Biopsia liquida nella diagnosi del tumore al polmone

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Istituto Nazionale dei Tumori

Regione Lombardia

DISCLOSURES

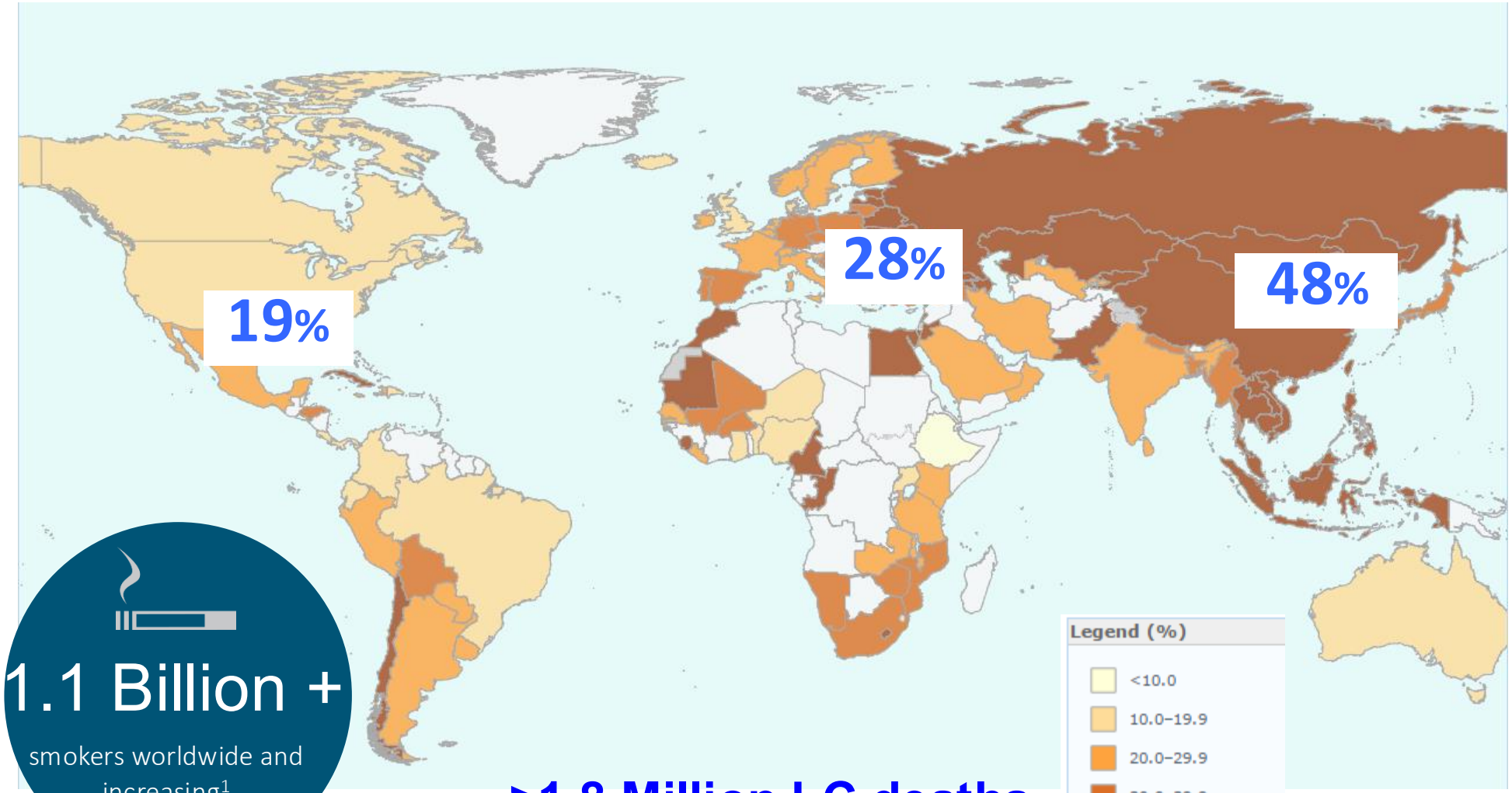
Commercial Interest	Relationship(s)
Co-inventor for three patent applications regarding the miRNA signature classifier.	These patents were licensed to a private company, ACCUSTEM, under regulations of Fondazione IRCCS Istituto Nazionale dei Tumori of Milan.

Outline

- Lung cancer screening and biomarkers: overview
- circulating miRNAs: the MSC test and the bioMILD screening trial
- MSC origin and immunity complementary biomarkers

Lung Cancer: The #1 Global Cause of Cancer Death

Prevalence of smoking any tobacco product among persons aged >= 15 years in 2015: male



1.1 Billion +

smokers worldwide and increasing[1]

>1.8 Million LC deaths

< 20% 5-year survival

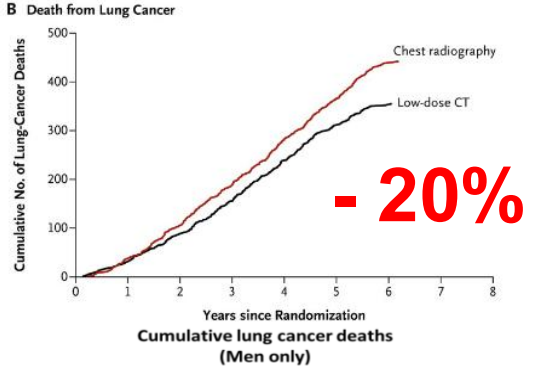
World Health Organization (WHO), WHO Report on the Global Tobacco Epidemic, 2017

LDCT screening trials results

lung cancer mortality can be reduced

NLST (USA) 2011

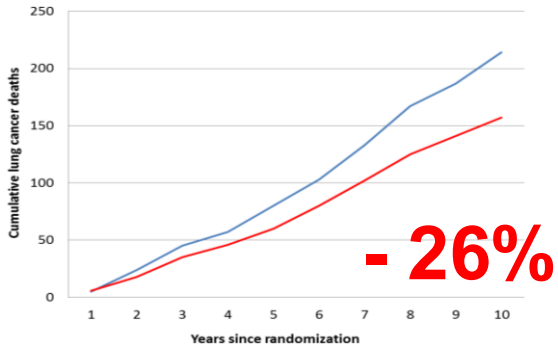
Volunteers: > 50,000
Nodule size : > 3 mm diameter
no risk modulation
LDCT: 3 rounds / 2 years



- 20%

NELSON (NL-BE) 2020

Volunteers: > 15,000
Nodule size : > 50 mm^3 volume + VDT
no risk modulation
LDCT: 4 rounds / 6.5 years

