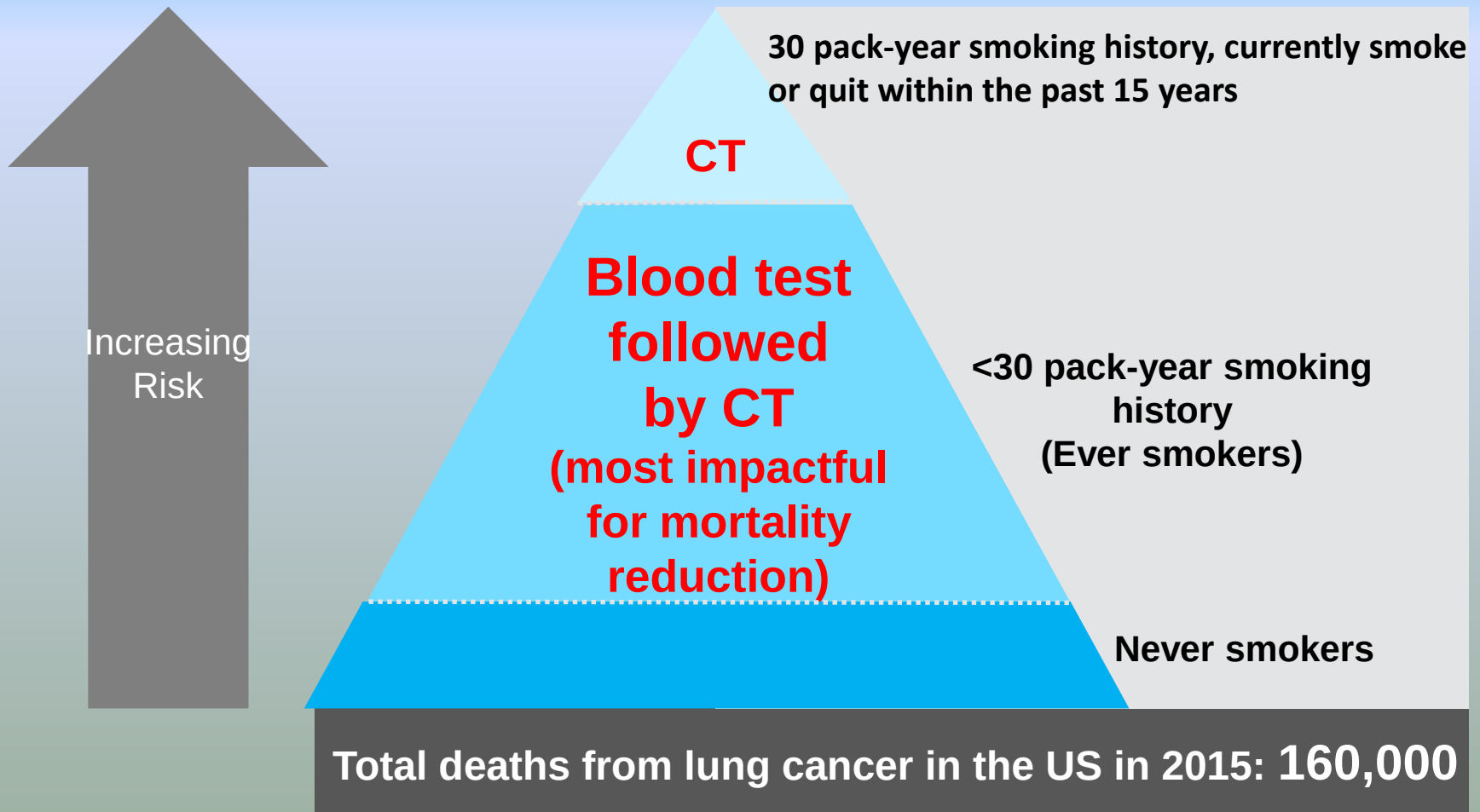


Development of a blood based marker panel for SCLC

S. Hanash

The US Preventive Services Task Force recommends low-dose CT annually only for high risk adults



Study Objectives

- A marker panel that signals a high risk for developing or harboring SCLC
- Take advantage of available cohorts to test the performance of known and other promising marker candidates
- The developed marker panel would complement a panel for NSCLC currently being validated intended to trigger CT based screening.

Investigators

- **MD. Anderson**

S. Hanash, I. Wistuba, H. Tran, A. Taguchi, Z. Feng,

- **UT Southwestern**

A. Gazdar, D. Gerber

- **U of Pittsburgh**

J M. Yuan

Challenges in the Development and implementation of a screening panel for SCLC

- Subjects at risk for SCLC are also at risk for NSCLC, thus there is a need to integrate markers across LC subtypes
- Substantial heterogeneity in both NSCLC and SCLC
- A blood based panel to trigger screening for LC among asymptomatic subjects has to pass the cost effectiveness requirements

Integrative discovery strategy

Mouse models

Genomics

Glycomics

Proteomics

Biomarker panels

Cancer cells

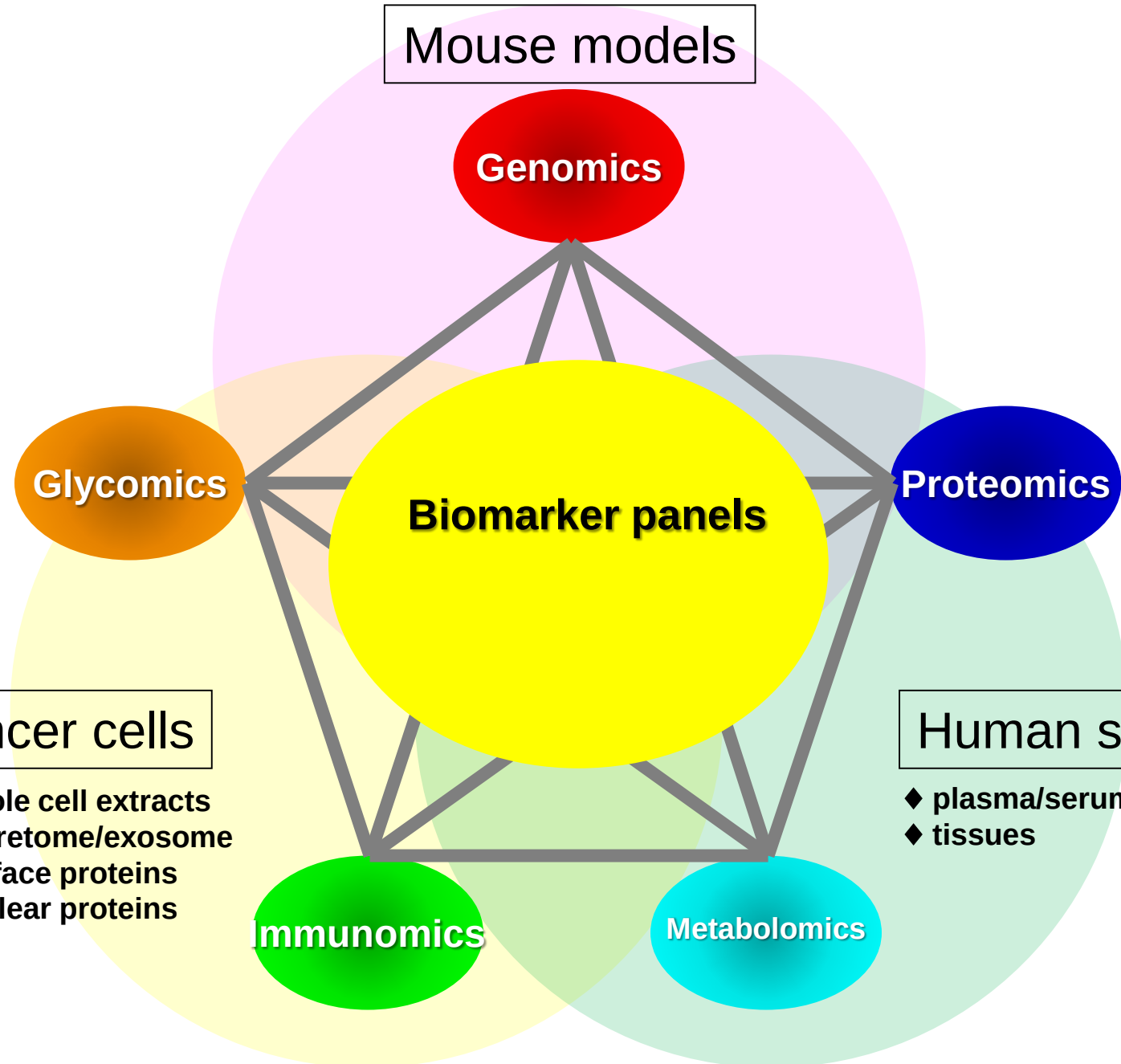
- ◆ whole cell extracts
- ◆ secretome/exosome
- ◆ surface proteins
- ◆ nuclear proteins

