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Potential biomarkers for screening

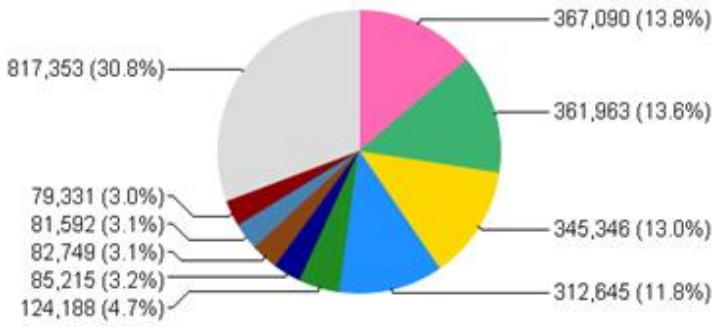
Gabriella Sozzi, Ph.D.

Disclosure slide

- Research Supported by:
- Italian Association for Cancer Research (AIRC) from 2005-2007, 2008-2010, 2011-2013
- Italian Ministry of Health (2005-2008; 2012-2014)
- National Cancer Institute (EDRN UO1 CA166905) from 2013
- Research funding was received from Gensignia from 2012

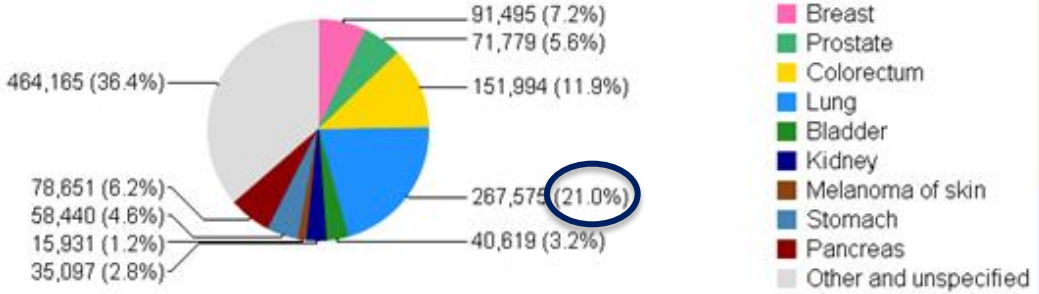
EUROPEAN UNION (EU 28)Estimated incidence, mortality: both sexes

Incidence



International Agency for Research on Cancer
World Health Organization

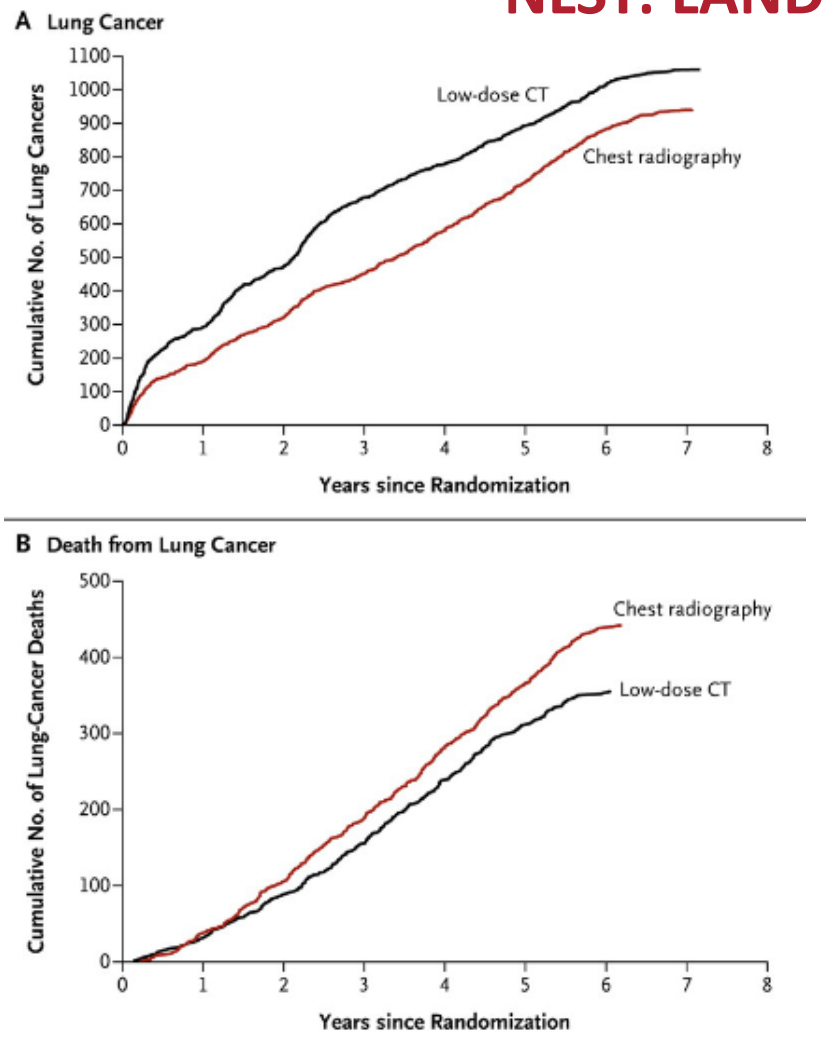
Mortality



- The overall 5-year survival rate for **Non-Small Cell Lung Cancer** which accounts for over 85% of total lung cancer cases, has risen from only 12% to 16% in the past four decades
- Only 30% of NSCLC diagnoses are caught before progressing to later stages of growth, contributing to the gap between the prevailing survival rate and the best case survival rate
- Properly treated **early stage** NSCLC has a 5-year survival rate variously cited to be as **high as 80%**
- Early diagnosis and proper treatment are more important for NSCLC than any other cancer

Early detection is essential to improve survivability through identification and therefore treatment of patients before their cancers become inoperable and lethal

NLST: LANDMARK TRIAL[1]



- randomized screening trial. 53,454 persons: 3 rounds of LDCT annual screening vs CXR
- 20% reduction of lung cancer mortality; 7% reduction all cause mortality
- 24.2% positive subjects
- 96.4% of these false positive
- need to screen 320 subjects to prevent 1 lung cancer death
- Overdiagnosis by LDCT[2]: > 18% number of cases of overdiagnosis in the 320 subjects above[2]: 1.38

Unmet clinical needs:

➢Reduce rate of false positives after initial screen

➢Reduction of overdiagnosis through more effcient prediction of aggressive disease