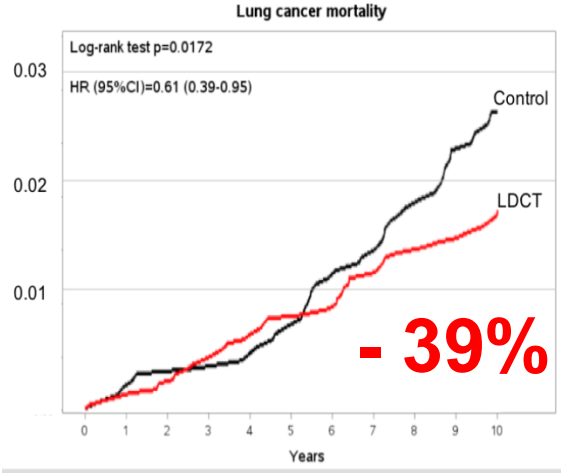


- 26%

MILD (ITALY) 2019

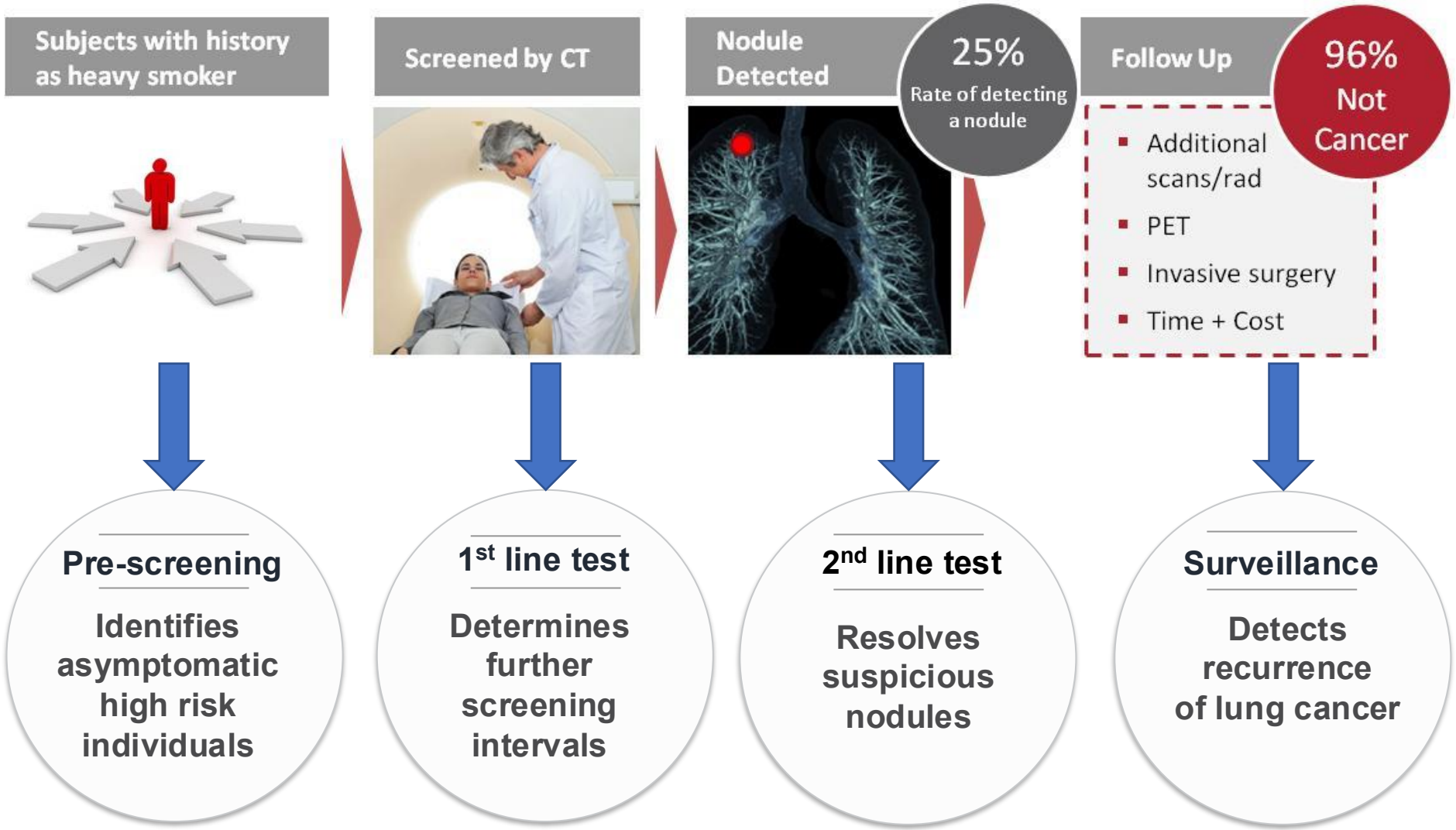
Volunteers: > 4,000
Nodule size : > 60 mm^3 volume + VDT + PET
LDCT risk modulation (1yr vs 2yrs)
5+ rounds / 8-10 years

Stage I-II > 50%

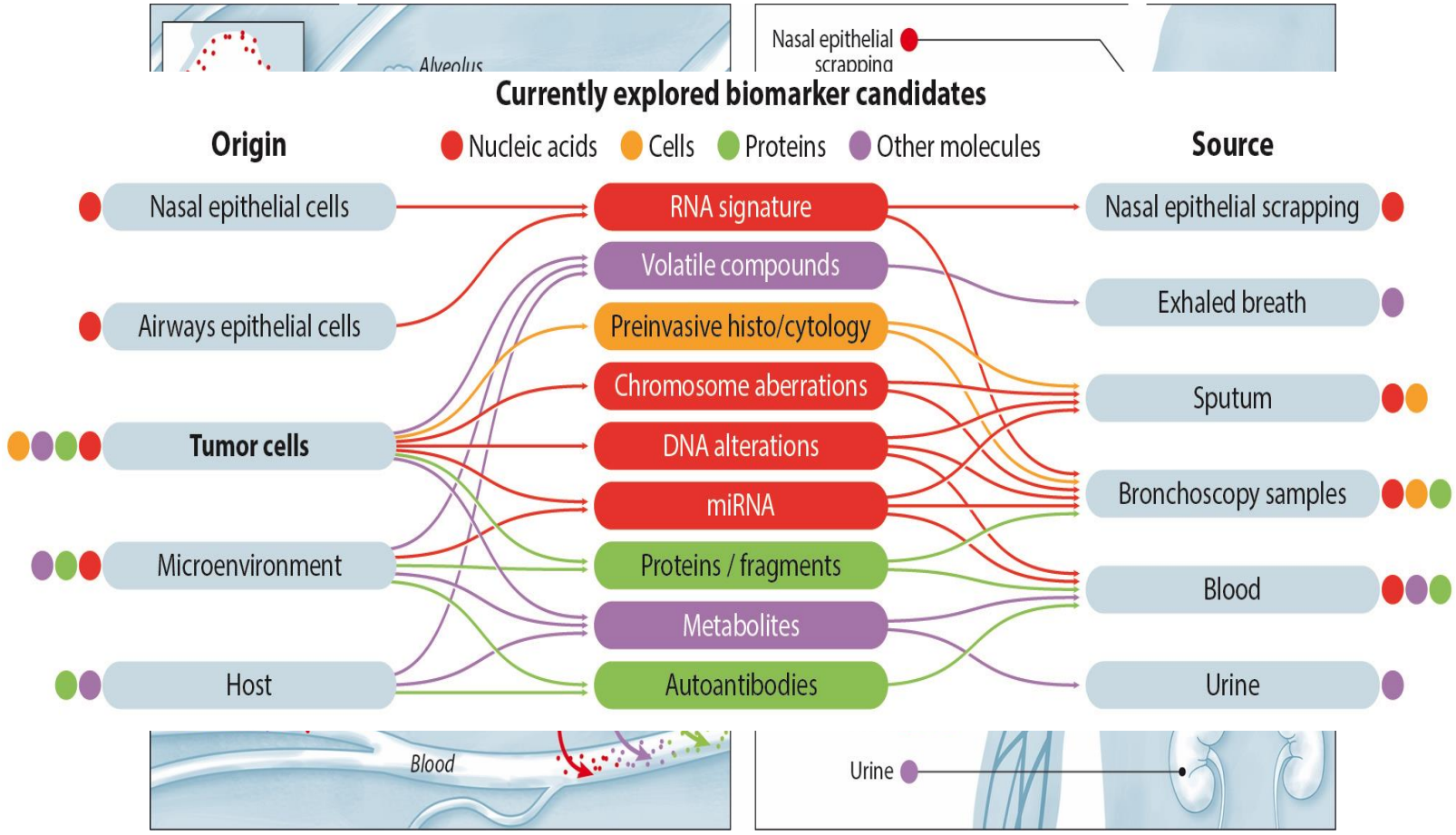


- 39%

Clinical utility of biomarkers in screening settings



Sources of lung cancer biomarkers



Seijo LM, et al. JTO. 2018

LUNG CANCER BLOOD BIOMARKERS

Targeted methylation (cfDNA) Galleri™ (Grail) tumor agnostic

- Sens. 21.9% Stage I: 95,2% Stage IV
  Retrospective Case-control LC — *Klein, Ann Oncol 2021*
- Sens. 8.7% Stage I: 96,3% Stage IV
  Prospective symptomatic pts LC — *Nicholson, Lancet Oncol 2023*
- Sens. 29% all st (19 solid,17 Lymph)
  Spec 99% Prosp PATHFINDER MCED — *Schrag, Lancet 2023;*
- NHS Galleri trial in UK
  142.000 adults aged 50-77,
  randomized test vs no test, 3yrs FU — The primary endpoint of statistically significant stage III-IV reduction was not observed Cohen, *BMJ 2026. Results will be presented at ASCO 2026*

Genome wide Fragmentome (cfDNA) DELFI tumor agnostic

- Sens 17% Stage I-II: 83% Stage III-IV
  symptomatic patients LC — *Leal, Chest 2023*
- Sens 71% Stage I (58%spec) prosp.
  case-control (DELFI-L101) LC — *Mazzone, Can Discovery 2024*

Circulating Tumor Cells (CTC) tumor-agnostic

- Baseline CTC: Sens 26.3%; Spec 96.2%
  AIR LC screening trial — *Marquette, Lancet Res Med 2020*
- CTC clusters: sens 41.4%, Spec 100%
  Prospective small LC screening study — *Manjunath, Lung Cancer 2019*