Human studies

- ◆ plasma/serum
- ◆ tissues

Immunomics

Metabolomics



Cohen J et al CancerSEEK, Science 2018

[CancerSEEK and destroy — a blood test for early cancer detection ...](#)

<https://www.nature.com> › nature reviews clinical oncology › research highlights

Search for blood based biomarkers through cohort studies

Blood collected up to 10 yrs
prior to Dx

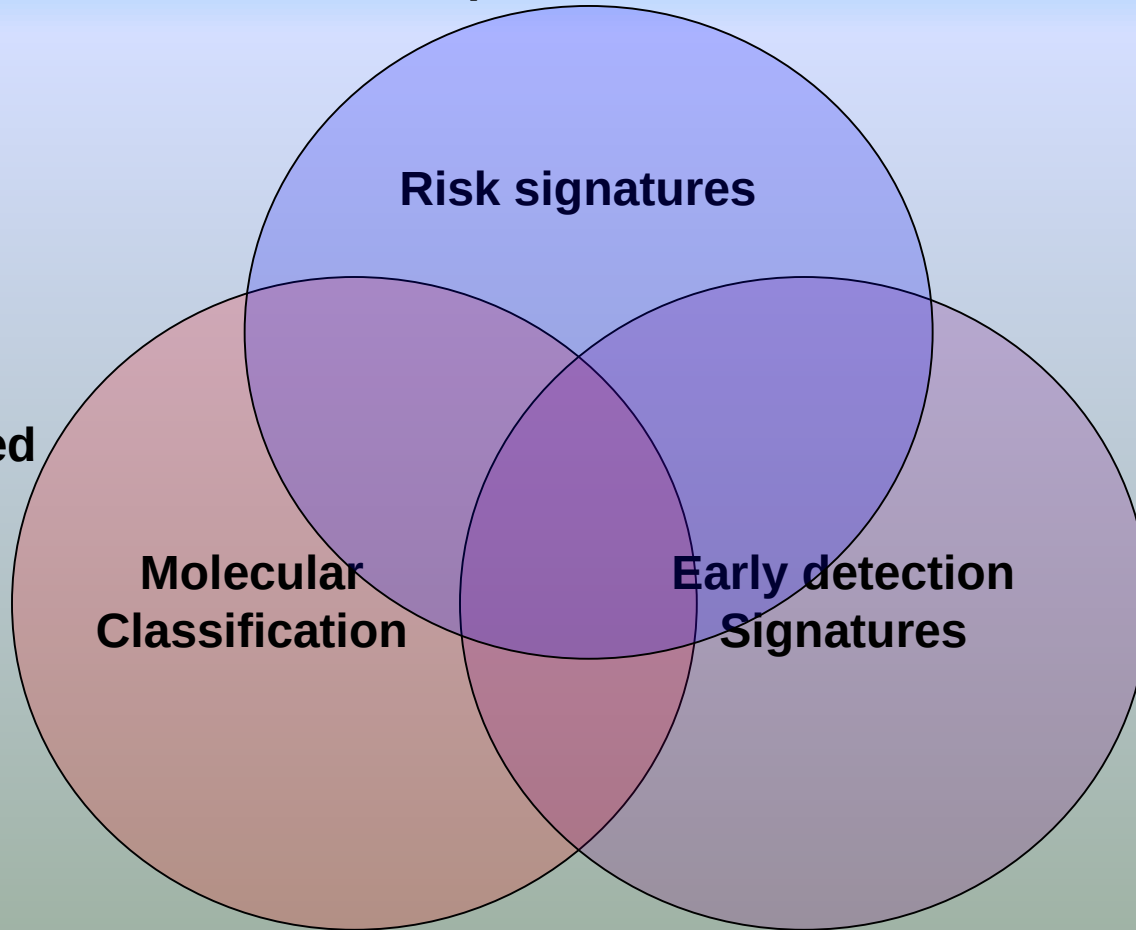
Risk signatures

Blood collected
at Dx

Molecular
Classification

Early detection
Signatures

Blood collected
0-24 months
prior to Dx





Metabolomics Platform

- Liquid-Chromatography-based Mass Spectrometry
- **Untargeted Profiling; 2 column Configuration**

