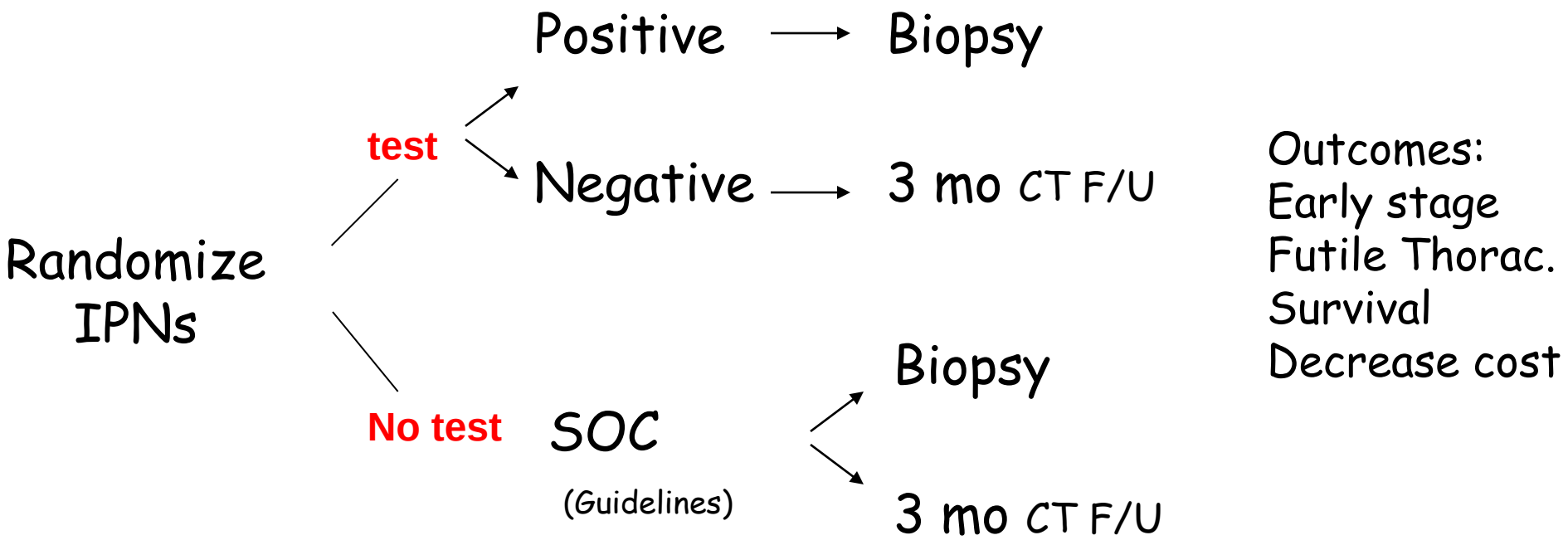
Clinical utility of a diagnostic biomarker: study design



Randomization of nodules based on the use of a biomarker test.
Proves that biomarker "+" affects patients outcome
Proves that biomarker test affects patients outcome when compared with unselected use of same Standard Of Care.

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