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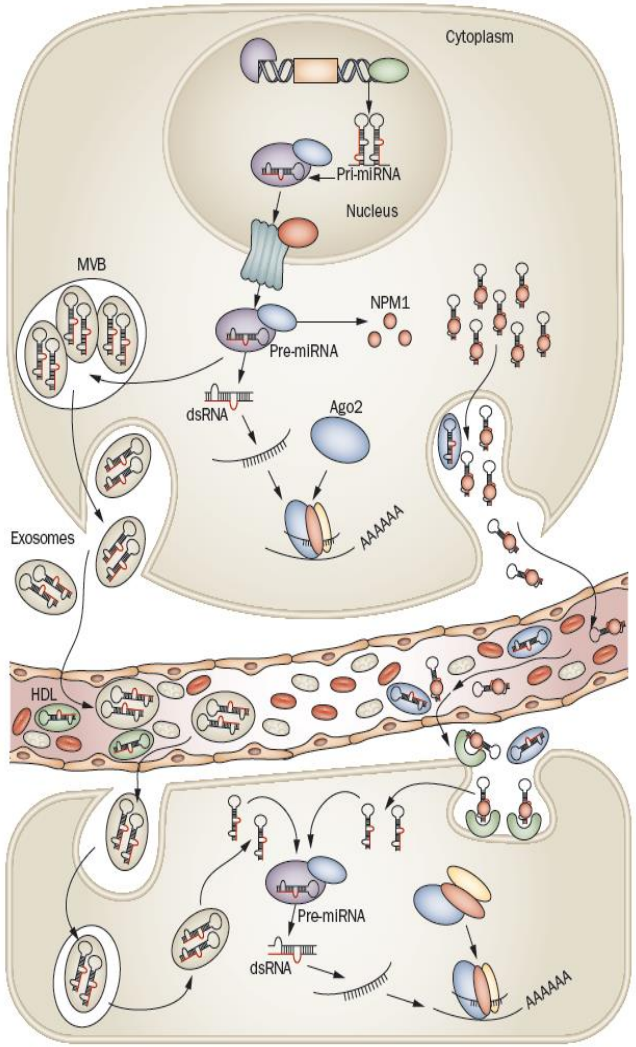
Circulating microRNAs: biomarkers of the tumor-host interplay

Small non-coding RNAs that regulate gene expression by binding complementary sequences of target mRNAs inducing their degradation or translational repression

Active release

Exosomes

High-density lipoproteins



RNA-binding proteins (Ago 2, NPM1)

Passive release

miRNAs remain rather **intact and stable** in plasma/serum

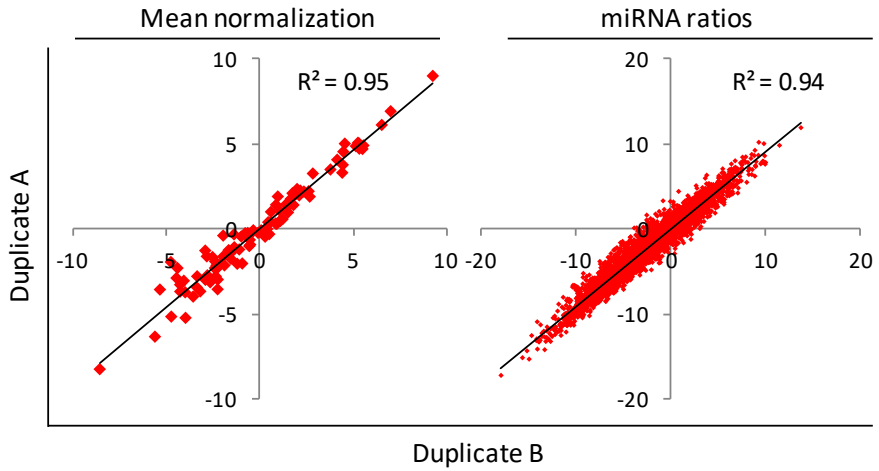
Easily **detectable** by common technique (RT-qPCR)

Cortez MA et al. Nat Rev Clin Oncol. 2011

miRNA SIGNATURES IN PLASMA OF SCREENING PARTICIPANTS

Since plasma miRNAs showed good statistical properties (i.e.gaussian distribution), but no housekeeping circulating miRNAs were found, we used miRNA ratios in logarithmic scale instead of single miRNA features

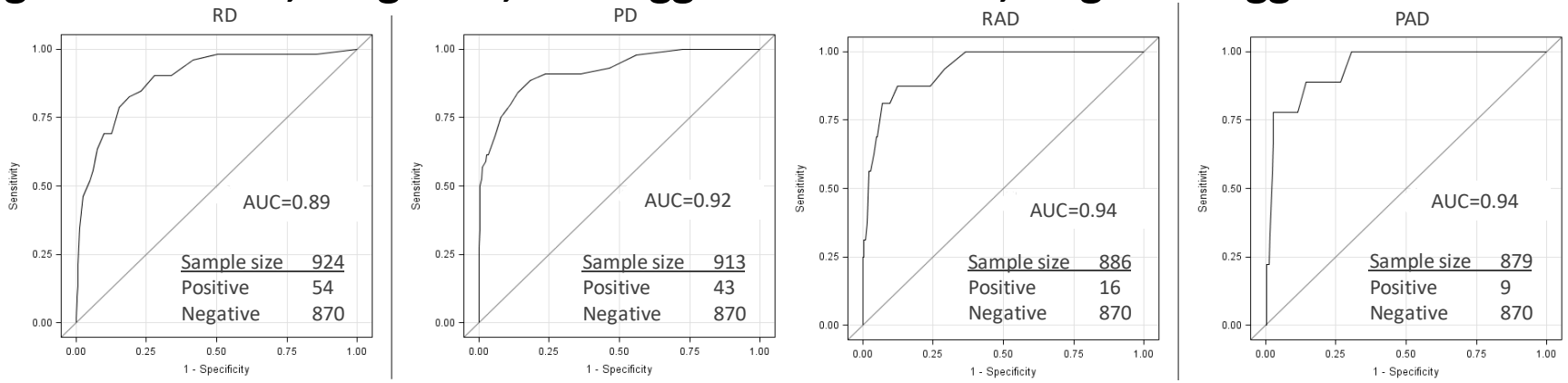
100 miRNAs circulating in plasma → $\frac{100 * 99}{2}$ = **4950 miRNA Ratios**



24 miRNAs composing ratios differentially expressed between cases and controls:

mir-16, mir-17, mir-21, mir-101, mir-126, mir-145, mir-197, mir-221, mir-320, mir-451, mir-660, mir-106a, mir-133a, mir-140-3p, mir-140-5p, mir-142-3p, mir-148a, mir-15b, mir-19b, mir-28-3p, mir-30b, mir-30c, mir-486-5p and mir-92a.

4 Signatures: Risk, Prognosis, Risk Aggressive Disease,Prognosis Aggressive Disease

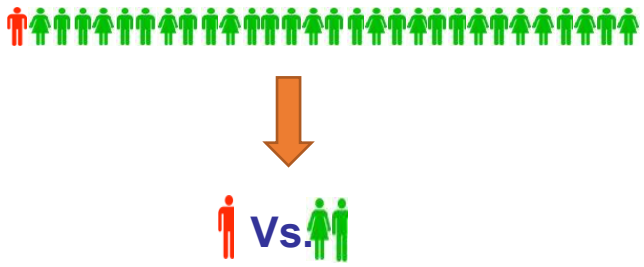


RNA-biomarkers: the microRNA signature classifier (MSC)

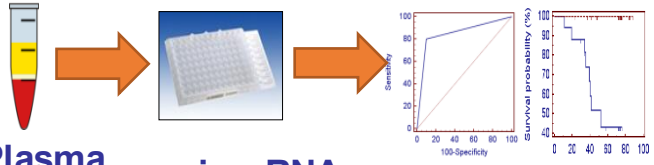
TRAINING SET

Pilot study: 1035 high risk individuals

38 LC within 5 years



Vs.



Plasma collection | **microRNA profiling** | **Signature generation**

MSC	Risk level
Positive	High
	Intermediate
Negative	Low

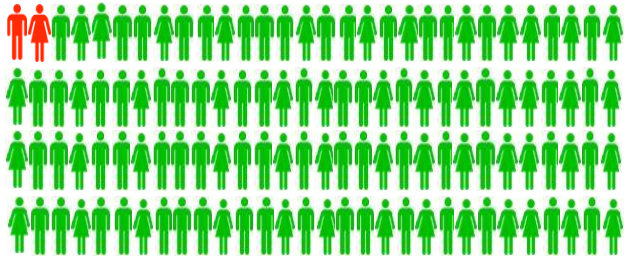
Ratios between 24 microRNAs

Boeri et al. PNAS, 2011

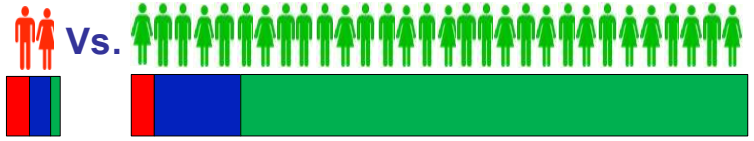
VALIDATION SET

MILD: 4099 high risk individuals

85 LC within 5 years



Vs.