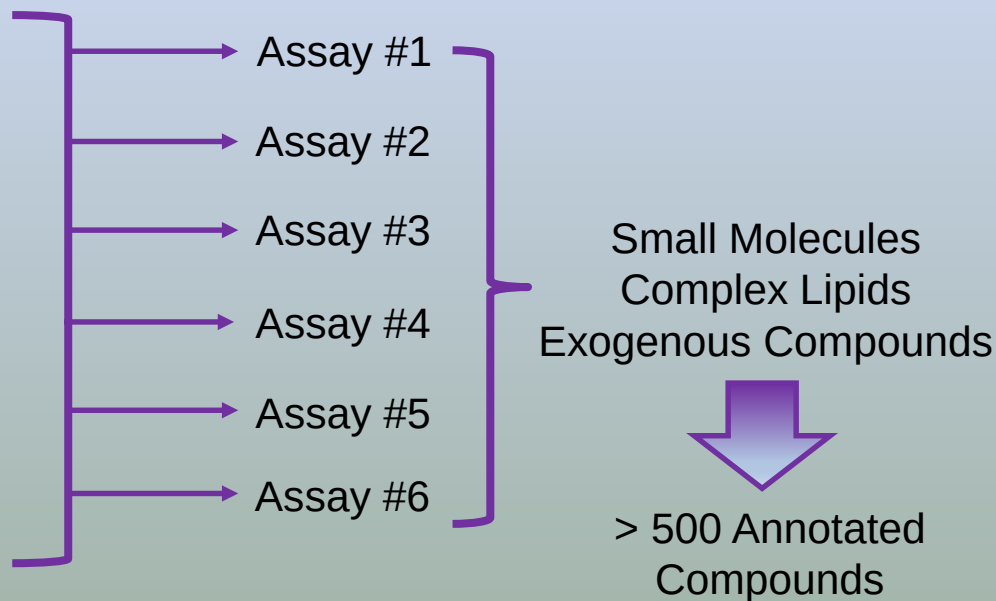
Four Instruments in Lab



ACQUITY
UPLC System



Xevo
G2-XS QToF



Search for proteins, genes, or peptide in IPAS experiments.

Search in

Identifiers ▼

Query

Search is case insensitive.
 Use % as wildcard for any number of character.
 Use _ as wildcard for a single character.

Quantitation

 Fold Change

 p-value <=

« Selected Experiments

IP0154_v313 - Human - Lung
 IP0155_v313 - Human - Lung
 IP0156_v313 - Human - Lung
 IP0157_v313 - Human - Lung
 IP0158_v313 - Human - Lung
 IP0159_v313 - Human - Lung
 IP0160_v313 - Human - Lung
 dIP0161_v313 - Human - Lung
 IP0161_v313 - Human - Lung
 IP0162_v313 - Human - Lung

12 IPASes selected.

Quick Selectors

Organism Cancer

 Human ▲
 Mouse ▼

 Breast ▲
 Colon
 Leukemia
 Lung
 Melanoma
 Ovarian ▼

Non-Cancer

 CHD ▲
 Confounder
 GVHD
 HT
 Kidney
 Stroke ▼

Control

Control ▲

Version

 v313 ▲
 v329
 v341
 v348
 v357
 v387 ▼

☒ Include IG Bound MS runs

By Gene

By IPI

Fractions

Peptides

BigSearch

File	Type	SampleDesc	Gene	Desc	MW	BestPr	TTotall	TNumI	MTotall	MNRat	MNum	STotall	SNRati	SNumI	NTotall	NNRat	NNumI
P0379_v357	Lung	H82 (SCLC)	CHGA	chromogranin-a.	50,688	1.00	14	1	443	39.15	1	16	1.67	1			
P0382_v357	Lung	DMS79 (SCLC)	CHGA	chromogranin-a.	50,688	1.00	35	1	411	23.49	1	17	0.49	1	0	0.00	0
P0397_v357	Ovarian	Ovca432	CHGA	chromogranin-a.	50,688	1.00	40	1	226	7.29	1	41	1.30	1	0	0.00	0
P0381_v357	Lung	H69 (SCLC) (E	CHGA	chromogranin-a.	50,688	1.00	34	1	193	7.83	1	9	0.31	1	0	0.00	0
P0325_v357	Lung	H1568 (Female	CHGA	chromogranin-a.	50,688	1.00	1	2	171	151.85	1	0	0.00	1	0	0.00	1
P0355_v357	Lung	H1385 (Female	CHGA	chromogranin-a.	50,688	1.00	80	1	112	4.21	1	18	0.31	2			
P0387_v357	Lung	H1793 (Female	CHGA	chromogranin-a.	50,688	1.00	0	1	63	168.63	1	2	2.99	1	0	0.00	1
P0399_v357	Ovarian	Skov3	CHGA	chromogranin-a.	50,688	1.00	2	2	27	24.33	1	0	0.00	1	0	0.00	0
P0348_v357	Lung	H522 (Male: sr	CHGA	chromogranin-a.	50,688	1.00	0	1	25	47.52	1	0	0.00	1			
P0302_v357	Colon	SW480	CHGA	chromogranin-a.	50,688	1.00	0	1	23	62.96	1	0	0.00	1	0	0.00	1
P0395_v357	Ovarian	Ovcar8	CHGA	chromogranin-a.	50,688	1.00	0	1	18	23.45	1	0	0.00	1	0	0.00	0
P0383_v357	Lung	H1573 TGFβ T	CHGA	chromogranin-a.	50,688	1.00	0	1	15	22.97	1	0	0.00	1			
P0352_run2	Lung	H2291 (Male: r	CHGA	chromogranin-a.	50,688	1.00	0	1	12	29.15	1	0	0.00	1	0	0.00	1
P0336_v357	Lung	H1573 (Female	CHGA	chromogranin-a.	50,688	1.00	0	1	11	16.83	1	0	0.00	1	0	0.00	1
P0318_run2	Lung	H838 (Male: S	CHGA	chromogranin-a.	50,688	1.00	0	1	8	8.81	1	0	0.00	1	0	0.00	1
P0394_v357	Ovarian	Ovcar3	CHGA	chromogranin-a.	50,688	1.00	0	1	8	15.86	1	0	0.00	1	0	0.00	0
P0361_v357	Pancrea	PANC-1 (epithe	CHGA	chromogranin-a.	50,688	1.00	0	1	5	9.25	1	0	0.00	1	0	0.00	1

IPAS	Fraction	AX	RP	Type	Stage	SampleDesc	Frac	Uniq	Tota	Num	100	200	300	400
▼	▼	▼	▼	▼	▼	▼	▼							
IP0381	SG01to21 AXs/Co-		SG01to21	Lung		H69 (SCLC) (Epitl	01x24	12	25	1				
IP0381	SG22to24 AXs/Co-		SG22to24	Lung		H69 (SCLC) (Epitl	01x24	9	25	1				
IP0381	SG25to26 AXs/Co-		SG25to26	Lung		H69 (SCLC) (Epitl	01x24	12	37	1				
IP0381	SG27to28 AXs/Co-		SG27to28	Lung		H69 (SCLC) (Epitl	01x24	16	53	1				
IP0381	SG29to30 AXs/Co-		SG29to30	Lung		H69 (SCLC) (Epitl	01x24	2	2	1				
IP0381	SG48to49 AXs/Co-		SG48to49	Lung		H69 (SCLC) (Epitl	01x24	2	2	1				
IP0381	SG50to51 AXs/Co-		SG50to51	Lung		H69 (SCLC) (Epitl	01x24	4	4	1				
IP0381	SG52to53 AXs/Co-		SG52to53	Lung		H69 (SCLC) (Epitl	01x24	4	5	1				
IP0381	SG54to55 AXs/Co-		SG54to55	Lung		H69 (SCLC) (Epitl	01x24	4	5	1				

1 Records from 1 to 462 of 462

Legends: IPAS - Non-Cys containing id'd peptide (Up-extreme/ Up-regulated/ No Quant/ No Change/ Down-regulated/ Down-extreme) Cys containing
Cell Line - id'd peptide (Up/ No Change/ Down) Media:TCE | Surface:TCE | ... @ protein level

