Challenges in Data-Driven Genomic Computing
March 6-8 2019

Lung Cancer: Circulating microRNAs for early lung cancer detection

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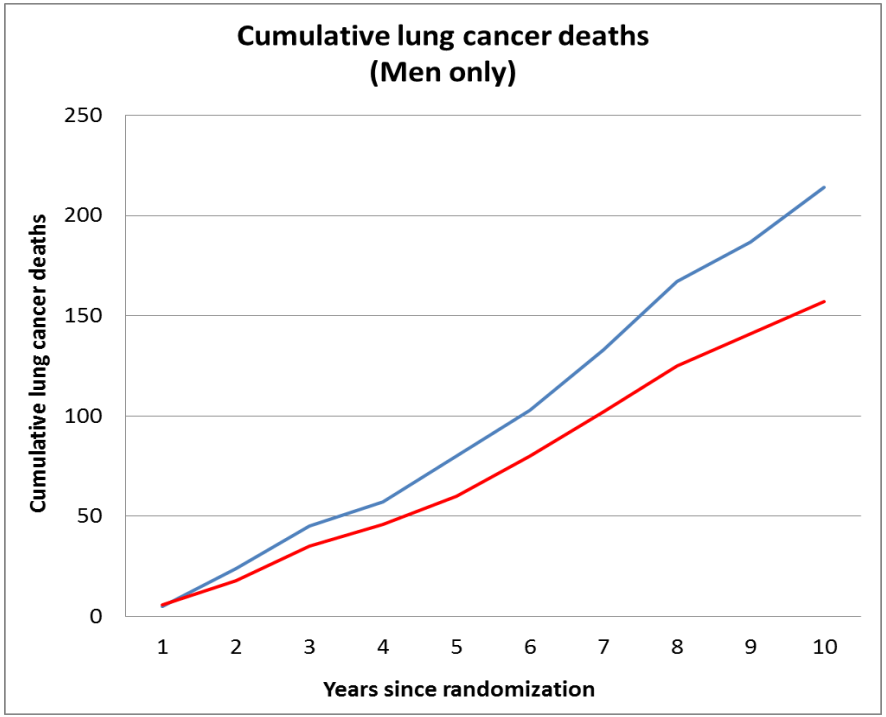
FONDAZIONE IRCCS
ISTITUTO NAZIONALE
DEI TUMORI