[1]*Aberle DR,. N Engl J Med 2011*
[2] *Patz EF., JAMA 2013*

Organisers

IASLC International Association for the Study of Lung Cancer

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE
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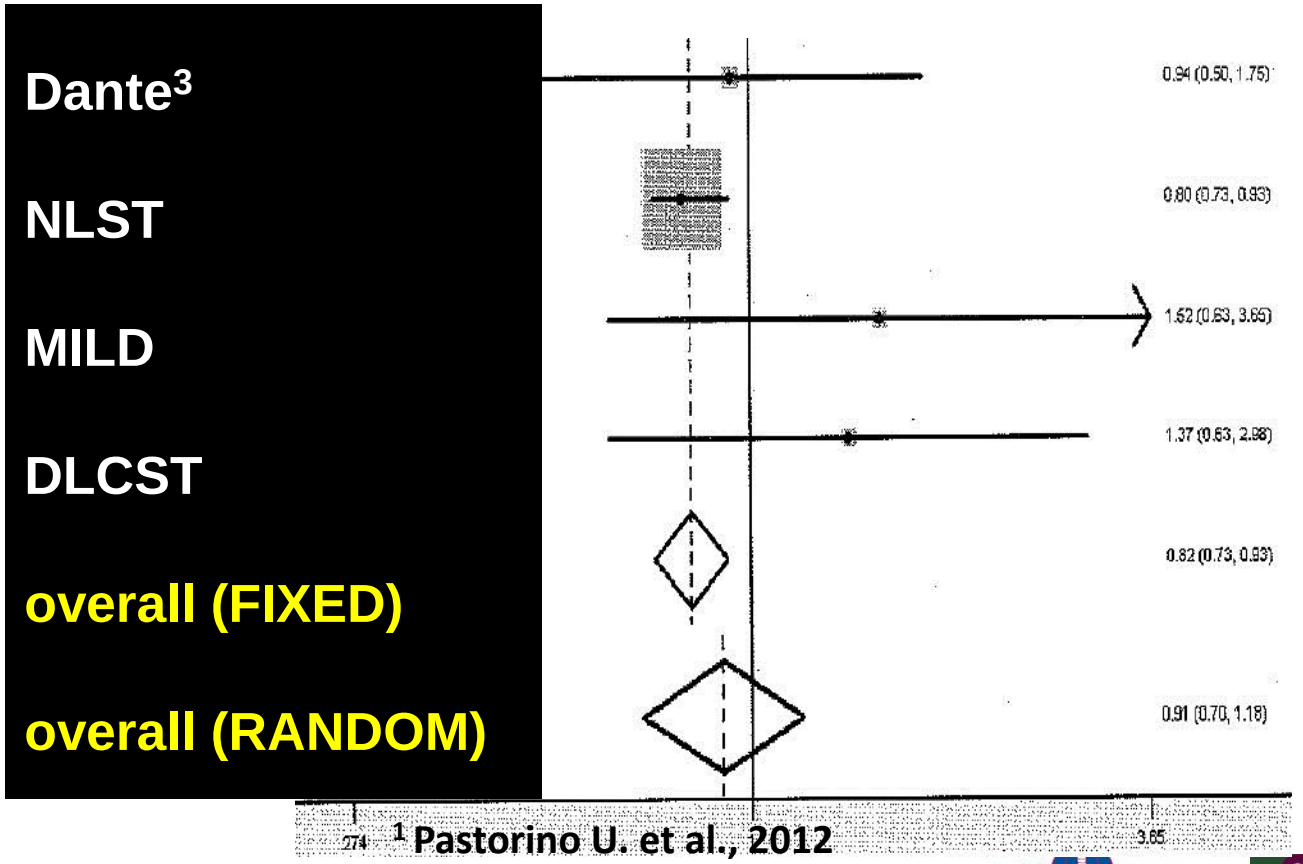
CT screening & mortality: meta-analysis of four published trials

MILD[1] + DLCST[2] = higher mortality
all four trials = small difference



[1] Pastorino U. et al., 2012
[2] Saghir Z.et al. 2012
[3] Infante M. et al, 2009

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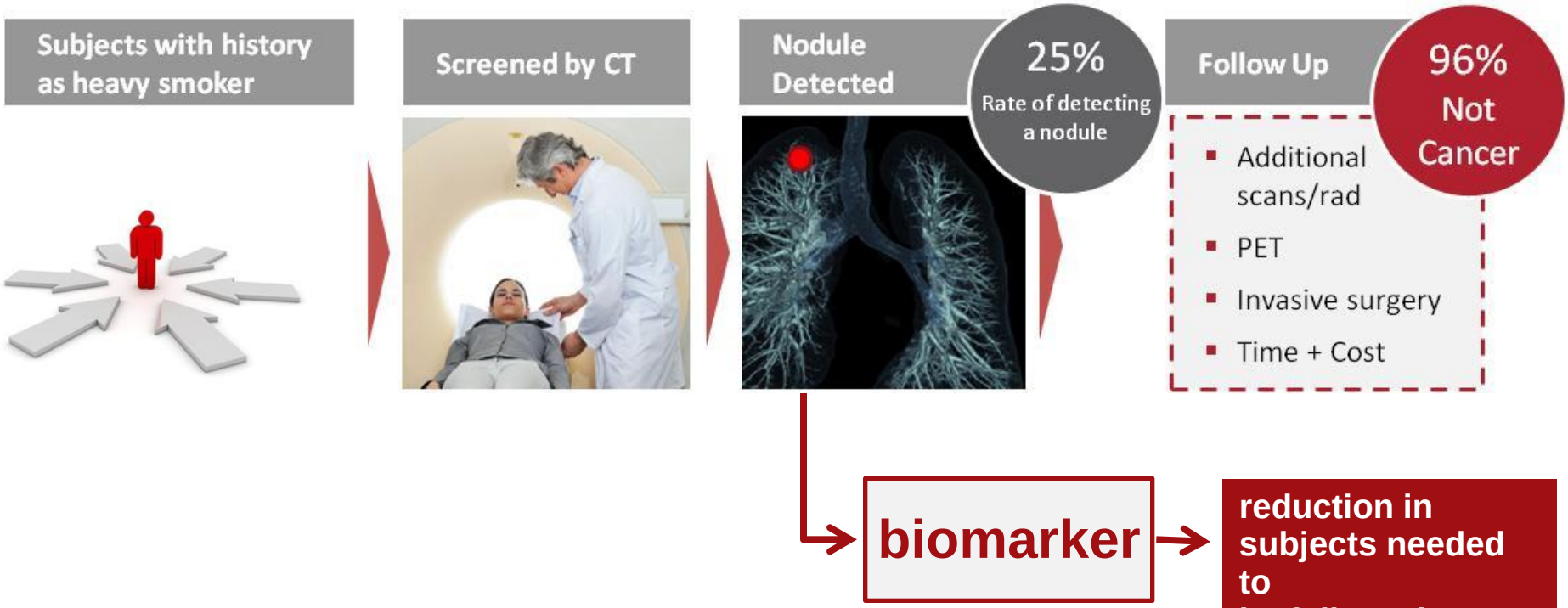
ESMO European Society for Medical Oncology

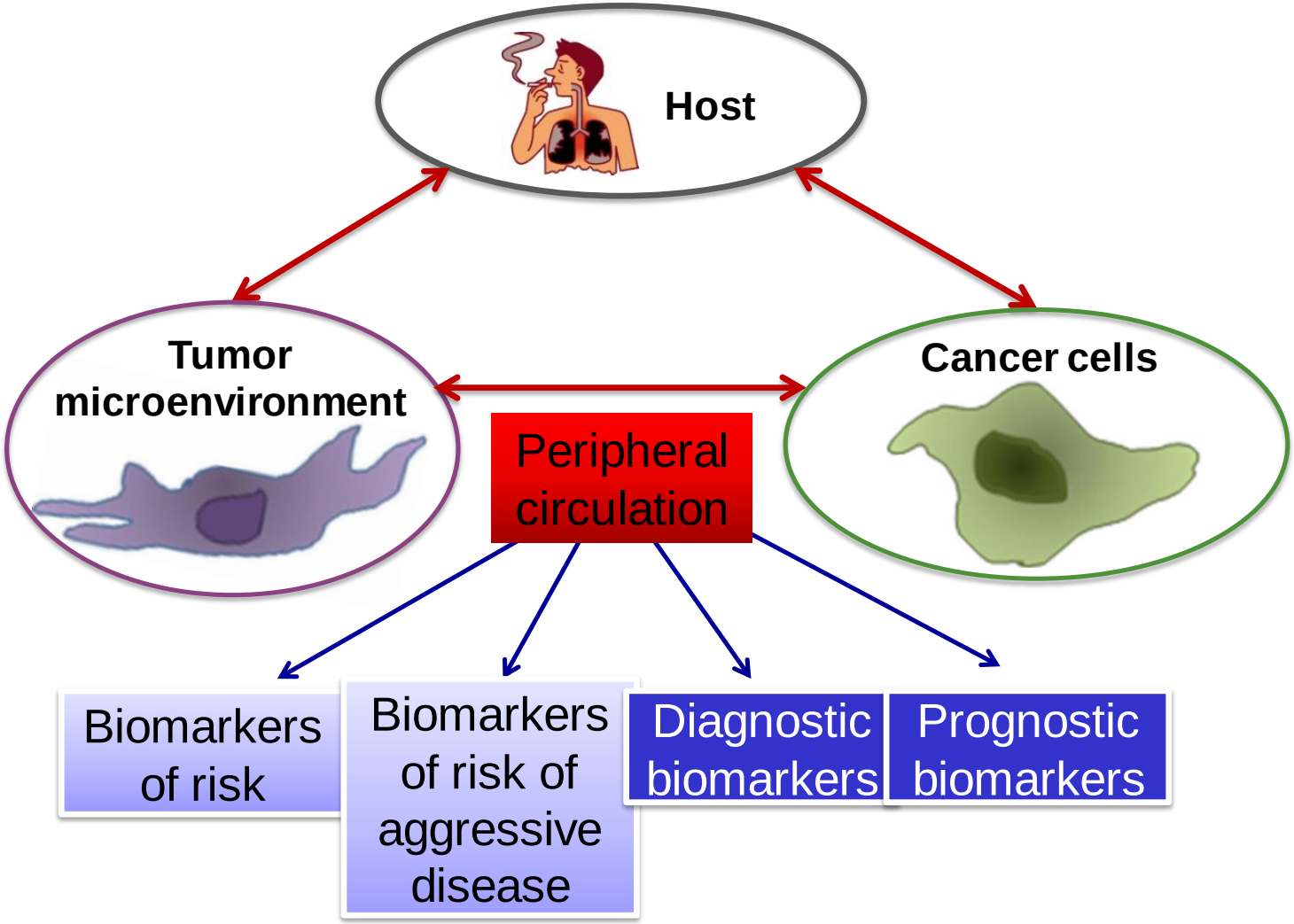
Clinical Utility of biomarkers



Decrease False Positive Rate of CT scans

Sources of blood-based biomarkers.