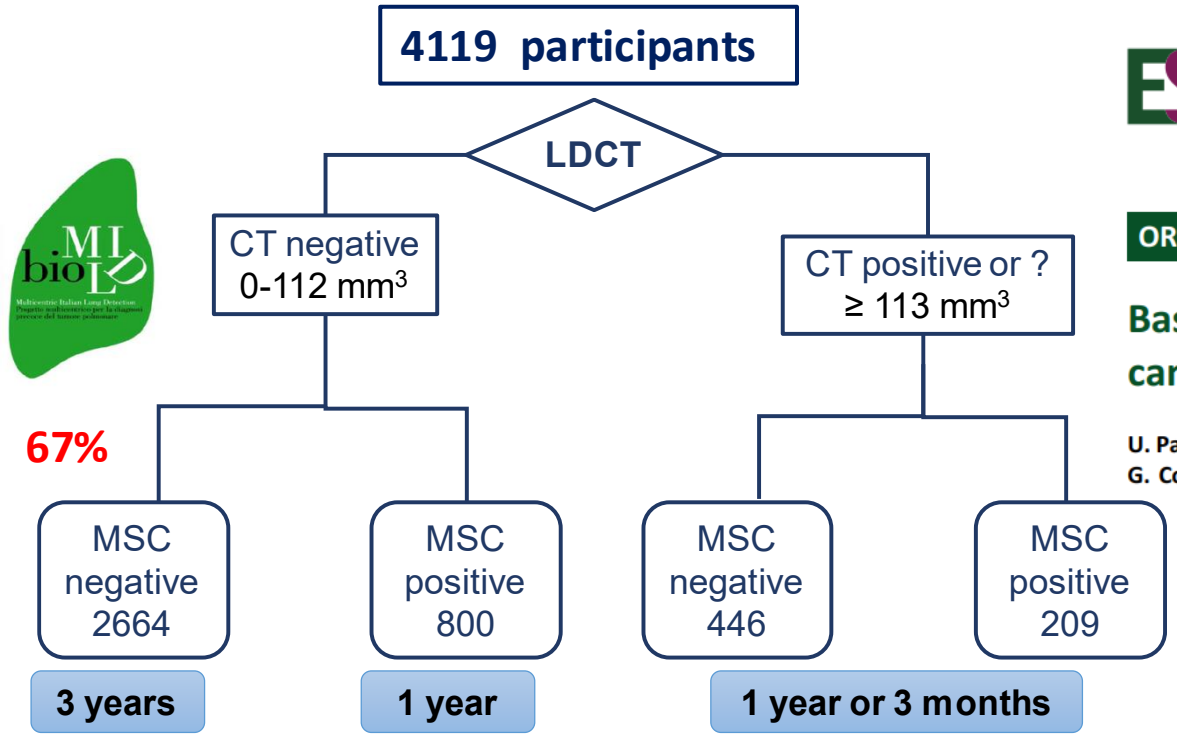
87% Sensitivity

81% Specificity

5 fold reduction of false-positives

Sozzi et al. JCO, 2014

The BioMILD screening trial



ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

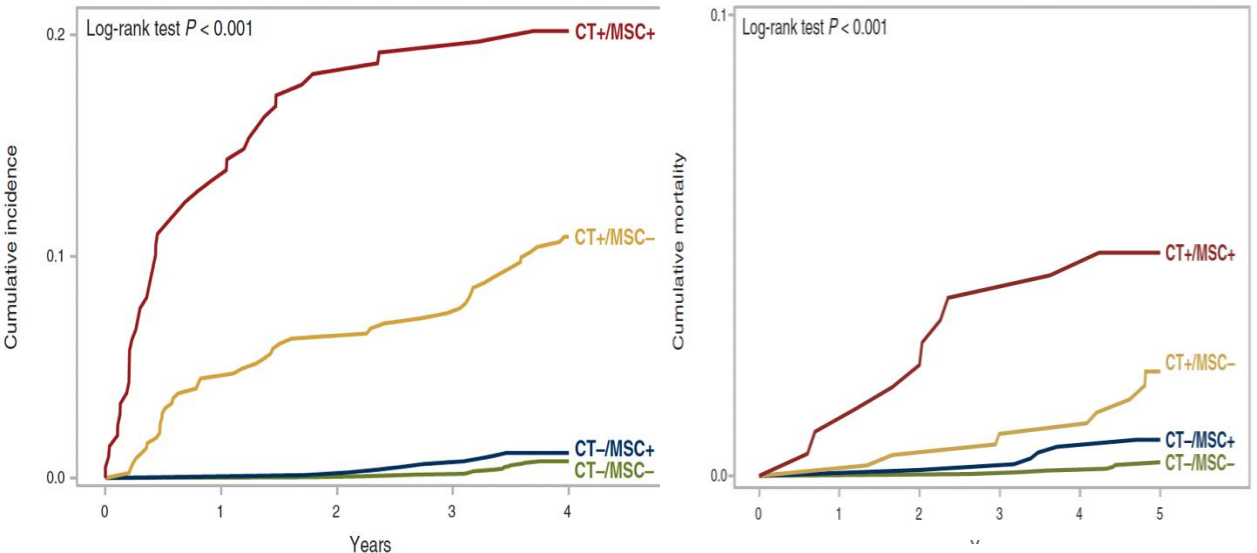
ANNALS OF ONCOLOGY driving innovation in oncology

2022

ORIGINAL ARTICLE

Baseline computed tomography screening and blood microRNA predict lung cancer risk and define adequate intervals in the BioMILD trial

U. Pastorino[1*], M. Boeri[2], S. Sestini[1], F. Sabia[1], G. Milanese[1,3], M. Silva[3], P. Suatoni[1], C. Verri[2], A. Cantarutti[4], N. Sverzellati[3], G. Corrao[4], A. Marchianò[5] & G. Sozzi[2]



- **Baseline** LDCT and blood microRNAs profile (MSC) define **individual lung cancer risk** profiles.
- Targeted LDCT intervals reduce **unnecessary repeat LDCT** without **detrimental effect (**stage I LC, resection rates, interval cancers)
- MSC risk test shows a **major added value for CT indeterminate/positive** participants

Saving >30% CT exams in 3 years

Blood microRNA testing in participants with suspicious low-dose CT findings: follow-up of the BioMILD lung cancer screening trial

Mattia Boeri,[a] Federica Sabia,[b] Roberta E. Ledda,[b,c] Maurizio Balbi,[b,d] Paola Suatoni,[b] Miriam Segale,[a] Anna Zanghì,[a] Anna Cantarutti,[e] Luigi Rolli,[b] Camilla Valsecchi,[b] Giovanni Corrao,[e] Alfonso Marchianò,[f] Ugo Pastorino,[b,g] and Gabriella Sozzi[a,g,*]

The Lancet Regional Health - Europe 2024;46: 101070

Published Online 16 September 2024

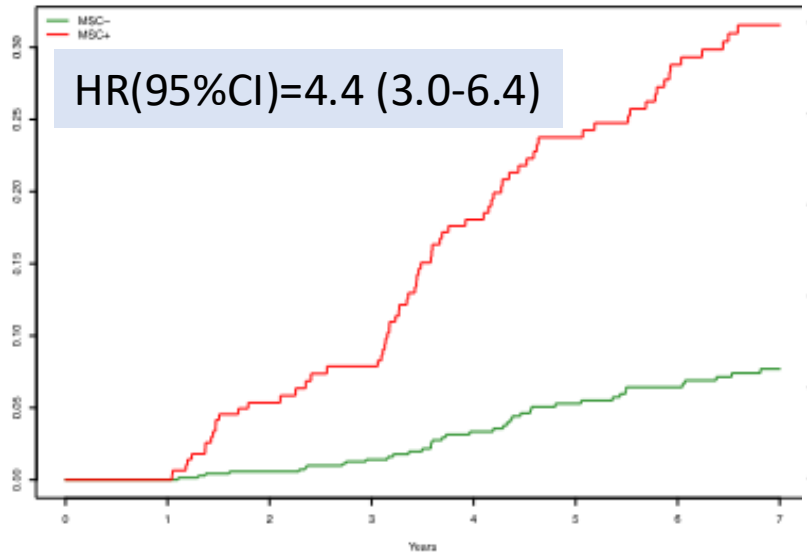
https://doi.org/10.1016/j.lanepe.2024.101070