Sotto l'alto patrocinio

Presidenza del Consiglio dei Ministri

10 years results of the european NELSON lung cancer screening trial



Control arm:
214 deaths

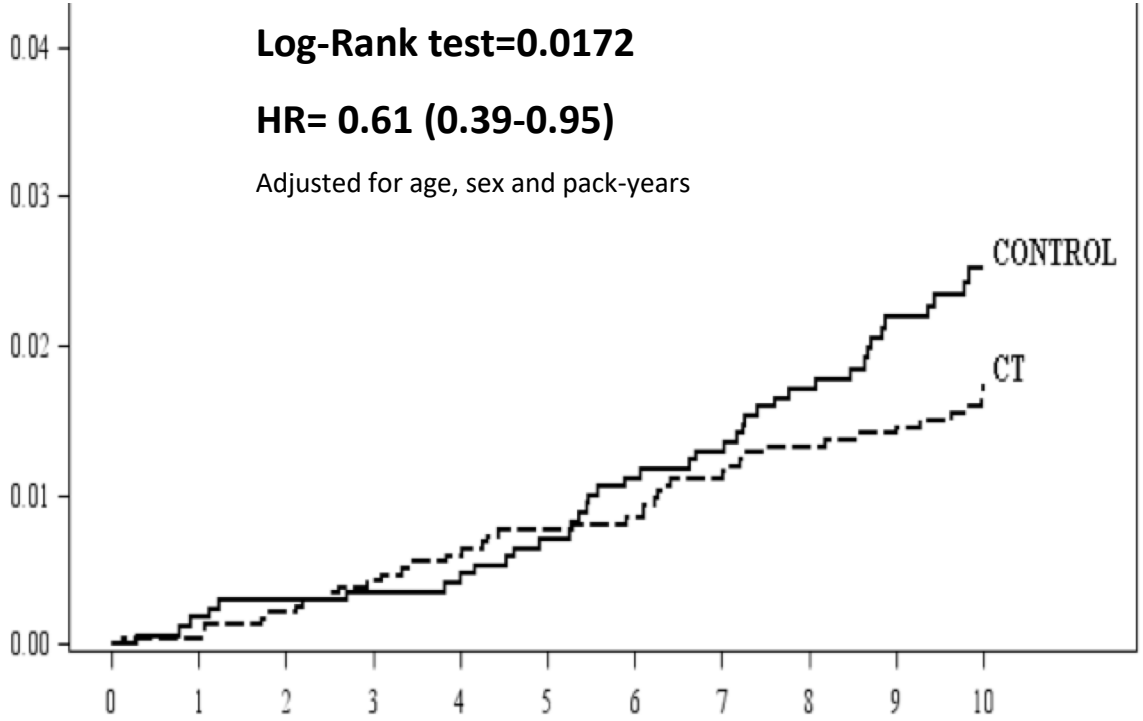
LDCT arm:
157 deaths

LDCT
- 26%

Erasmus MC
University Medical Center Rotterdam

Effects of Volume CT Lung Cancer Screening: Mortality Results of the NELSON Randomised-Controlled Population Based Trial, De Koning et al, IASLC 2018

10 years results of the Italian MILD lung cancer screening trial



LDCT
- 39%

Progetto MILD

Ugo Pastorino, Prolonged LDCT screening from 5 to 10 years

Unresolved issues of lung cancer screening trials

The NLST (USA)