Novel promising biomarkers are generated by cancer cells, tumor microenvironment, the host response and their dynamic interaction.



Early Lung cancer diagnostic biomarkers

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Sullivan-Pepe, JNCI 2001- EDRN

Candidates	Phase 1 Discovery, Prediction	Phase 2 Assay validation	Phase 3 Retro-longitudinal	Phase 4 Prospective screening	Phase 5 Cancer Control
SERUM/PLASMA					
MALDI TOF MS profiling	x	x	x		
Autoantibodies	x	x	x	x	
Specific antigens /proteins	x	x	x		
miRNAs	x	x	x	X	
DNA methylation Blood	x	x			
Circulating Tumor cells	x				
TUMOR/airway epith					
Preinvasive histo/cytology	x	x	x		
DNA methylation	x	x	x		
RNA airway signature	x	x	x		
MALDI MS profiling	x	x			
Chromosome aberrations	x	x			
SPUTUM/EBC					
DNA Methylation Sputum	x	x	x		
DNA CN -FISH	x	x			
VOCs	x	x			

Disconnect between promise and product
What makes it so hard?

- Discovery methods are often neither reliable nor efficient; rapidly changing technology.
- Selection of candidates: tumor-specific or highthroughput approaches (genetic heterogeneity of tumors)
- Reproducibility: overfitting, cross-validation and external validation.
- Poor design, case-control studies, not in the clinical context.
- Low concentration of signals; we are not sure at what concentration the signals are.
- Very few prospective collections available.
- Relative infrequent outcome.

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Ruben Pio and Luis Montuenga,
Pamplona, Spain

Biomarker 1.