N= 1024 participants with CT ind (1403)/pos (584) results and 1987 MSC test

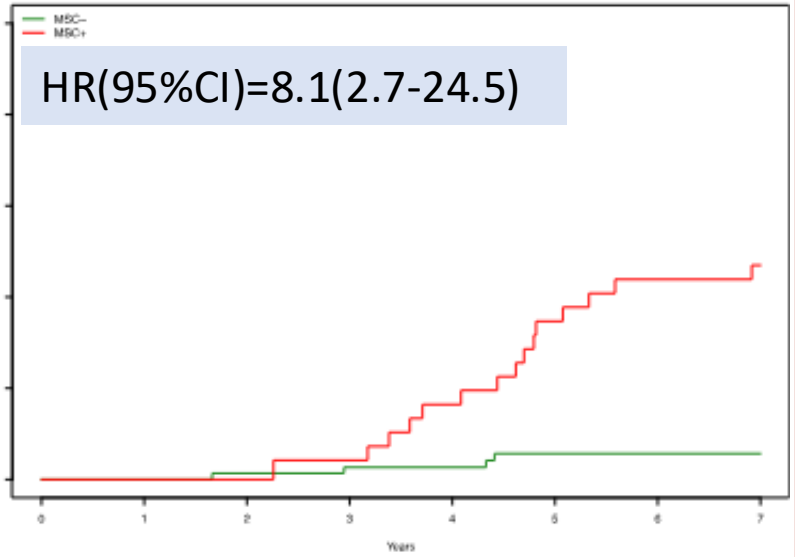
N= 168 incident LC within 2 years (3.4% CTind; 20.5% CT+)

Median follow-up: 8.5 years

LC Incidence



LC Mortality



−	706	669	705	652	510	451	427	316
+	318	314	230	222	220	203	191	147
−	706	696	750	737	680	655	648	639
+	318	326	265	270	319	326	324	321

	TOTAL exams		MSC−		MSC+		Sens	Spec	PPV	NPV
	N	% of total exams	N	% of total exams	N	% of total exams				
CTind	1403		894		509					
LC within 1 year	9	0.6%	2	0.2%	7	1.4%	78%	64%	1%	100%
LC within 2 years	48	3.4%	20	2.2%	28	5.5%	58%	65%	6%	98%
Total LC	136	9.7%	78	8.7%	58	11.4%	43%	64%	11%	91%
CT+	584		296		288					
LC within 1 year	90	15.4%	24	8.1%	66	22.9%	73%	55%	23%	92%
LC within 2 years	120	20.5%	34	11.5%	86	29.9%	72%	56%	30%	88%
Total LC	156	26.7%	50	16.9%	106	36.8%	68%	57%	37%	83%

CTind, computed tomography indeterminate; CT+, computed tomography positive; LC, lung cancer; MSC, 24-microRNA signature classifier; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity.

***Table 2*: Performance of the MSC test in the CTind and CT+ groups in terms of sensitivity, specificity, positive predictive value and negative predictive value.**

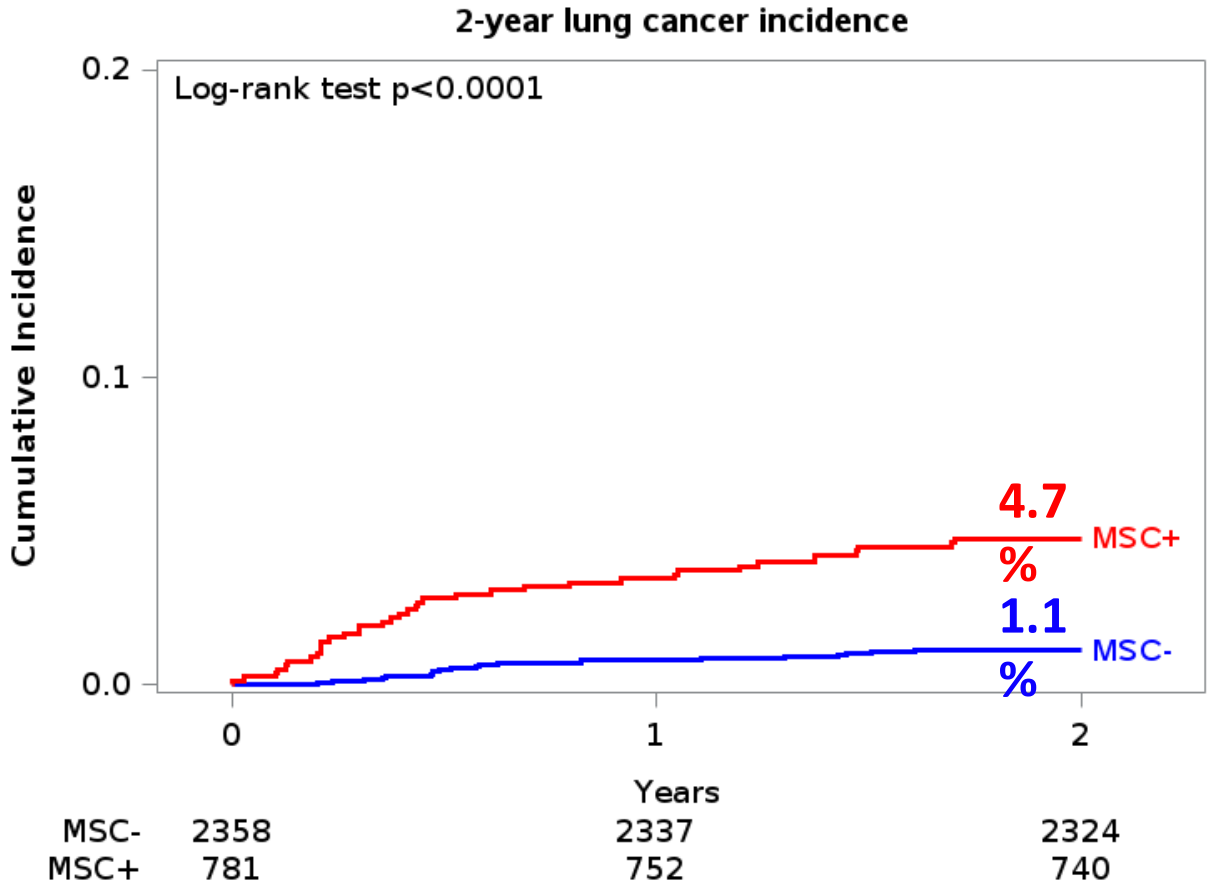
Sozzi et al. Journal of Experimental & Clinical Cancer Research (2025) 44:168
https://doi.org/10.1186/s13046-025-03424-5

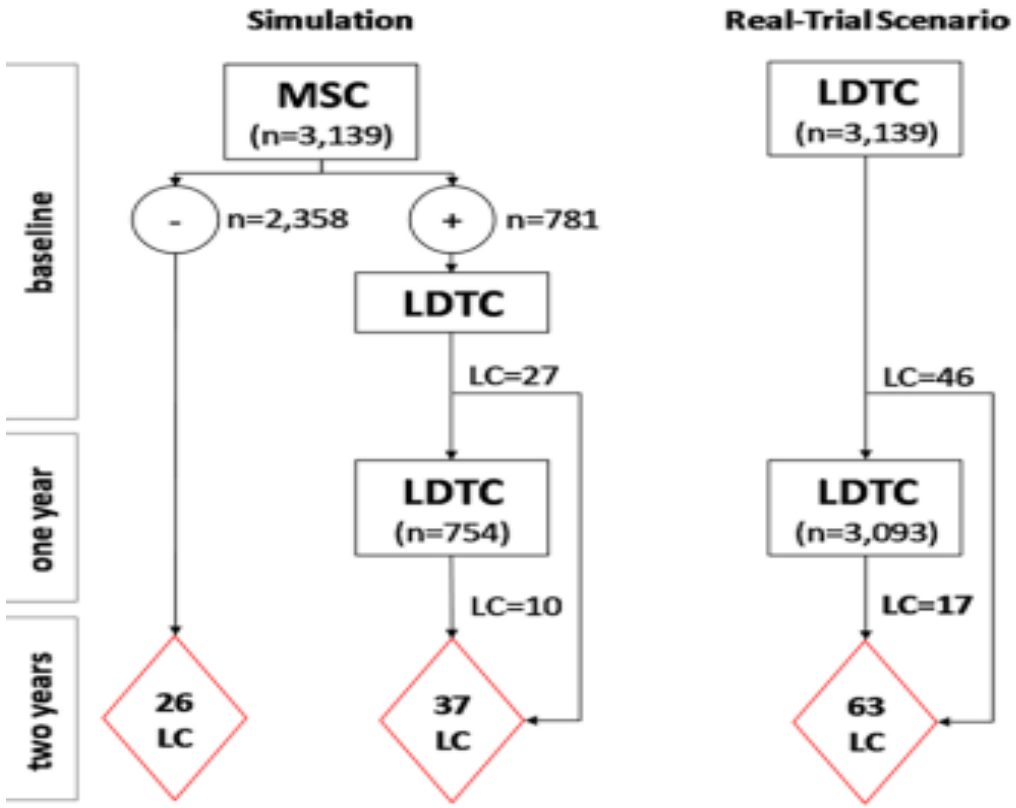
Journal of Experimental & Clinical Cancer Research

COMMENT Open Access

Upfront blood microRNA test in LDCT-reluctant individuals: insights from the biomild trial

Gabriella Sozzi[1†], Federica Sabia[2†], Luigi Rolli[2], Miriam Segale[1], Paola Suatoni[2], Anna Zanghi[1], Margherita Ruggirello[3], Alfonso Marchianò[3], Mattia Boeri[1*] and Ugo Pastorino[2]





Upfront MSC in LCS eligible (n=3139): Sensitivity 58.7%, 56.5% stage I;

Per-cas… for the bio… for standa… screening.