Randomized screening trial on 53,454 persons: LDCT vs CXR

20% reduction of lung cancer mortality
7% reduction all cause mortality

Need to screen 320 subjects to prevent 1 lung cancer death

24% positive subjects
96% of these false positive

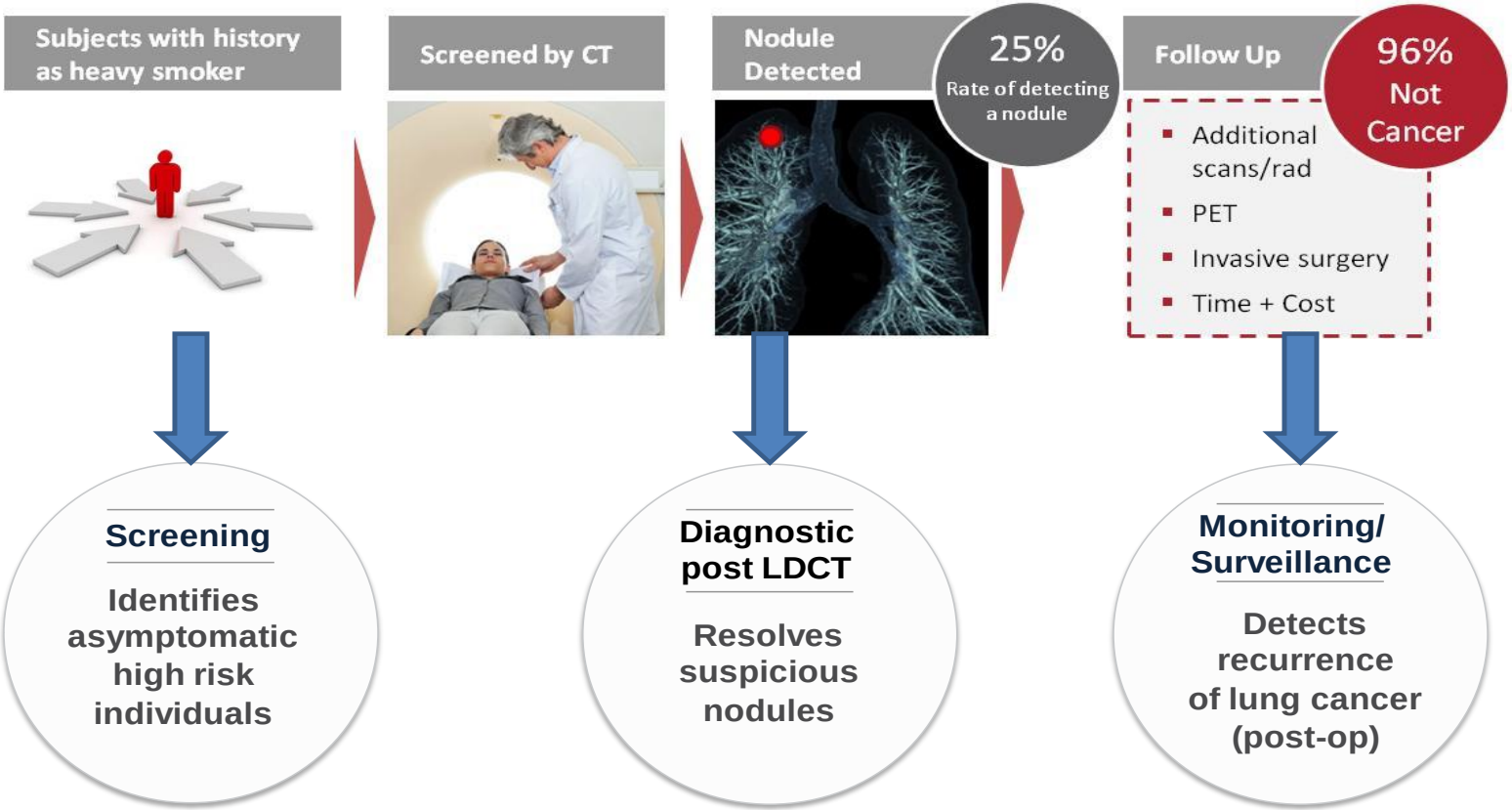
Overdiagnosis by LDCT: > 18%

Number of cases of overdiagnosis in the 320 subjects above: 1.38

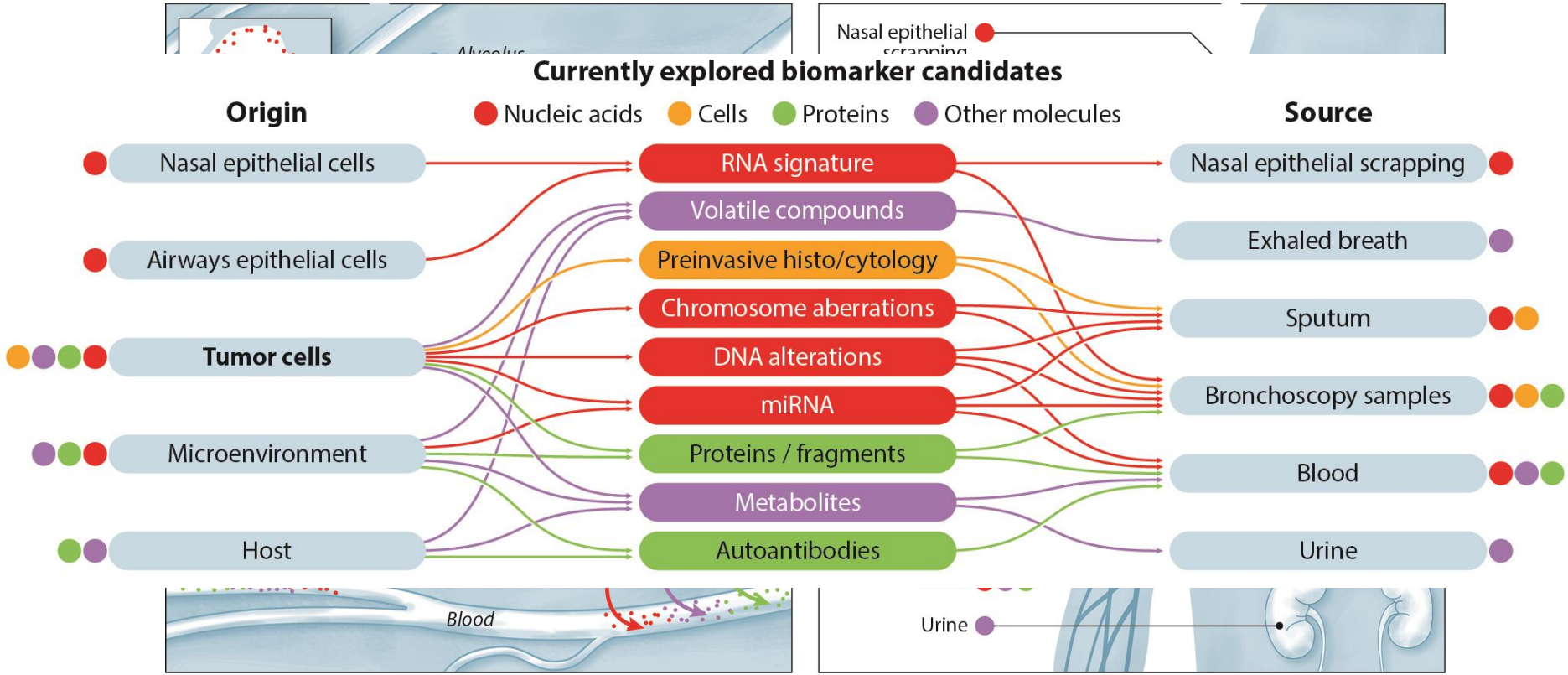
Unmet clinical needs:

- Better definition of the “at risk population”
- Reduction of false positives after initial screen
- Reduction of overdiagnosis through more effcient prediction of aggressive disease

Clinical utility of biomarkers



Sources of biomarkers in lung cancer screening



Seijo LM, et al. JTO. 2018

Blood based biomarkers in lung cancer screening

Test	Retrospective		Prospective		Sources
	Study population	Sens –Spec	Study population	Sens –Spec	
Plasma C4d levels	190 high risk volunteers	AUC = 0.74			Ajona, JNCI 2013
EarlyCDT-Lung (7 AAbs)	55 ptz vs 234 ctrls	40% - 90%	1613 high risk volunteers	41% – 87%	Jett, Lung Cancer 14
			ECLS Study: 12000 high risk vol	Ongoing	
Xpresys Lung (2 proteins)	Indet Nodules: 353 ptz	82% - 32%	Indet Nodules: 178 ptz	97% - 44%	Silvestri, Chest 18
CAPP-Seq	13 ptz vs 5 ctrls	85% - 96%			Newman, NatMed14
mPCR-NGS	96 ptz (stage I-III)	48% - NA			Abbosh, Nature 17
CCGA (tNGS-WGS-methylome)			15000 volunteers	Ongoing	Oxnard, ASCO 18
CancerSEEK	104 ptz (stage I-III) vs 812 ctrls	59% - 99%			Cohen, Science 18
MSC (24 microRNA)	1069 high risk volunteers	87% - 81%	BioMild: 4119 high risk volunt.	Ongoing	Sozzi G, JCO 2014
			SMILE: 2000 high risk volunt.	In 2019	
MiR-Test (19 microRNA)	1008 high risk volunteers	78% - 75%	COSMOS2: 10000 high risk vol.	Ongoing	Montani, JNCI 2015

Proteins | DNA | DNA+proteins | RNA

The EarlyCDT-Lung test (7 AAbs)

The marker: Autoantibodies (AAbs) develop in response to an abnormal tumor antigens (p53, NY-ESO-1, CAGE, GBU4-5, SOX2, HuD, and MAGE A4) in some patients with lung cancer.

The clinical setting: Individuals deemed by their clinician to be at an increased risk of developing lung cancer due to age and smoking history or other factors.

	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.