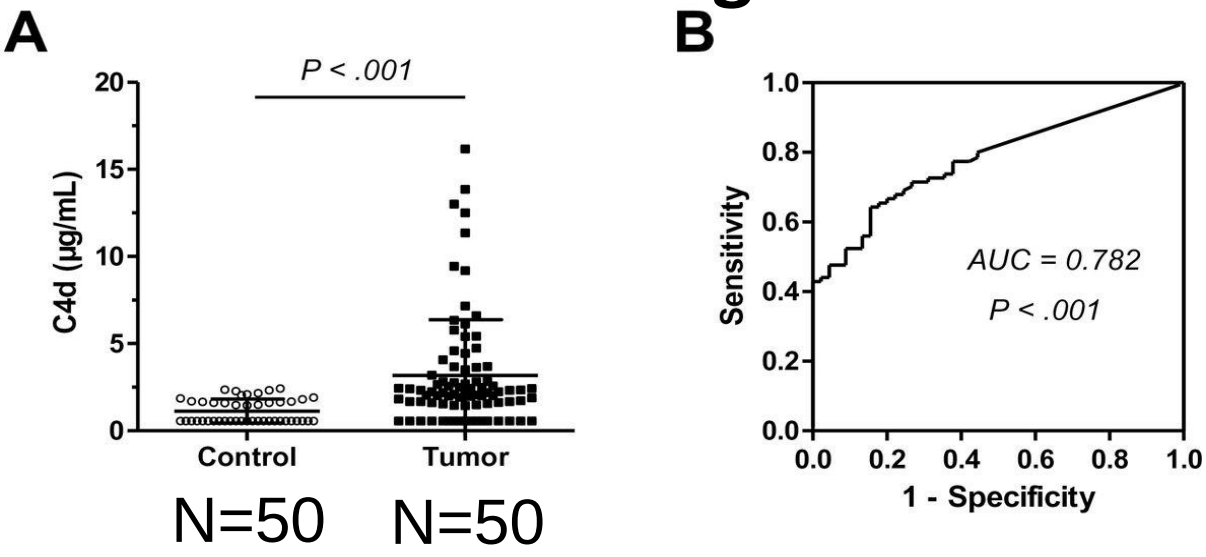
C4d

a stable complement split product

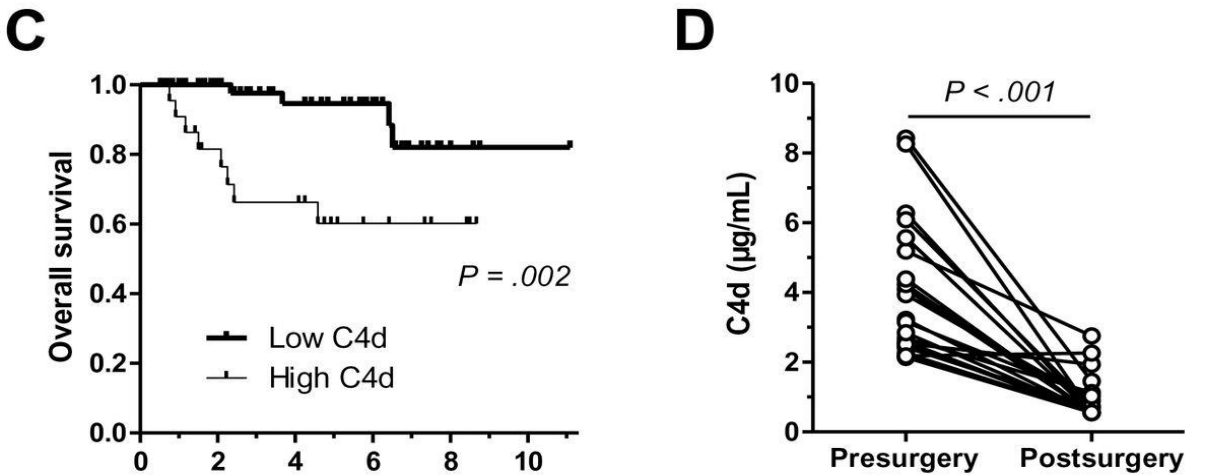
Phase 2

Plasma C4d levels in early stage lung cancer

Ajona et al, JNCI 2013

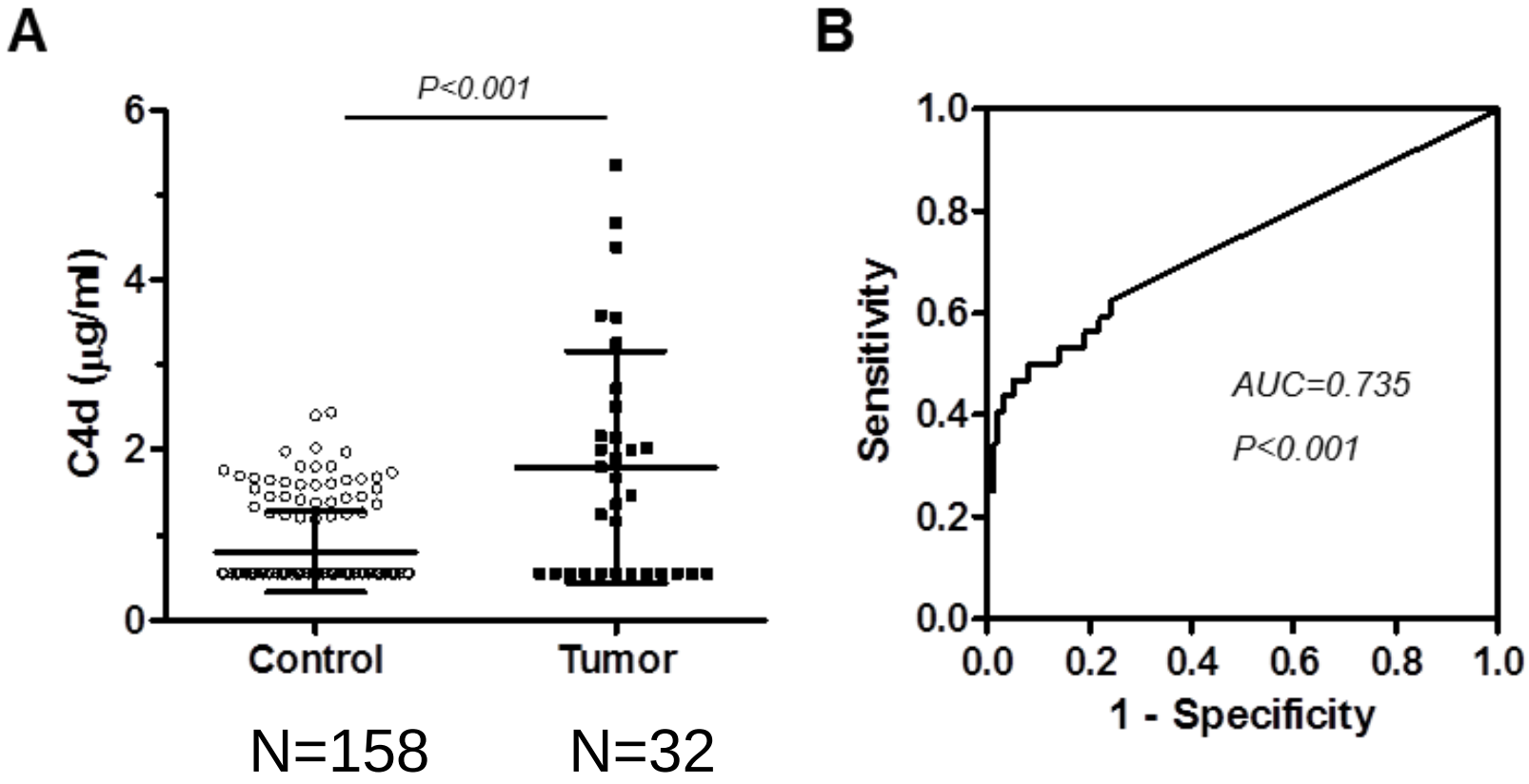


Stage I –II patients



Post-surgery samples were obtained between 2 and 44 months after surgery (median: 7 months).

C4d levels in screening detected lung cancer



Ajona et al, JNCI 2013

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Biomarker 2.

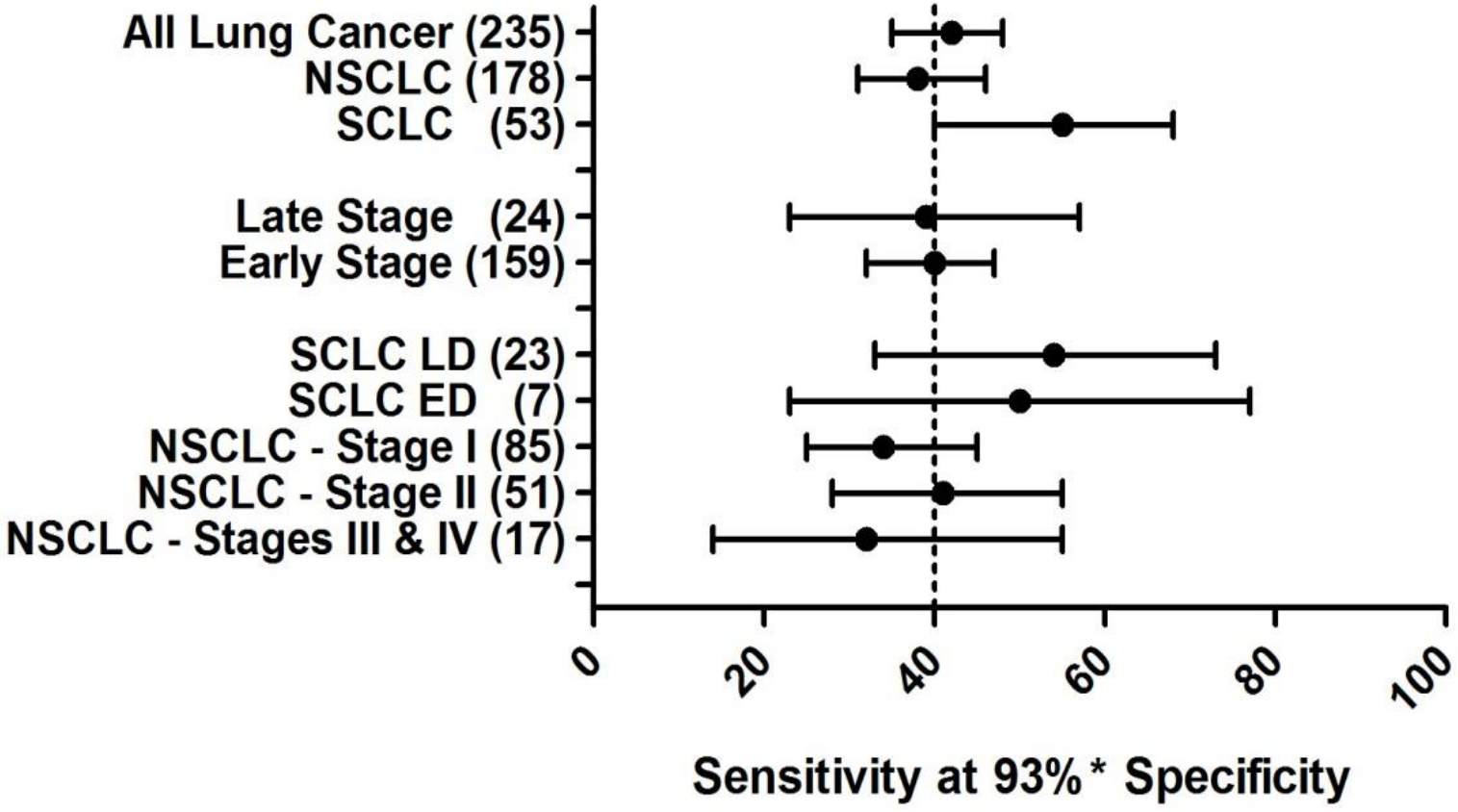
Robertson JF et al.
Oncimmune USA LLC

6/7 Autoantibody signature

Phase 4

7 Autoantibody signature
EarlyCDT-Lung test

CAGE, GBU 4–5, HER2, p53, c-myc, NY-ES0-1 and MUC1



Boyle, Annals of Oncology 2010
Lam, Cancer Prev Res 2011
Chapman Tum. Biol. 2012
Jett, Lung Cancer 2013 in press

Audit of the autoantibody test,EarlyCDT®-Lung, in 1600 patients: An evaluation of its performance in routine clinical practice

James R.Jett et al. Lung Cancer 83 (2014) 51–55

Clinical performance of the 6AAB and 7AAB panels, calculated from the clinical audit dataset with 6 month follow-up for all patients.

	Specificity (%; 95% CI)[a]	Sensitivity (%; 95% CI)[b]	PPV
Overall	1341/1538 (87%; 85–89%)	25/61 (41%; 29–54%)	1 in 8.9 (11%)
6AAB	599/726 (83%; 79–85%)	12/26 (46%; 27–67%)	1 in 11.6 (9%)
7AAB	742/812 (91%; 89–93%)	13/35 (37%; 21–55%)	1 in 6.4 (16%)

95% CI: 95% confidence interval, calculated in SAS using the Clopper-Pearson exact method.

[a] The 7AAB panel shows a highly statistically significant improvement in specificity of EarlyCDT-Lung ($p < 0.0001$).

[b] The sensitivities of the 6AAB and 7AAB panels were not statistically different ($p = 0.5$).