Median costs in Italy of non-screen detected advanced NSCLC

~ 52000€ x LC

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- Lung cancer screening and biomarkers: overview
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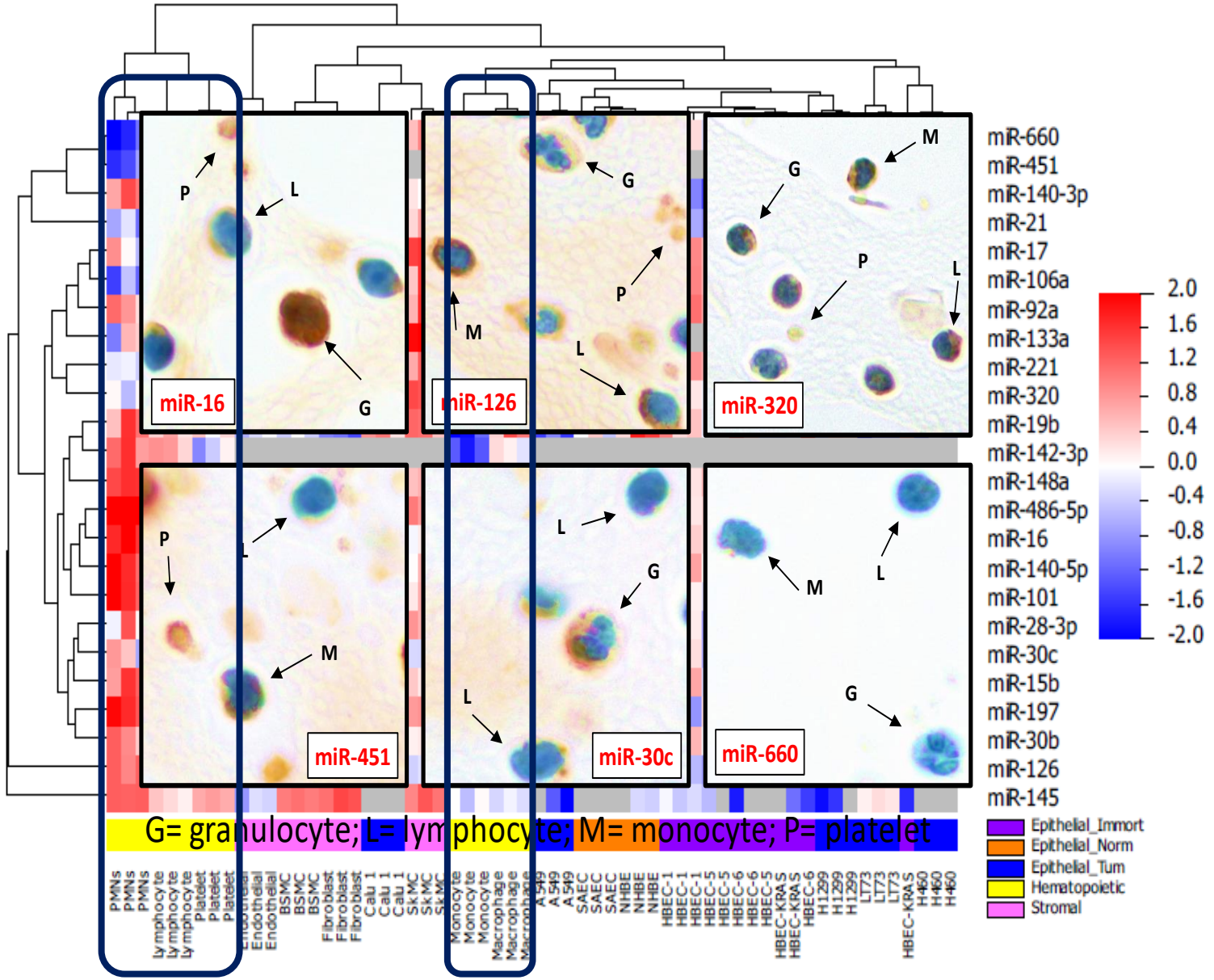
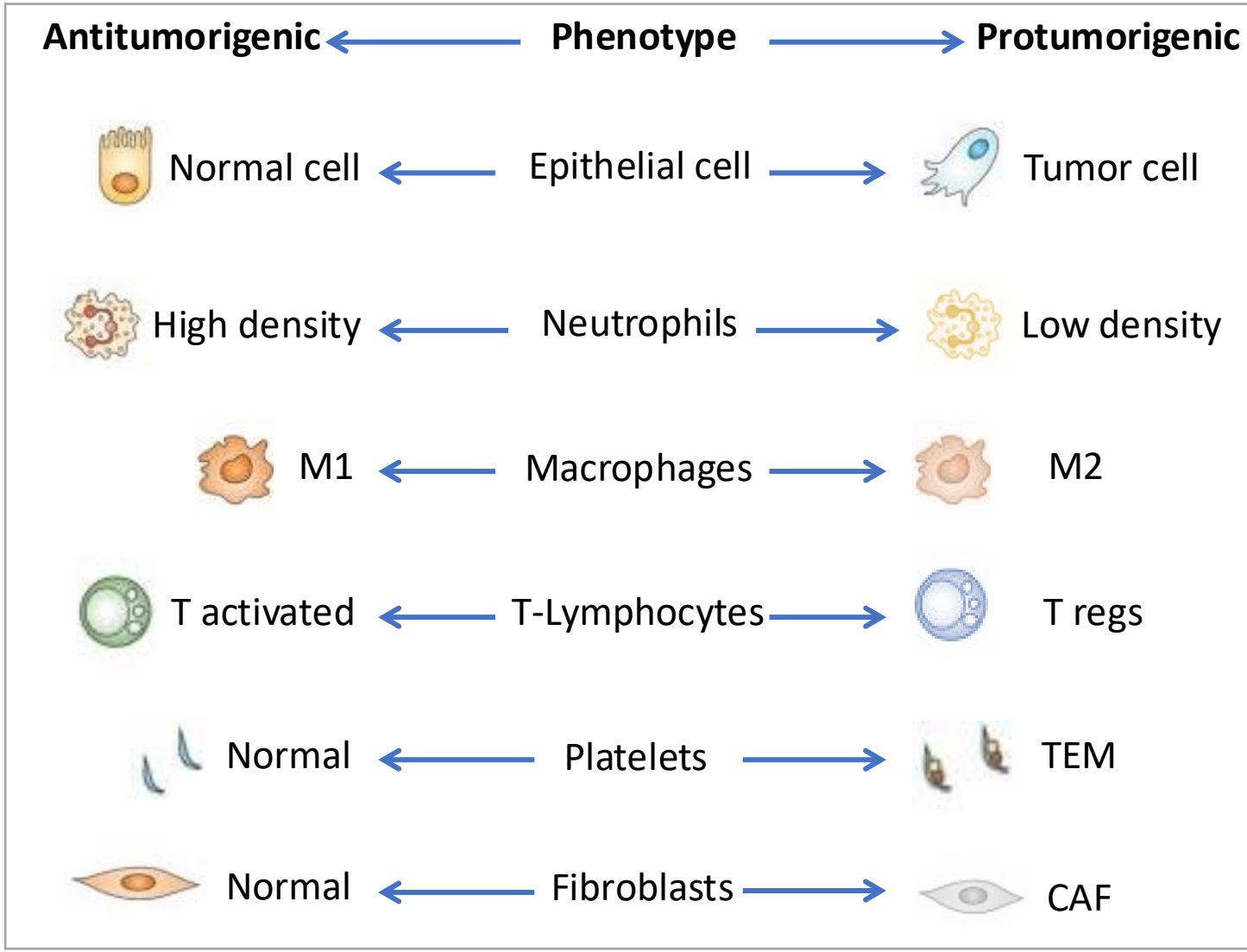
microRNAs composing the MSC derive from hematopoietic cells