Lam S et al. CanPrevRes, 2011 – Chapman CJ et al. TumBiol, 2012 – Jett JR et al. Lung Cancer, 2014

The Circulating Cell-free Genome Atlas Study (CCGA)

The marker: circulating free DNA can be released in blood by tumor cells.

The technology: Illumina platforms

1) Paired cfDNA and white blood cell (WBC) targeted sequencing (60,000X, 507 gene panel)
2) Paired cfDNA and WBC whole-genome sequencing (WGS; 35X)
3) cfDNA whole-genome bisulfite sequencing (WGBS; 34X) for abnormally methylated fragments

The clinical setting: Blood was collected from participants with newly diagnosed therapy-naive cancer (cases) and participants without a diagnosis of cancer (controls) for a total of 15,000 individuals.

The Circulating Cell-free Genome Atlas Study (CCGA)

Results of the test in patients with lung cancer (n=127) and a subset of controls (580) :
-Similar ages (mean±SD yrs: 67±9, 60±13)
-85% and 43% were ever-smokers
-46% and 22% were men

Fixed **95% specificity**

Sensitivity in 63 stage I-IIIA pts was:
48% (targeted NGS), **54%** (WGS), and **56%** (WGBS)

Sensitivity in 54 stage IIIB-IV pts was:
85% (targeted NGS), **91%** (WGS), and **93%** (WGBS)

Oxnard et al, ASCO 2018

The CancerSEEK test: ctDNA + proteins

The markers: - Circulating tumor DNA released in blood by tumor cells
- A combination of tumor-expressed and host response proteins

The technology:

1) PCR–based sequencing assay that could simultaneously assess multiple regions of 16 commonly mutated genes
2) 8 proteins evaluated through a single Luminex bead based immunoassays platform (Millipore)

The clinical setting: 812 Controls vs. 1005 patients who had been diagnosed with stage I to III cancers of the ovary, liver, stomach, pancreas, esophagus, colorectum, lung or breast.

The CancerSEEK test: ctDNA + proteins

Results of the test:
Specificity >99% (7/812 controls)

Median age:
-Controls 55 years
-All patients: 64 years
-LC patients: 69 years

Lung Cancer	CancerSEEK positive /all LC cases	Sensitivity
Stage I	20/46	43%
Stage II	18/27	67%
Stage III	23/31	74%

Cohen et al, Science 2018

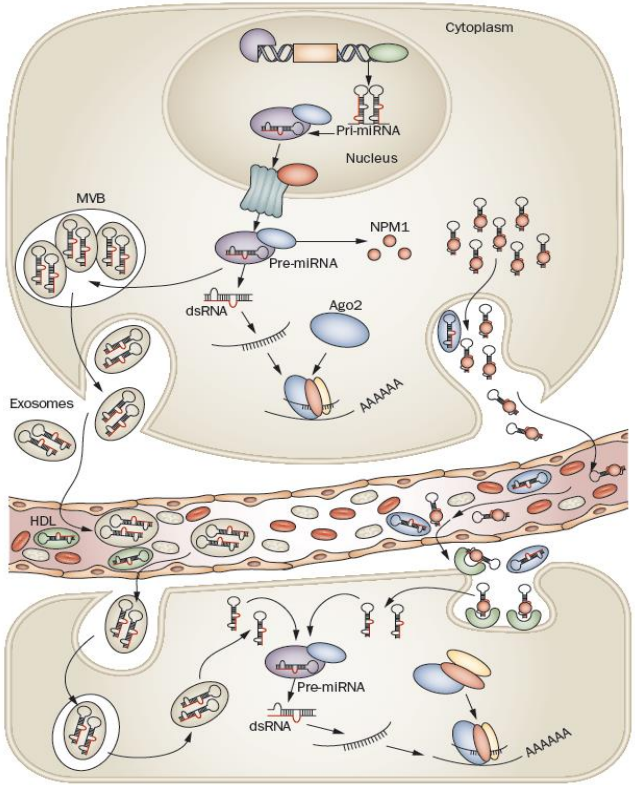
Circulating microRNAs

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)