7 Autoantibody signature EarlyCDT- Lung Oncimmune

189 nodules tested with the 7 AAB test

	Cases	Controls			
				Sensitivity	44.2
Profile +	19	17	36	Specificity	88.4
Profile -	24	129	153	PPV	52.8
	43	146		NPV	84.3
				Prevalence	0.23
				RR	3.36

In nodules 8-20 mm, the RR is 4.6

Massion P. WCLC 2013 : Autoantibodies to a panel of lung cancer-associated antigens can provide significant discrimination between malignant and non-malignant lung nodules

Assessing the value of the EarlyCDT-Lung test as a pre-CT screening tool

- **10,000 people** (50-75 yrs, smokers or ex-smokers) from Glasgow and the surrounding areas.
- Half of those taking part will be offered the **EarlyCDT-Lung test** (lung cancer test group). The other half (non-test group) will also have their blood taken, but it will not be tested as part of this study.
- People who have **a positive lung cancer blood test will get a chest X-ray and a lung scan and 6 monthly scans for 2 years**: only 1 in 9 people with a +test is likely to develop LC within 2 yr.
- People with a **negative lung cancer blood test and those in the non-test group will not get any X-rays or scans** will be monitored by their GP as normal: 98-99/100 people with a -test do not have LC at that time.

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Fondazione IRCCS Istituto Nazionale dei Tumori

Istituto Europeo di Oncologia
Istituto di Ricovero e Cura a Carattere Scientifico

Biomarkers 3,4.

Serum & Plasma circulating miRNAs

Phase 4

Circulating microRNA as biomarkers for cancer detection

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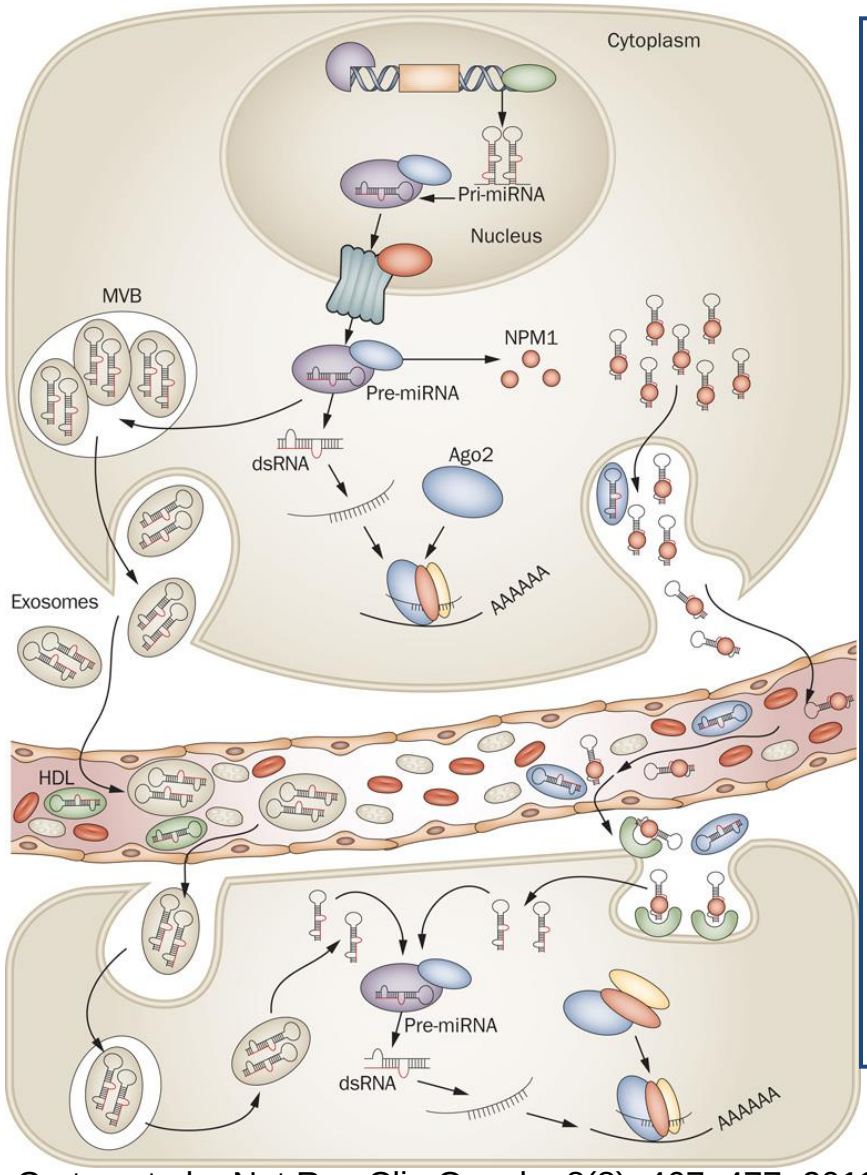

Cortez et al. , Nat Rev Clin Oncol. ; 8(8): 467–477, 2011

miRNA remain rather intact and stable in plasma/serum

packaged in exosomes, microvescicles, or bound to specific proteins such as Ago-2 (core catalytic component of the miRNA-induced silencing complex RISC), NPM1, HDL

robust universally applicable assays for quantification (i.e. qRT-PCR)

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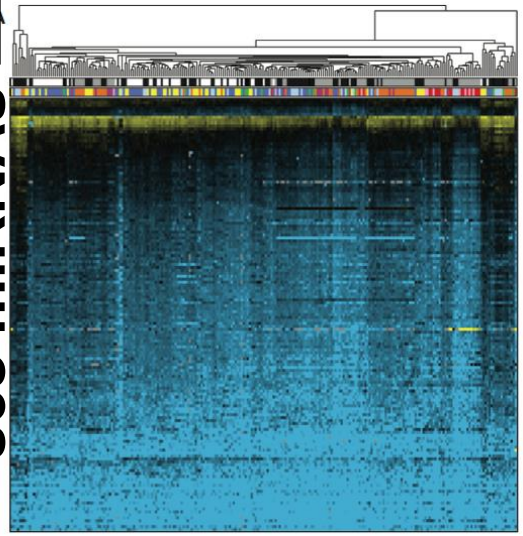
COSMOS study
(5201 individuals LD-CT screened)

DISCOVERY PHASE

Serum <0.5ml

Global Serum miRNA Expression Profile



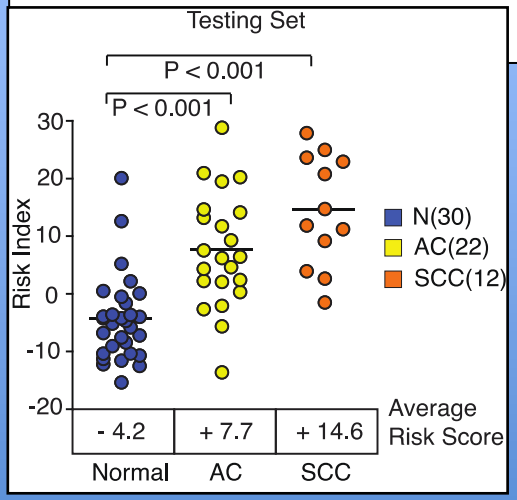
365 miRNAs

253 samples

Quantity
High
Low

IEO serum miRNA-test
34-miRNA signature

$$\text{Risk-Index} = \Sigma x_i w_i$$



- Normal
- Adenocarcinoma (AC)
- Squamous Cell Carcinoma (SCC)

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Bianchi et al. EMBO Mol Med 3(8) 2011

Performance of IEO miRNA-test

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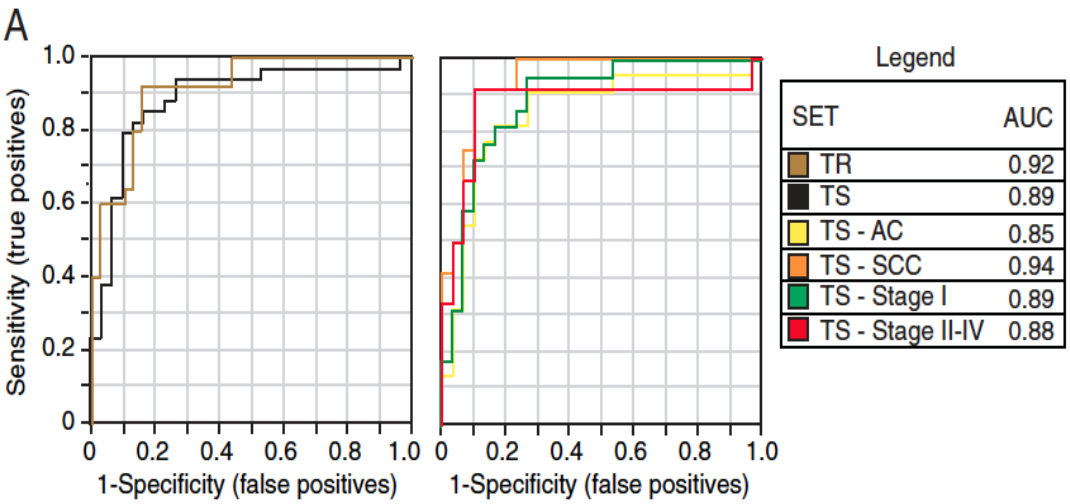
Table 1. Clinical characteristics of patients of the training and testing sets