Fortunato O, Int J of Cancer 2018

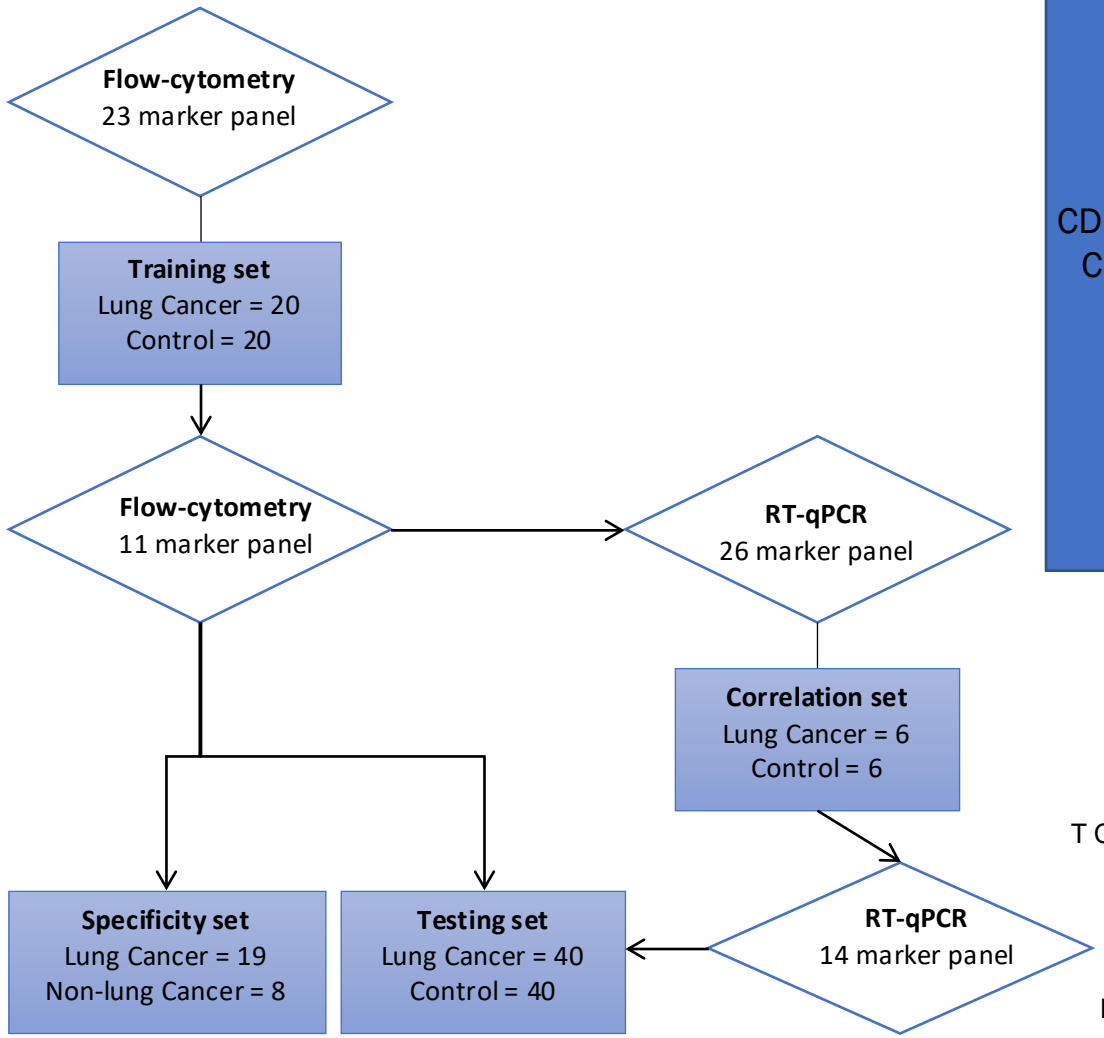
Modulation of the 24 miRNAs in the transition toward a protumorigenic/immunosuppressive phenotype

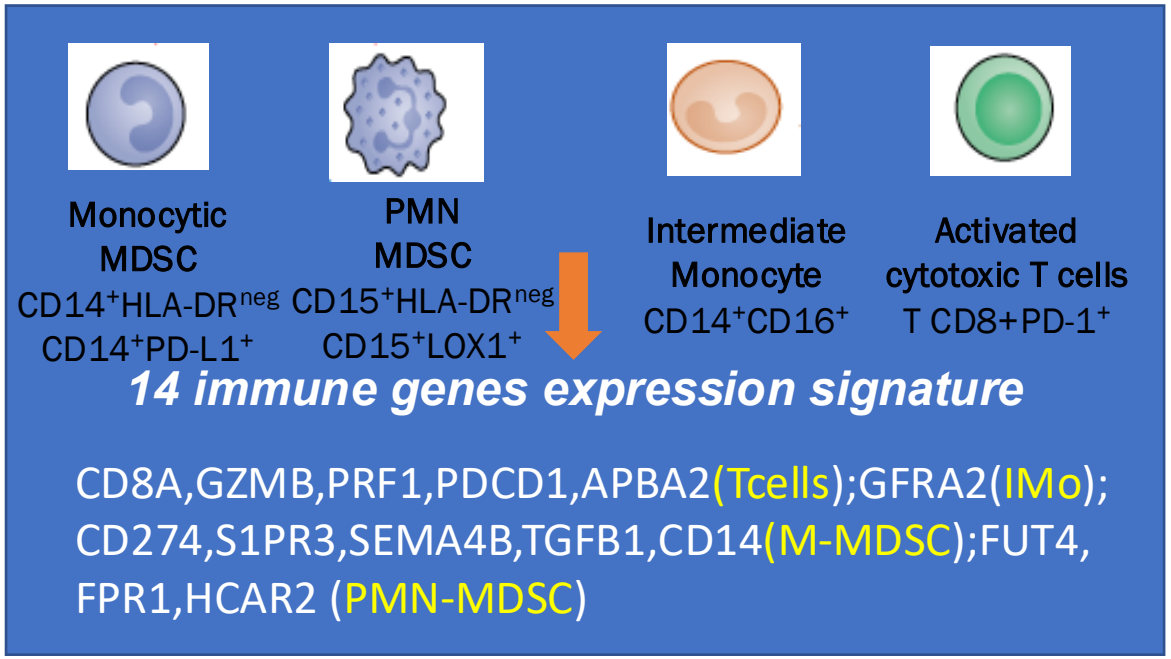


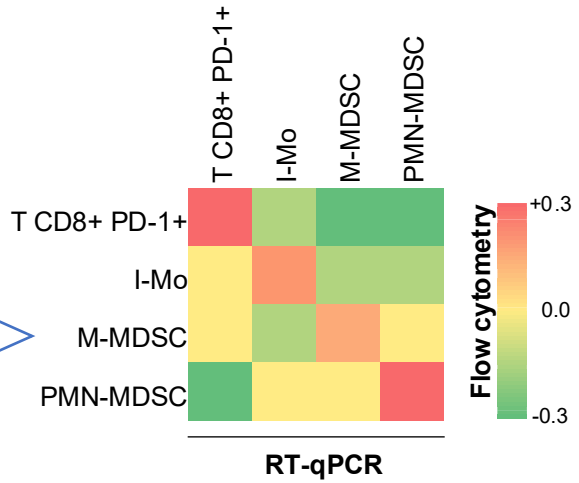
24 miRNAs
mir-16, mir-21, mir-221, mir-30c and mir-451
mir-221, mir-320, mir-101, mir-451 and mir-660
mir-142-3p, mir-15b, mir-145, mir-17, mir-197, mir-320…
mir-106, mir-197, mir-28-3p, mir-17, mir-19b, mir-15b…
mir-126, mir-140-5p, mir-142-3p, mir-28-3p, mir-486-5p…
mir-145, mir-106a, mir-17 and mir-16