By Gene

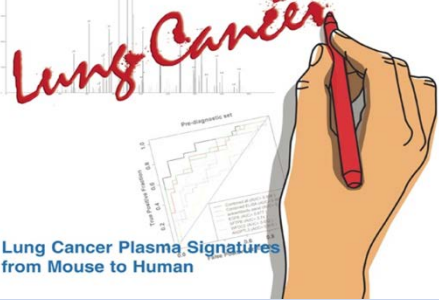
By IPI

Fractions

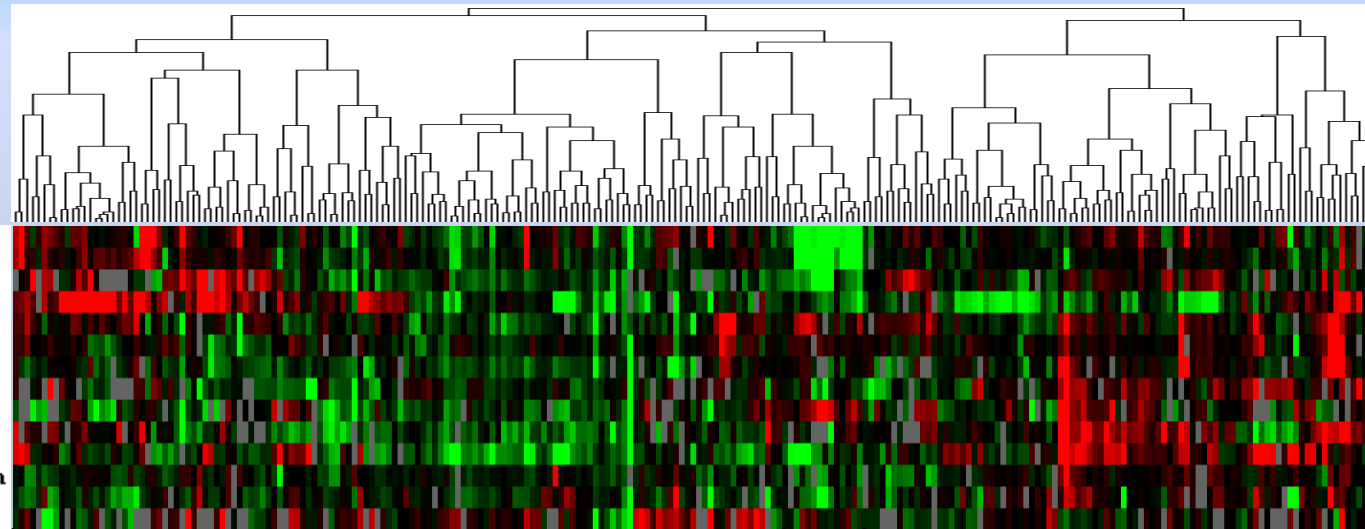
Peptides

BigSearch

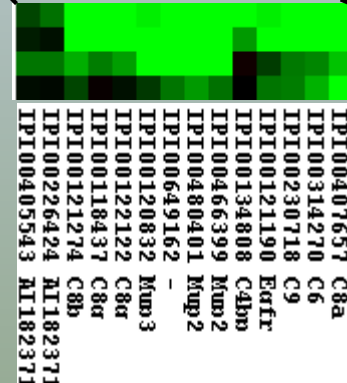
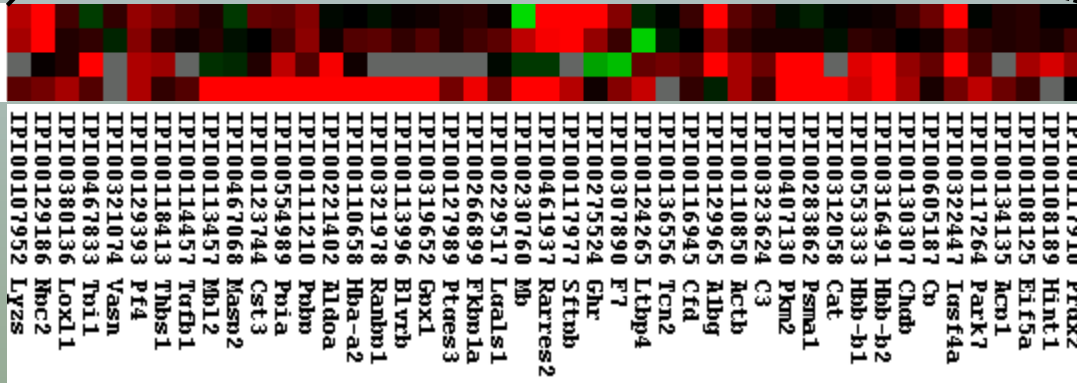
File	Type	SampleDesc	Gene	Desc	MW	BestPr	TTotall	TNumI	MTotall	MNRat	MNum	STotall	SNRati	SNumI	NTotall	NNRati	NNumI
0394_v357	Ovarian	Ovcar3	CD47, IAF	isoform oa3-323 of leukocyt	35,214	1.00	7	1	20	4.77	1	29	5.03	1	0	0.00	0
0385_v357	Lung	H3255 TGFb Tre	CD47, IAF	isoform oa3-323 of leukocyt	35,214	1.00	0	1	4	6.71	1	21	27.11	1	0	0.00	0
0357_v357	Lung	H920 (Male: Cau	CD47, IAF	isoform oa3-293 of leukocyt	31,743	1.00	2	5	0	0.00	1	20	7.42	1	4	1.98	1
0397_v357	Ovarian	Ovca432	CD47, IAF	isoform oa3-323 of leukocyt	35,214	1.00	0	1	8	11.54	1	12	16.32	1	0	0.00	0
0396_v357	Ovarian	Ovcar10	CD47, IAF	isoform oa3-293 of leukocyt	31,743	1.00	2	5	12	7.32	1	11	6.17	1	0	0.00	0
0351_v357	Lung	H820 (Male: nor	CD47, IAF	isoform oa3-293 of leukocyt	31,743	1.00	4	5	8	2.51	5	10	2.87	1	0	0.00	1
0339_v357	Lung	HCC2279 (Fema	CD47, IAF	41 kda protein.	41,047	0.96	2	1	0	0.00	1	8	4.13	1	0	0.00	1
0354_v357	Lung	HCC2935 (Male:	CD47, IAF	isoform oa3-293 of leukocyt	31,743	1.00	0	1	7	23.99	5	7	9.84	5	0	0.00	1
0356_v357	Lung	H1703 (Male: sn	CD47, IAF	isoform oa3-293 of leukocyt	31,743	1.00	0	1	4	12.18	5	6	8.03	5	0	0.00	1
0333_v357	Lung	HCC827 (Female	CD47, IAF	isoform oa3-293 of leukocyt	31,743	1.00	0	1	4	7.09	5	6	9.46	5	0	0.00	1
0393_v357	Lung	H1838 (Female:	CD47, IAF	isoform oa3-293 of leukocyt	31,743	1.00	1	5	6	10.65	5	4	2.78	5	0	0.00	0
0338_v357	Lung	HCC4011 (Male:	CD47, IAF	isoform oa3-293 of leukocyt	31,743	0.98	0	1	2	3.55	5	4	4.93	1	0	0.00	1
0395_v357	Ovarian	Ovcar8	CD47, IAF	isoform oa3-293 of leukocyt	31,743	1.00	0	1	4	5.99	5	3	5.28	1	0	0.00	0
0373_v357	Pancre	Hypoxia CFPAC-	CD47, IAF	isoform oa3-293 of leukocyt	31,743	1.00	0	1	1	3.08	5	3	4.59	5	0	0.00	0
0370_v357	Pancre	Hypoxia BxPC-3	CD47, IAF	isoform oa3-293 of leukocyt	31,743	0.93	0	1	1	3.59	5	3	4.65	5	0	0.00	0
0342_v357	Lung	H1792 (Male: sn	CD47, IAF	41 kda protein.	41,047	0.82	0	1	0	0.00	1	3	5.17	1			
0311_v357	Ovarian	OVCA3 (differe	CD47, IAF	isoform oa3-293 of leukocyt	31,743	1.00	9	1	3	0.98	1	3	2.05	5			