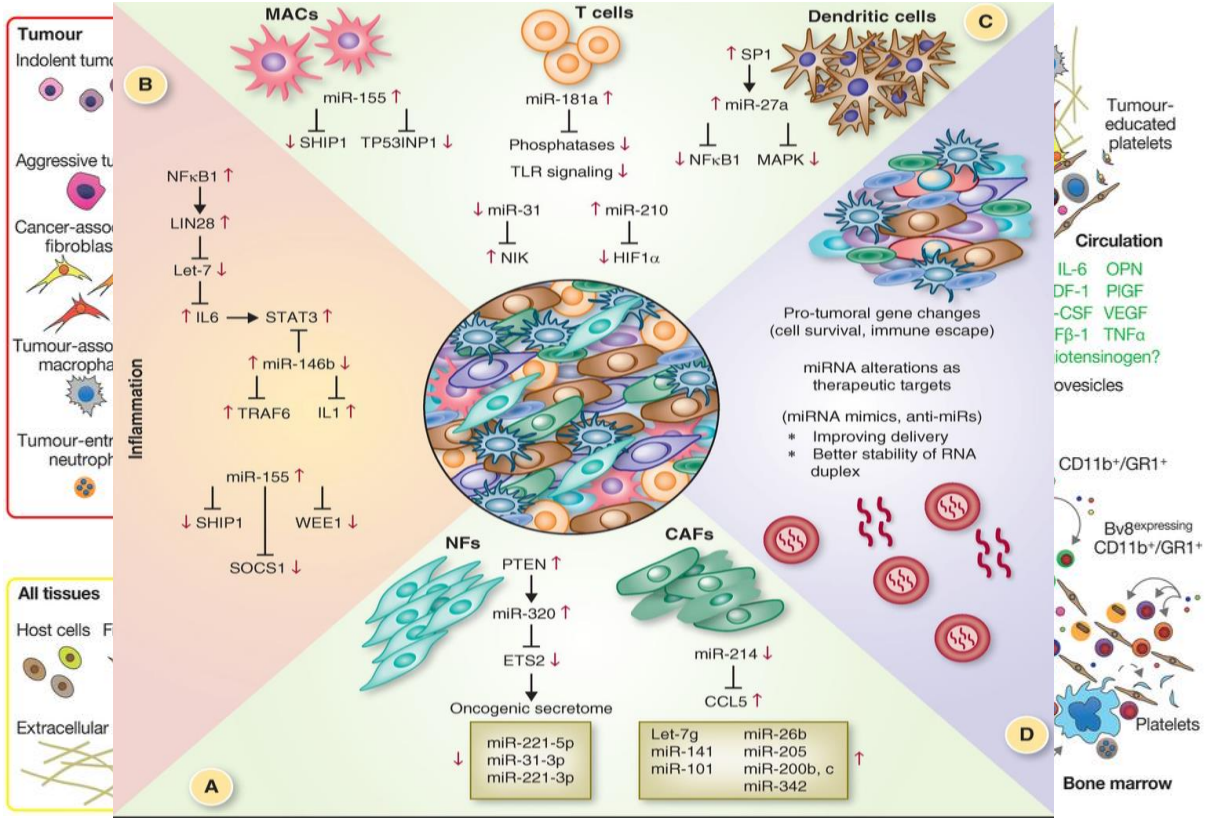
Passive release

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

MicroRNAs: biomarkers from the tumor-host interplay



Cancer progression is driven not only by a tumour's underlying genetic alterations and paracrine interactions within the tumour microenvironment, but also by complex **systemic processes.**

The microRNA signature classifier (MSC)