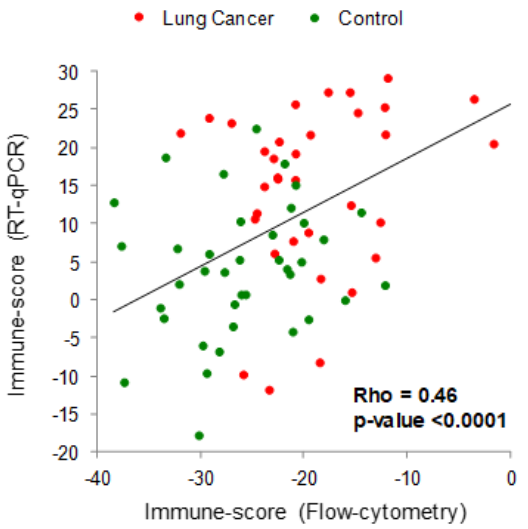
Fortunato O, Int J of Cancer 2018

A complementary immune signature classifier for LC risk

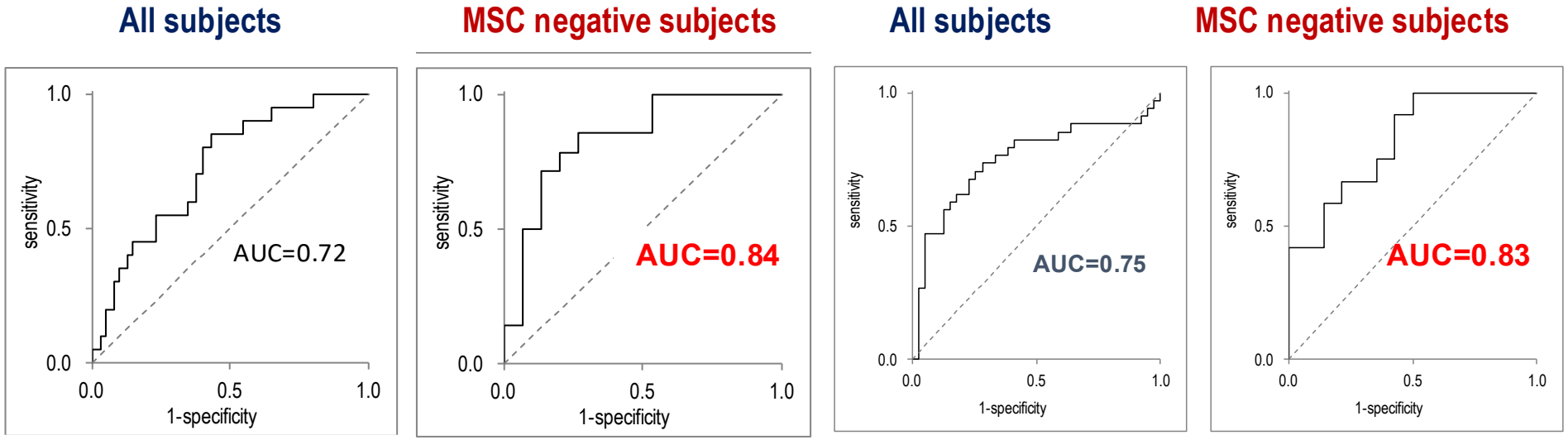




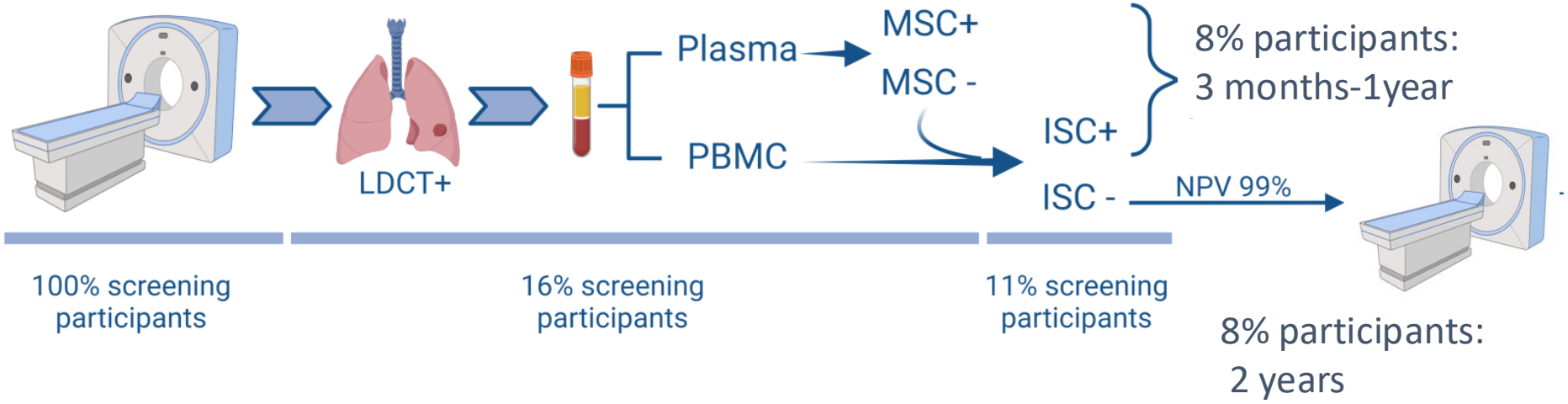




Fortunato O. et al. Cancer Epidemiol Biomarkers Prev 2022 AACRJournals.org

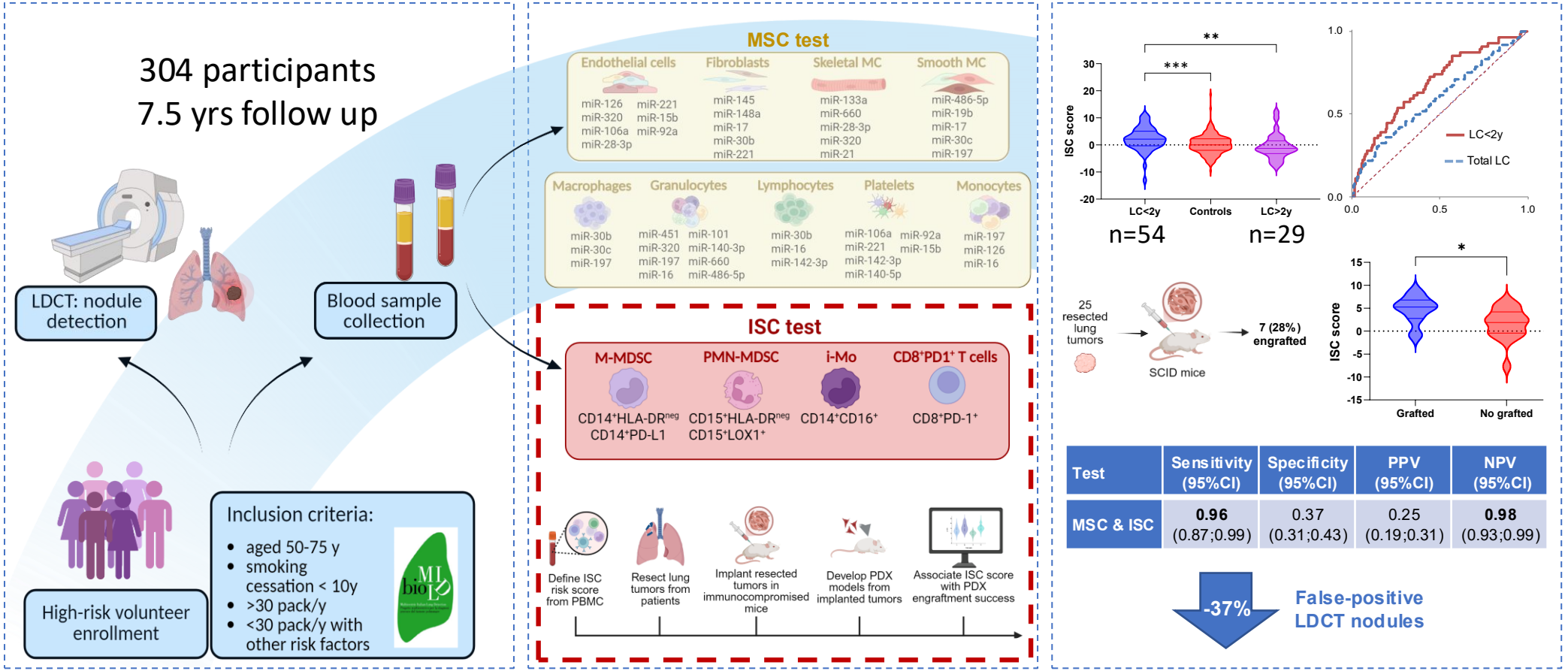


A chained-rule approach in lung cancer screening may reduce 48% unnecessary procedures



Clinical Value of Combining Molecular and Immune Blood Tests to Safely Reduce False Positives in Low-Dose CT Lung Cancer Screening

Journal of Thoracic Oncology



Test	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
MSC & ISC	**0.96** (0.87;0.99)	0.37 (0.31;0.43)	0.25 (0.19;0.31)	**0.98** (0.93;0.99)

-37% False-positive LDCT nodules

CONCLUSION: Integration of immune and molecular markers significantly enhances LDCT screening accuracy, possibly reducing unnecessary follow-up exams and invasive procedures.

Zanghì A, Moro M, Fortunato O, et al. ***Journal of Thoracic Oncology***
https://doi.org/10.1016/j.jtho.2026.103614

IASLC

R.I.S.P.
Rete Italiana Screening Polmonare

Blood bio-bank

1	1277 / 2041
2	1064 / 1064
3	422 / 812
4	335 / 394
5	312 / 590
6	244 / 326

total 3654 volunteers / 5227 samples

G. Sozzi M. Boeri P. Suatoni C. Borzi M. Segale A. Zanghi' O. Fortunato

Epigenomics & Biomarkers of Solid Tumors Unit

F. Sabia C. Valsecchi

Statistics and Data Management

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Thoracic Surgery Unit

FONDAZIONE IRCCS ISTITUTO NAZIONALE DEI TUMORI

Sotto l'alto patrocinio
Presidenza del Consiglio dei Ministri