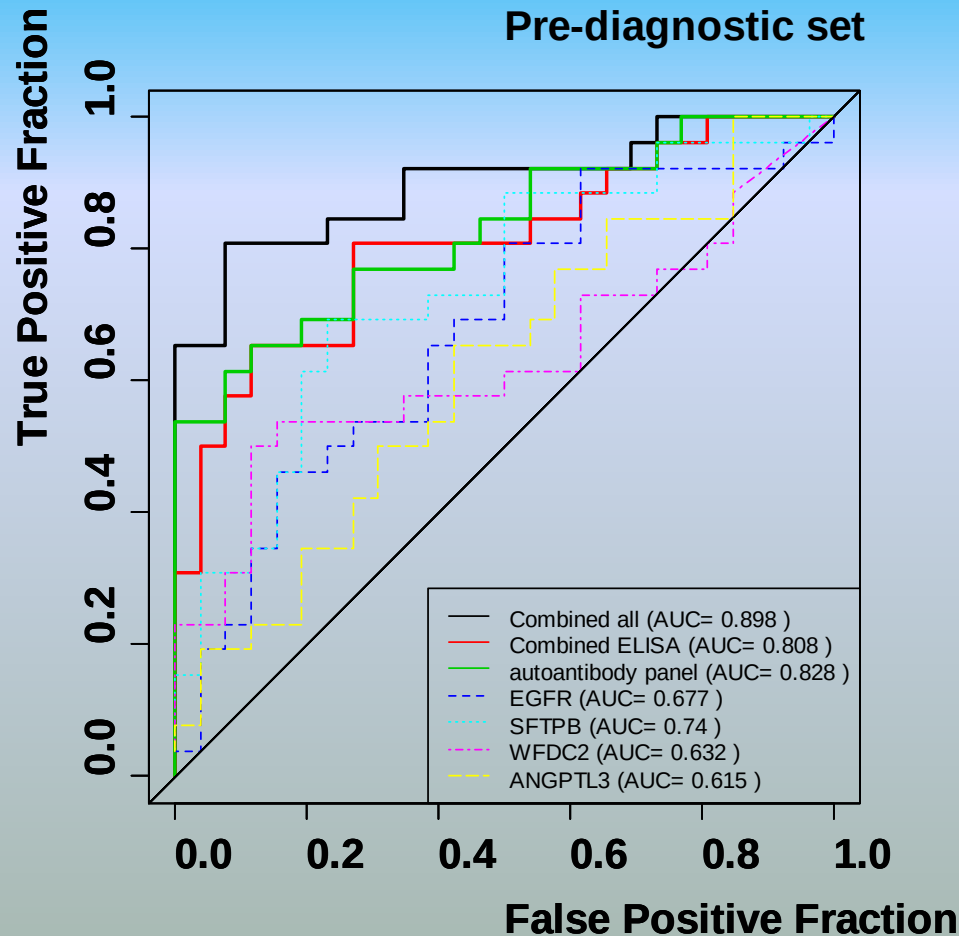
NKX2-1 signature proteins in the blood of lung cancer mouse models



Lung-EGFR
Lung-Kras
Lung-SCLC
Lung-Urethane



Plasma levels of potential biomarkers in pre-diagnostic lung cancer subjects



Pre-diagnostic set				
Assay	Normal	Cancer	T-test	Mann Whitney test
EGFR	1.00 ± 0.048	0.87 ± 0.051	0.0635	0.0287
SFTPb	1.00 ± 0.110	1.57 ± 0.144	0.0027	0.0031
WFDC2	1.00 ± 0.120	1.54 ± 0.237	0.0489	0.1049
ANGPTL3	1.00 ± 0.075	1.17 ± 0.080	0.1192	0.1561

Autoantibody panel (Qiu et al. JCO 2009):
ANXA1
YWHAQ
LAMR1

SFTPb in lung cancer mouse models

Lung cancer mouse models

IPAs	Fraction	AX	RP	Type	Stage	SampleDesc	Frac	Uniq	Total	Num	1	100	200	300	377
IP0058-		-	-	Lung		Varmus EGFR	08x12	6	35	8					
IP0085-		-	-	Lung		Varmus Kras	08x12	5	8	3					
IP0129-		-	-	Lung		Urethane	08x12	6	18	4					
IP0131-		-	-	Lung		Varmus EGFR Reg	08x12	6	17	5					

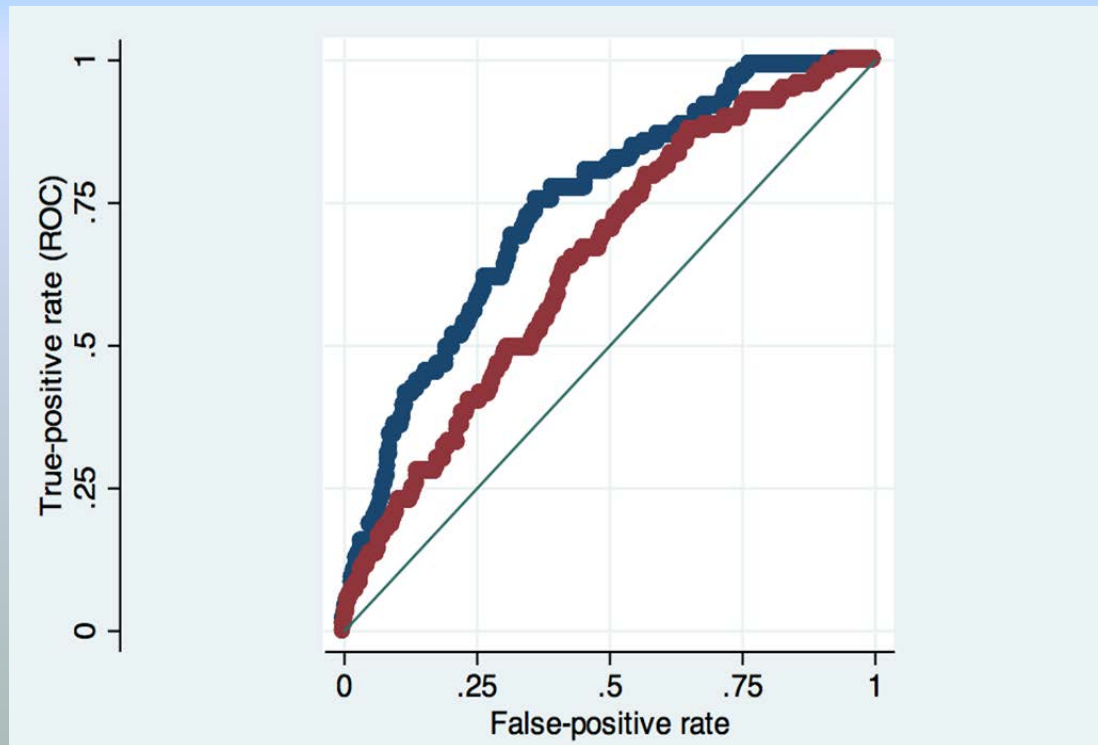
Concordant predominance of pro-SFTPb in Human lung cancer plasma