Categories	Training set ($N = 64$)	Testing set ($N = 64$)	Symptomatic set ($N = 36$)
Tumour	25	34	36
Normal	39	30	0
Male	45	43	30
Female	19	21	6
Non-smokers	0	0	8
Age (years)	58 ± 6	60 ± 6	66 ± 8
Pack-years	54 ± 18	49 ± 17	45 ± 37
Tumour subtype			
AC	25	22	23
SCC	0	12	13
Tumour Stage			
IA	19	18	7
IB	2	4	9
II–IV	4	12	20

AC, adenocarcinoma; SCC, squamous cell carcinoma.

The clinical and pathological characteristics are reported for the training and testing sets of patients. The average and relative standard deviation (±SD) is indicated for age (years) and smoking status (pack-years).



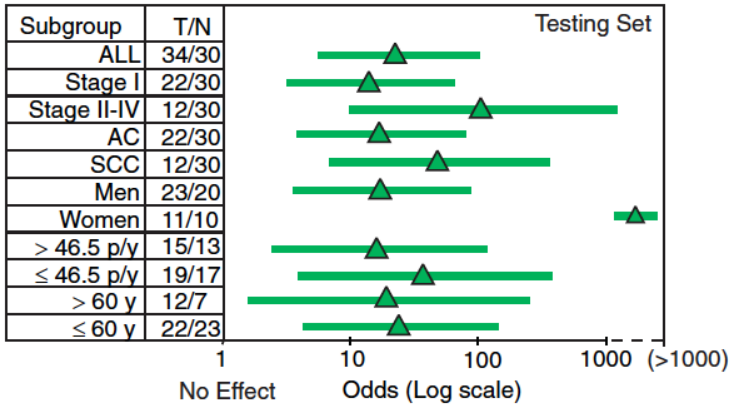


Table 2. Performance of the predictive model in various sets

Set	AUC	ACC %	SEN %	SPE %
Training set (39 N, 25 AC)	0.92	78[a]	69[a]	84[a]
Testing set (30 N, 22AC, 12 SCC)	0.89	80	71	90
Testing set—AC only (30 N, 22 AC)	0.85	79	64	90
Testing set—SCC only (30 N, 12 SCC)	0.94	88	83	90
Testing set—stage I only (30 N, 22 T)	0.89	77	59	90
Testing set—stage II–IV only (30 N, 12 T)	0.88	90	92	90

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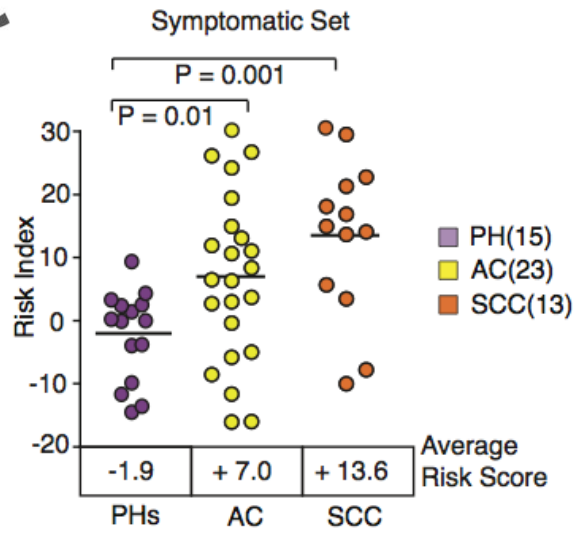
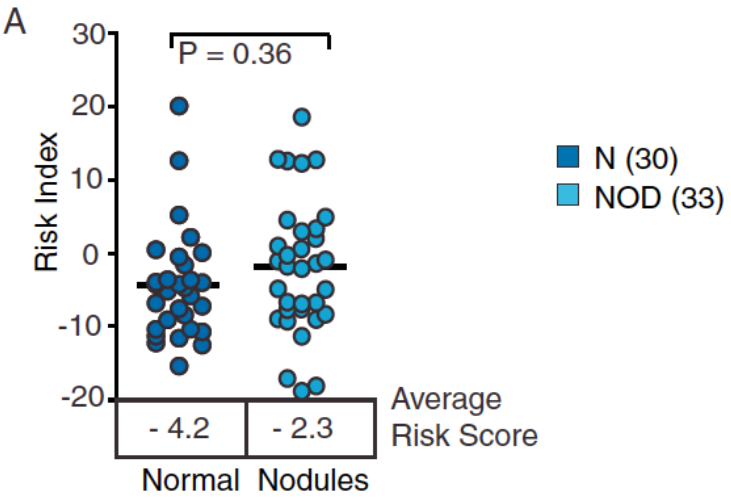
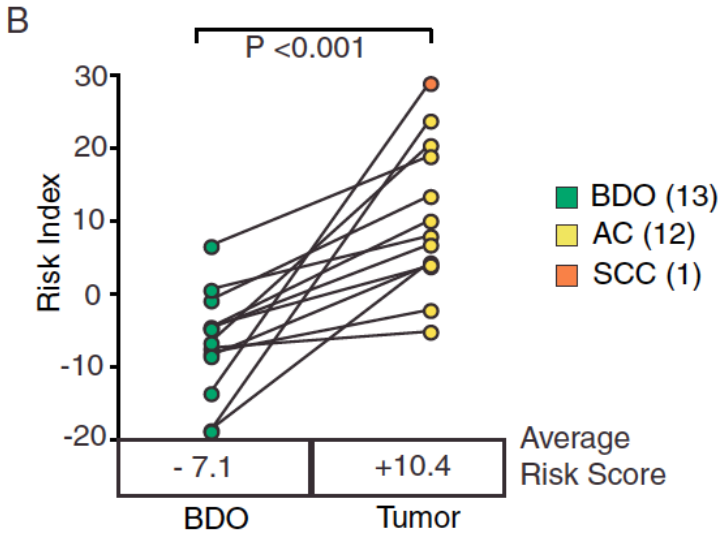
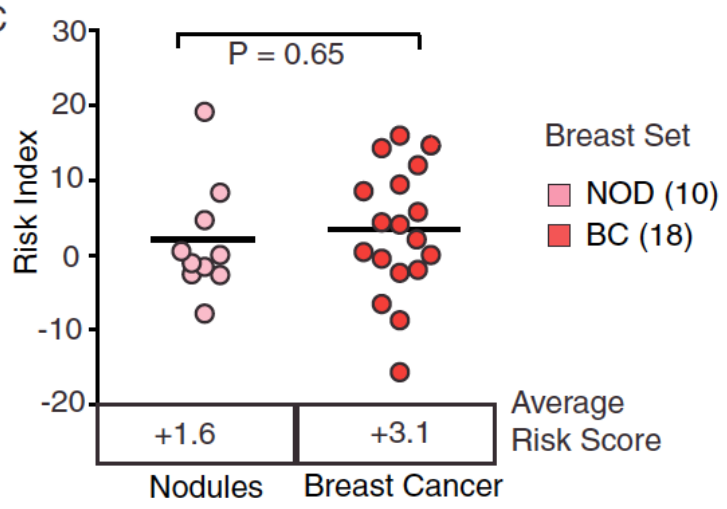
Performance of IEO miRNA-test









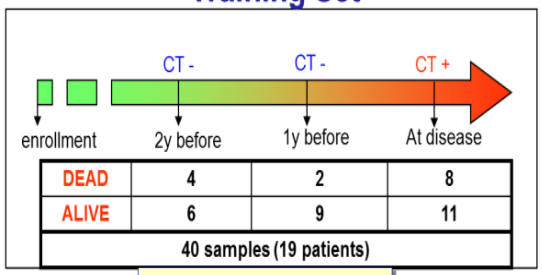
Bianchi et al. EMBO Mol Med 3(8) 2011

FONDAZIONE IRCCS
ISTITUTO NAZIONALE
DEI TUMORI

miRNA Signature Discovery & Initial Validation

Four miRNA ratios signatures (24 miRNAs)
Used to develop a single miRNA Classifier