pro-SFTPb vs mature SFTPb assays

ELISA	Newly diagnosed (28 case/ 39 control)			Pre-diagnostic (26 case/ 26 control)		
	p-value		AUC	p-value		AUC
	Unpaired t test	Mann Whitney test		Unpaired t test	Mann Whitney test	
mature SFTPb	0.0708	0.0332	0.654	0.0027	0.0031	0.74
proSFTPb	0.0004	0.0002	0.77	< 0.0001	0.0001	0.811

Obs	variable	group	Control N	Control Median	Control 25th %tile	Control 75th %tile	LC case N	LC case Median	LC case 25th %tile	LC case 75th %tile	AUC	P value (Wilcoxon test)
1		Total	216	2.5E-01	1.4E-01	3.7E-01	108	4.2E-01	2.6E-01	6.8E-01	0.70	0.0000
2	case_exp	Asbestos	70	2.5E-01	1.4E-01	3.4E-01	35	4.4E-01	2.2E-01	8.4E-01	0.72	0.0003
3	case_exp	Heavy smoker	146	2.5E-01	1.3E-01	3.9E-01	73	4.0E-01	2.7E-01	6.7E-01	0.69	0.0000
4	sex	Female	66	2.6E-01	1.6E-01	3.9E-01	33	5.2E-01	2.8E-01	7.5E-01	0.69	0.0018
5	sex	Male	150	2.4E-01	1.4E-01	3.5E-01	75	4.2E-01	2.6E-01	6.2E-01	0.70	0.0000
6	smstatus	Current smoker	144	2.6E-01	1.7E-01	3.9E-01	72	4.8E-01	2.8E-01	7.4E-01	0.71	0.0000
7	smstatus	Former smoker	72	2.1E-01	1.1E-01	3.2E-01	36	3.5E-01	2.0E-01	5.8E-01	0.69	0.0011
8	case_stag e	I and II	52	2.6E-01	1.5E-01	3.7E-01	26	4.3E-01	2.8E-01	6.2E-01	0.69	0.0062
9	case_stag e	III and IV	128	2.5E-01	1.3E-01	3.8E-01	64	3.9E-01	2.2E-01	7.1E-01	0.69	0.0000
10	case_his	Adenocarinoma	80	2.5E-01	1.6E-01	3.9E-01	40	3.3E-01	2.1E-01	6.8E-01	0.63	0.0177
11	case_his	Squamous cell	76	2.3E-01	1.4E-01	3.5E-01	38	4.5E-01	3.1E-01	7.5E-01	0.75	0.0000
12	case_his	Other NSCLC	60	2.5E-01	1.3E-01	3.7E-01	30	5.1E-01	2.6E-01	6.6E-01	0.72	0.0008
13	lt6ge6mos	0 to 6 months	80	2.5E-01	1.4E-01	3.8E-01	40	4.3E-01	3.2E-01	7.4E-01	0.74	0.0000
14	lt6ge6mos	6 to 12 months	136	2.5E-01	1.4E-01	3.5E-01	68	4.0E-01	2.2E-01	6.7E-01	0.67	0.0001

Is there evidence that blood markers could be useful? Validation in the pan Canadian Lung Ca screening study