Training Set

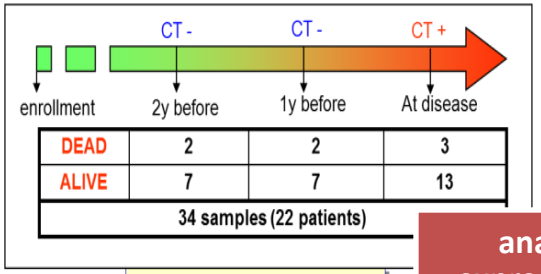


DEAD	4	2	8
ALIVE	6	9	11

40 samples (19 patients)

CONTROLS
5 POOLS
(28 individuals)

Validation Set



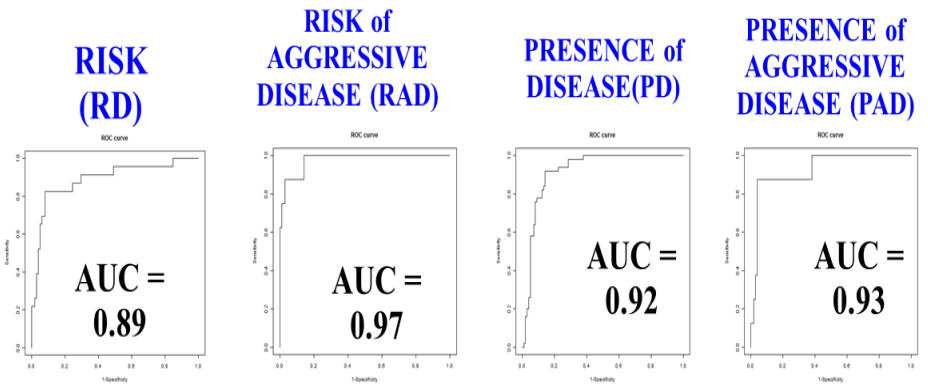
DEAD	2	2	3
ALIVE	7	7	13

34 samples (22 patients)

CONTROLS
10 POOLS
(54 individuals)

analysis of relative expression ratios of 100 miRNAs

(starting from 378) detectable in plasma



Generation of the three-level miRNA signature classifier (MSC)

MSC	RD	RAD	PD	PAD
Low risk	-	-	-	-
Intermediate risk	+	-	+/-	-
	+/-	-	+	-
High risk	+/-	+	+/-	+/-
	+/-	+/-	+/-	+

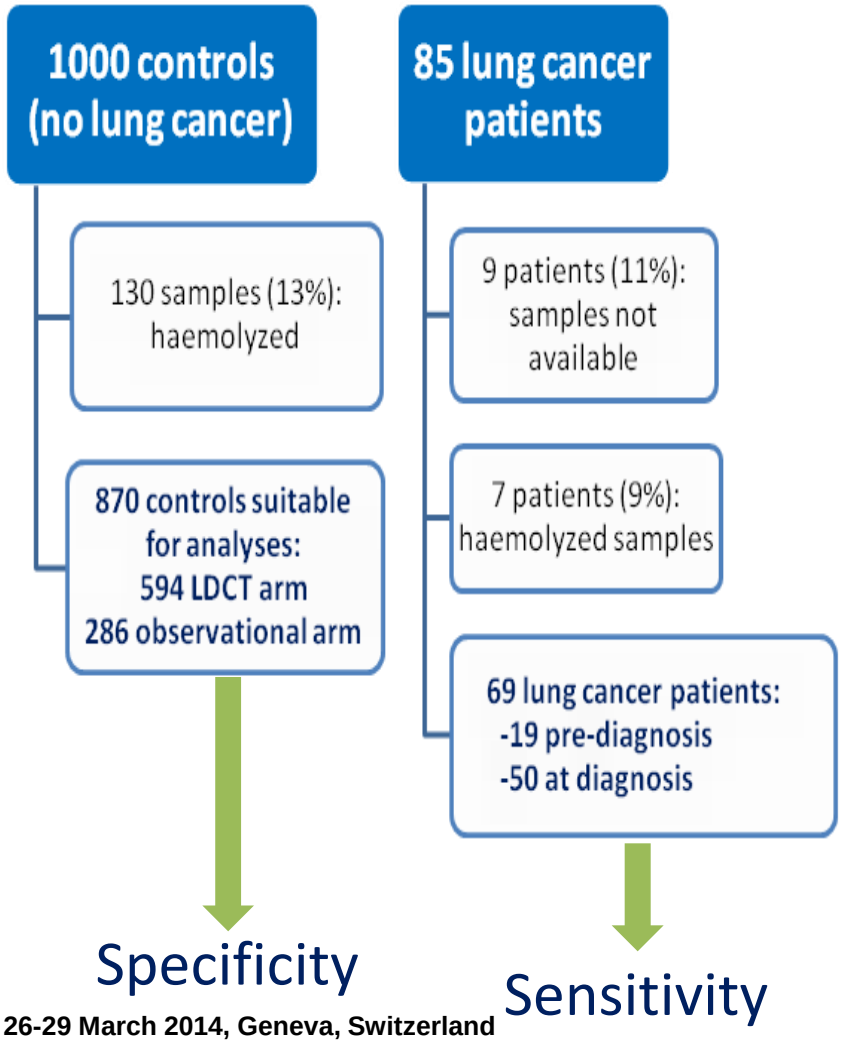
PNAS

MicroRNA signatures in tissues and plasma predict development and prognosis of computed tomography detected lung cancer

Mattia Boeri[a,1], Carla Verri[a,1], Davide Conte[a,1], Luca Roz[a,1], Piergiorgio Modena[b], Federica Facchinetti[a], Elisa Calabrò[c], Carlo M. Croce[d,2,3], Ugo Pastorino[c,2], and Gabriella Sozzi[a,2,3]

[a]Tumor Genomics Unit, Department of Experimental Oncology and Molecular Medicine, and [c]Unit of Thoracic Surgery, Fondazione IRCCS Istituto Nazionale Tumori, 20133 Milan, Italy; [b]Unit of Experimental Oncology 1, Centro di Riferimento Oncologico, 33081 Aviano (PN), Italy; and [d]Ohio State University Comprehensive Cancer Center, Ohio State University, Columbus, OH 43210

Clinical Validation Study – Multicentric Italian Lung Detection (MILD) Trial (2005-2012) [1]



- Specificity
  - 870 subjects **in both arms which did not have cancer** were examined to determine specificity of MSC
  - 594 subjects **in the LDCT arm which did not have cancer** were examined to assess the ability of MSC to reduce the false positive rate of LDCT
- Sensitivity

  69 patients **with lung cancer** from both arms of the trial were used to determine the sensitivity of MSC to detect cancer

[1]**Sozzi et al JCO, 2014**

Overall Diagnostic Performance of MSC[1]

	Total	MSC (risk of lung cancer)		
		High (%)	Intermediate (%)	Low (%)
All subjects	939	63 (6.7)	159 (16.9)	717 (76.4)
No lung cancer	870	32 (3.7)	130 (14.9)	708 (81.4)
Lung cancer	69	31 (44.9)	29 (42.0)	9 (13.0)

Sensitivity: 87%
Specificity: 81%
NPV: 99%

MSC risk groups not significantly associated with tumor stage (p=0.40) and histology (p=0.45)

[1]Sozzi et al JCO, 2014

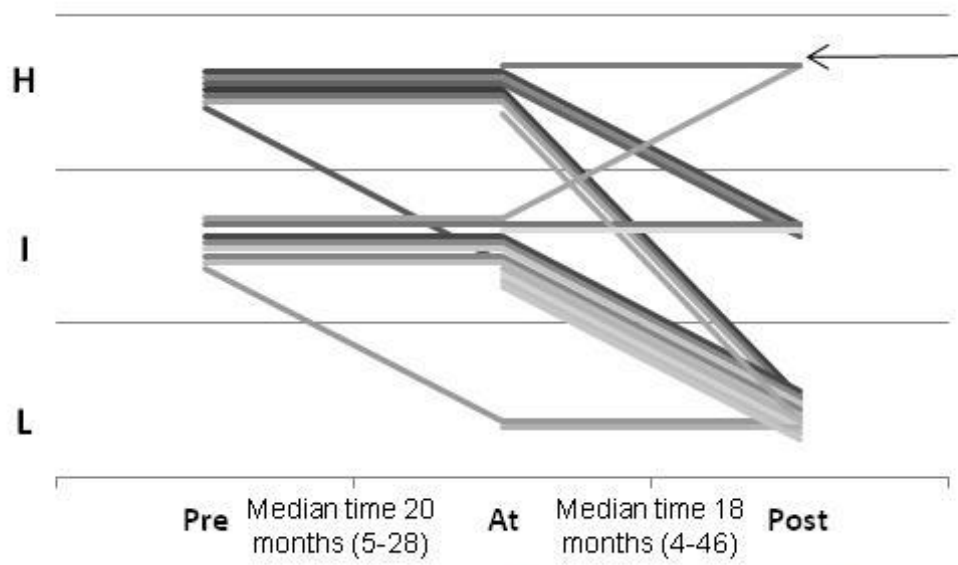
Time dependency analysis of diagnostic performance of MSC, at 6, 12, 18 and 24 months intervals between blood sampling and lung cancer diagnosis[1]

Months from blood sampling to lung cancer detection	SE	SP	PPV	NPV
6	83%	80%	18%	99%
12	86%	81%	22%	99%
18	86%	81%	23%	99%
24	87%	81%	25%	99%

[1] Heagerty PJ., Biometrics 2000, 2007

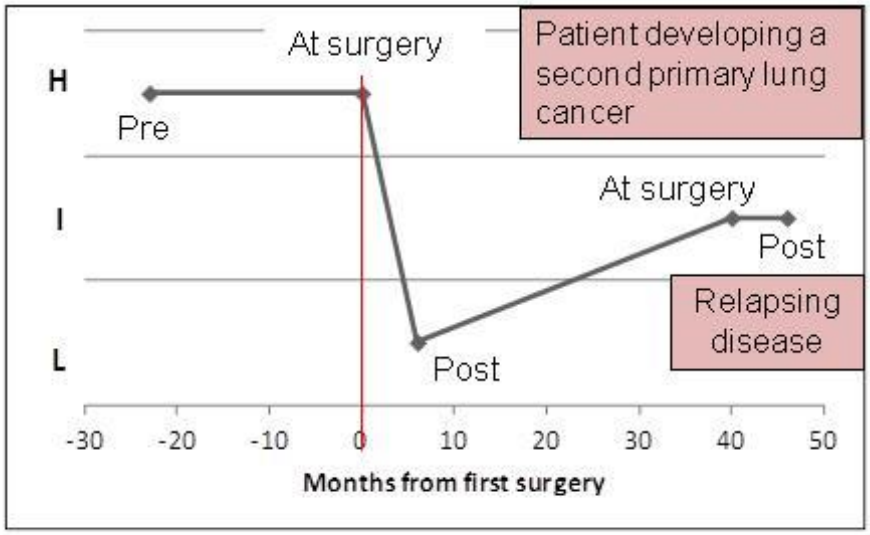
[1]Sozzi et al JCO, 2014

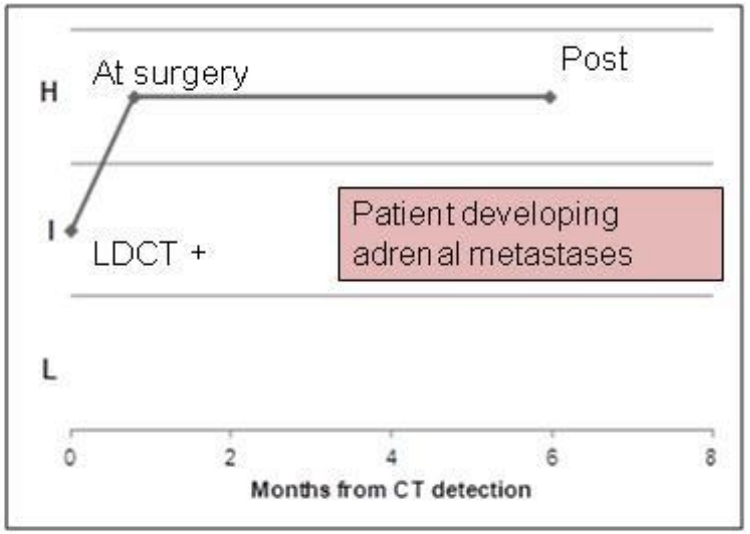
Disease Monitoring: Preliminary Data[1]



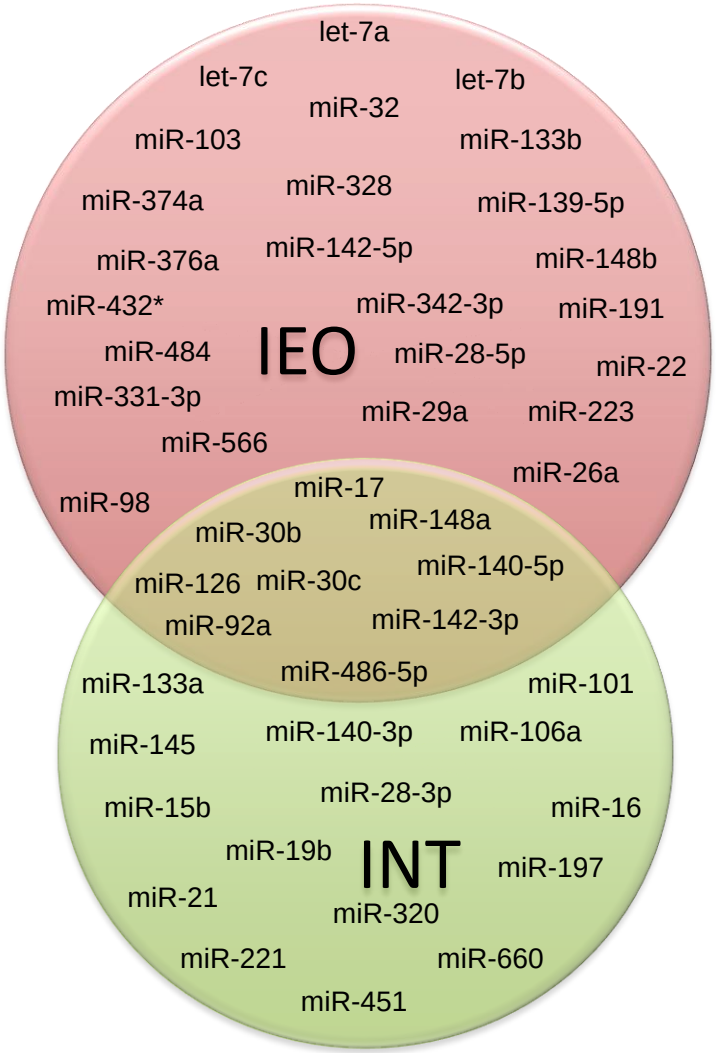
20 months after surgery. Two following samples collected 4 and 6 years after surgery, resulted I and L, respectively.

Samples	Tot	H	I	L
Pre	15	47%	53%	0%
At	23	35%	56%	9%
Post	23	9%	21%	**70%**





[1]Sozzi, Boeri, and Pastorino; Personal Communication



Nine out of the 34 miRNAs composing the IEO test are common to the 24 miRNAs of the INT test

Differences:

- serum (IEO) vs Plasma (INT)
- INT signature trained in plasma samples of patients before and at disease detection (earlier, microenvironment –related changes)
- IEO signature trained in serum samples of patients at the time of lung cancer diagnosis (tumor-specific changes)

Both tests in prospective screening trials:

- bioMILD (INT)
- COSMOS II (IEO)

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Conclusions

- early detection candidate biomarkers exist
- Few are validated or tested in screening settings. Priority to validate existing candidates.
- BM should provide knowledge about added value and therefore should be integrated to clinical, laboratory and imaging (LDCT) routine data.
- To demonstrate clinical utility requires significant investment in effort and resources towards prospective biomarkers driven clinical trial.