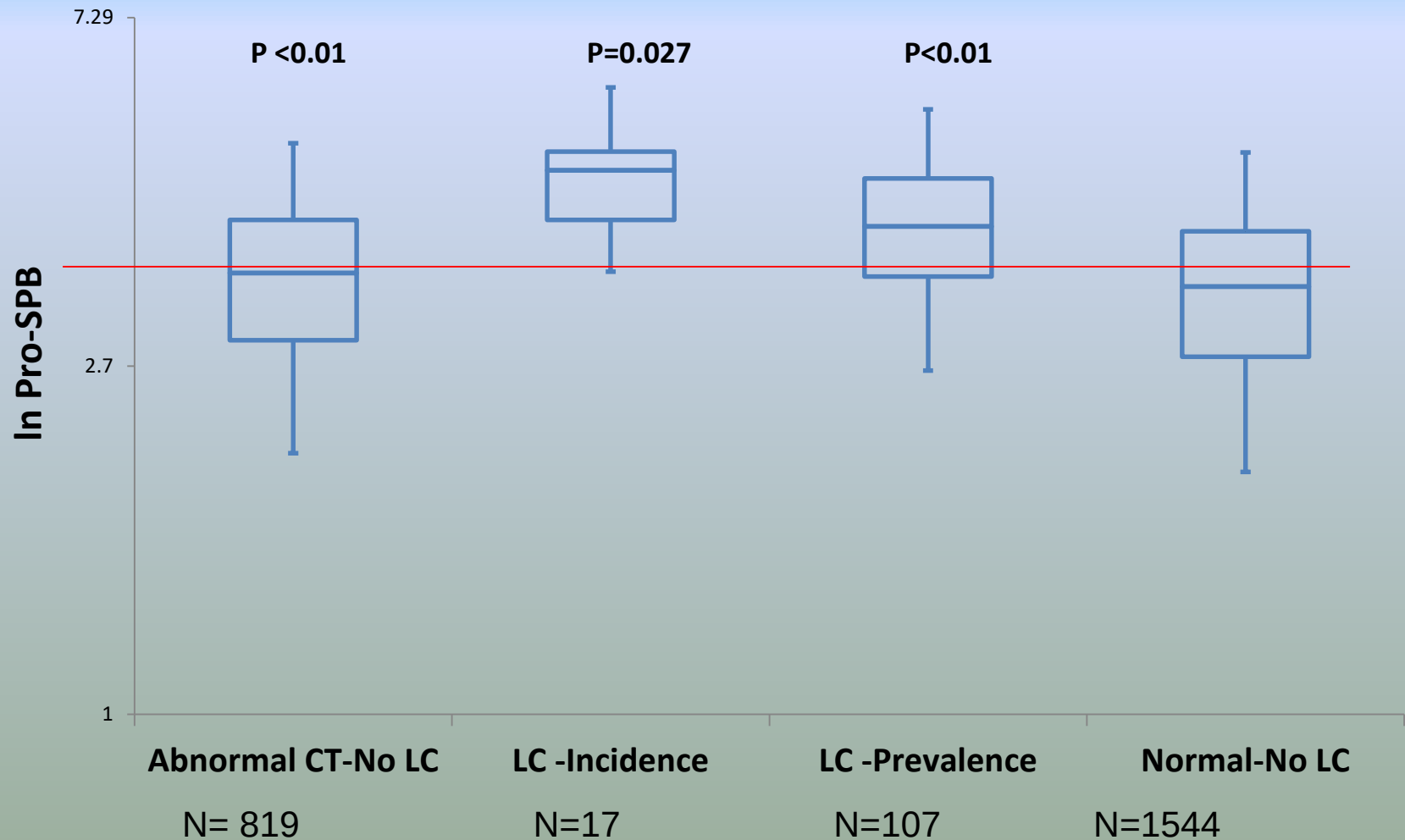
Red: Base model: AUC = .642

Blue: Base model + 1 marker (AUC = .736)

Difference in AUCs $p = .0002$

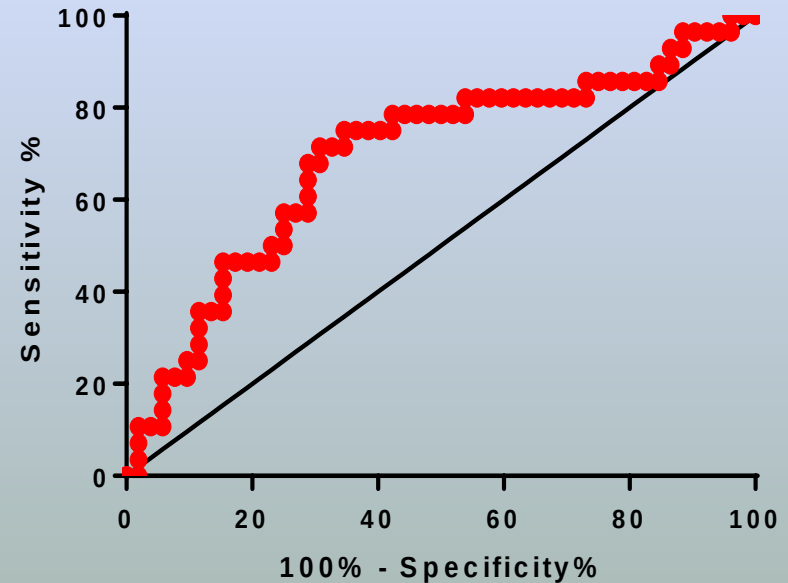
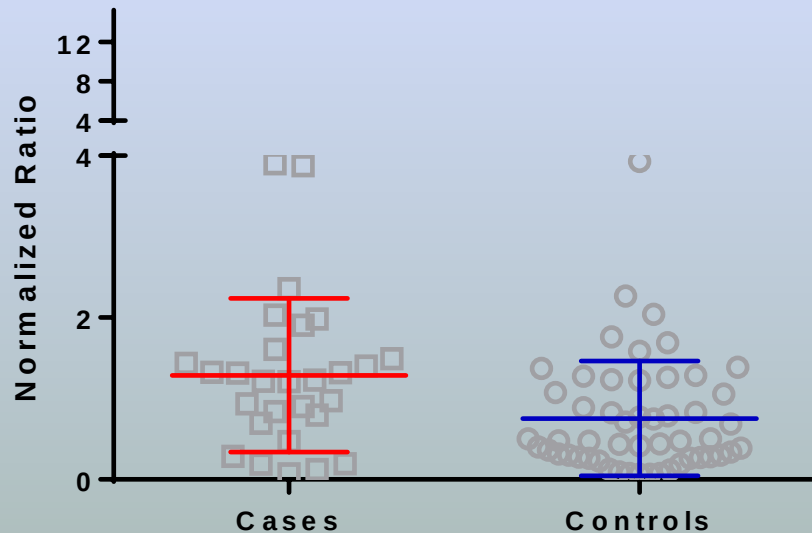
Sin D et al JCO 2015

Pro-Surfactant Protein B Identifies Incident Lung Cancers



Diacetylspermine (DAS)

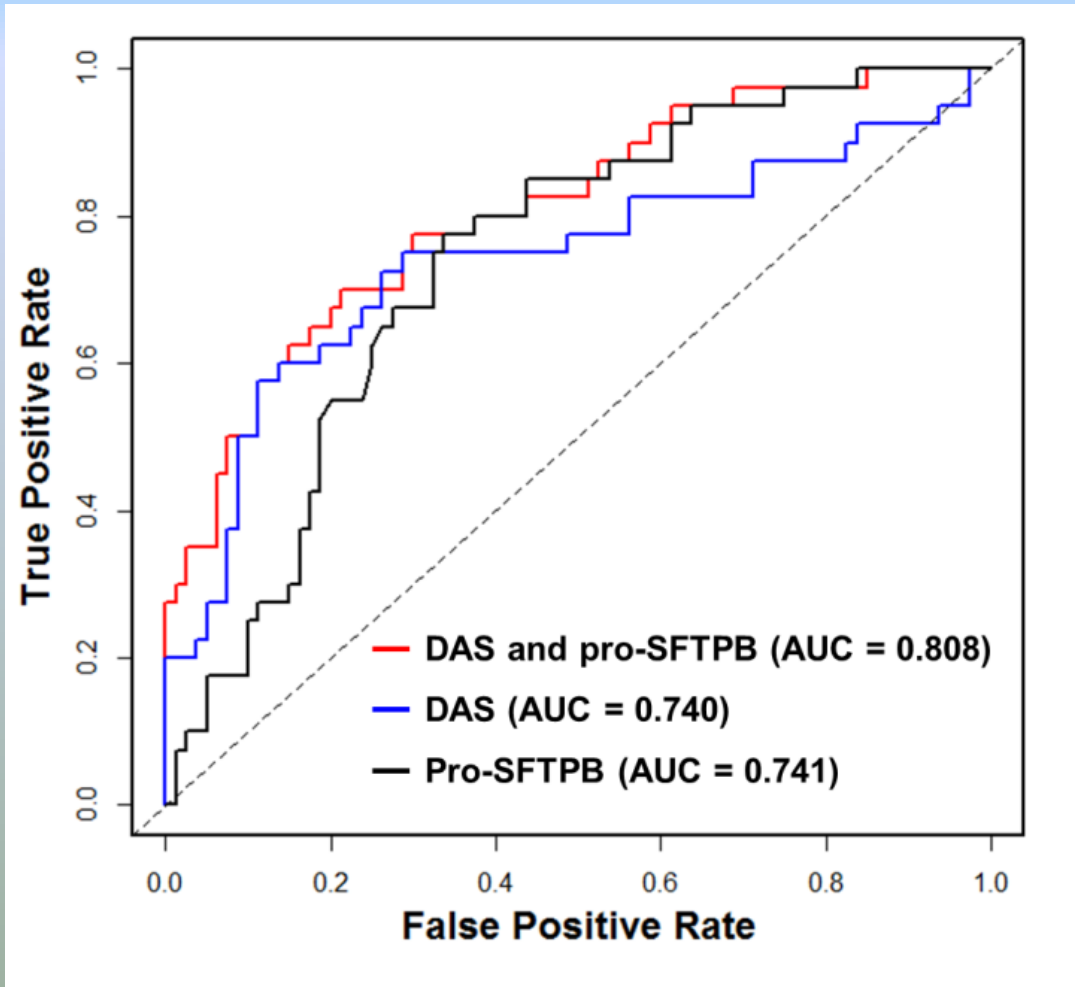
Performance based on PanCan screening cohort



AUC: 0.6916 (0.5649 to 0.8184)
P: 0.0049

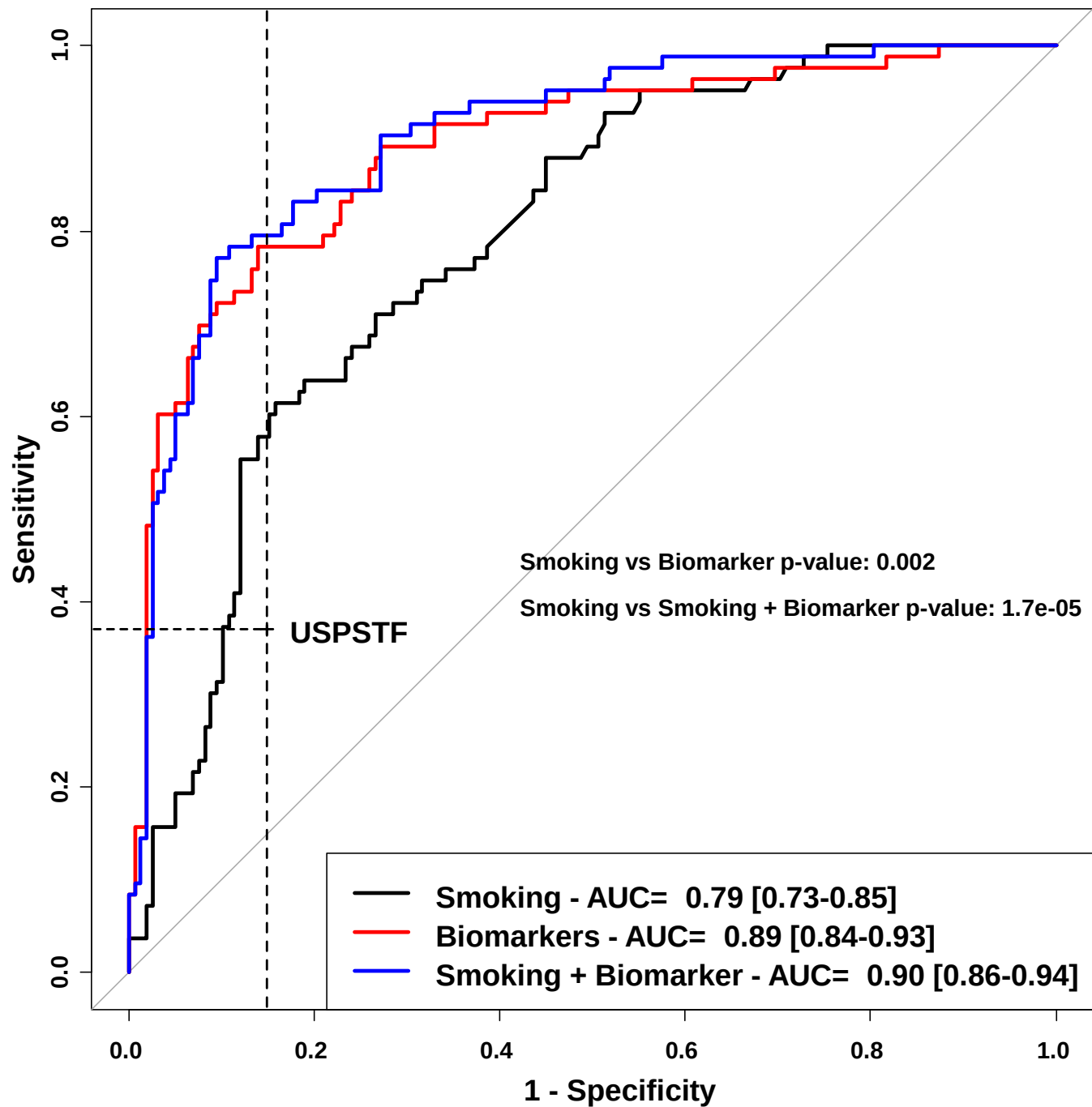
Testing of combinations of markers in pre-diagnostic samples

Sample collection: 0-6 months prior to diagnosis



$P < 0.0001$; vs. pro-SFTPb alone

Wikoff et al. JCO 2015



Prospective Screening Study

**US: MD Anderson, UT Tyler, USC,
Stanford, Pittsburgh,**

MD Anderson Regional Centers

China (three provinces)

Europe (France, Spain,)

Latest Accrual Figures

As of March 3, **8,614**

Ongoing SCLC focused studies

- Blinded validation of SCLC marker candidates using European EPIC/Umea pre-diagnostic SCLC samples (proteins, metabolites, autoantibodies)
- New mass spec based discoveries using the same samples
- Exhaustive mass spec analysis of SCLC cell lines representing disease heterogeneity

**Consortium Collaborations are
welcome!**

Acknowledgements

- **Lab group:** Mitzi Aguilar, Clemente Aguilar, Michela Capello, Julian Casabar, Muge Celiktas, Juan Chen, Jennifer Dennison, Dilsher Dhillon, Corinthia Emery, Johannes Fahrman, Hiroyuki Katayama, Makoto Kobayashi, Deepali Kundnani, Jon Ladd, Vivuen liu, Amin Momin, Eunice Murage, Nikul Patel, Nan Sun, Ayumu Taguchi, Saty Tripathi, Peiling Tsou, Nese Unver, Jody Vykokal, Hong Wang, Peng Wang, Hanasen, Xu, B. Yang, Chuan-Yih Yu.
- **Lung Ca Collaborators:** C. Amos, L. Bantis,, P. Brennan, A. G. Calin, K. Do, Z. Feng, O. Fiehn, D. Gandara, S. Gambhir, A. Gazdar, G. D. Gerber, Goodman, M. Johansson, M. Katz, J. Kim, K. Kinzler, H. Levine, S. Moghaddem, V. Nair, E. Ostrin, F. Perera, R. Prentice, A. Rundle, H. Sesso, E. Szabo, A. Taguchi, M. Tammemagi. M. Thornquist, H. Varmus, B. Vogelstein, D. Wilson, I. Wistuba, H. Ying, J. Yuan.