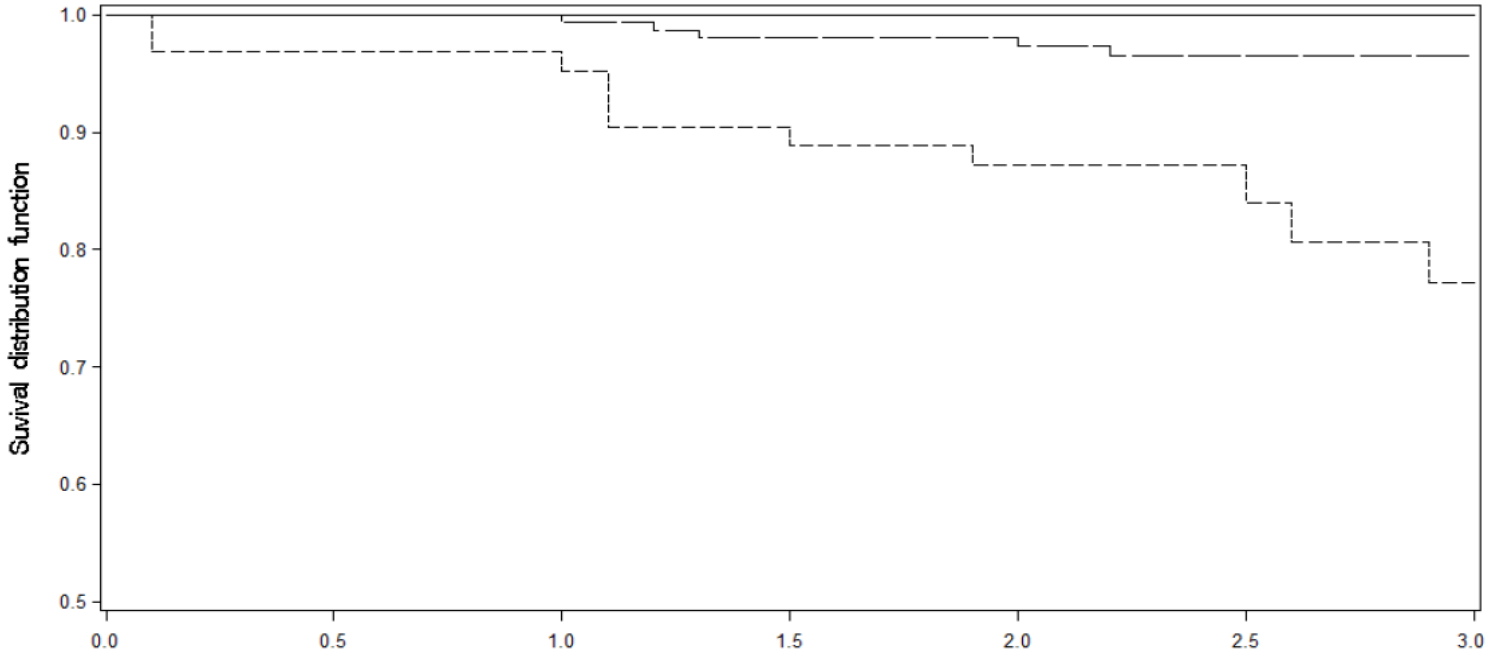
Screening Trials: LDCT every one or two years

PRE-DISEASE **DIAGNOSIS**

CONTROLS:
DISEASE FREE INDIVIDUALS WHIT AT LEAST 5 YEARS OF FOLLOW-UP

Boeri et al., PNAS 2011; Sozzi et al., JCO 2014

The microRNA signature classifier (MSC)

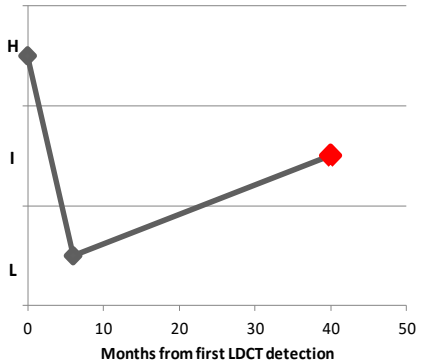


High	63	61	53	22
Intermediate	159	156	149	39
Low	717	715	710	83

The microRNA signature classifier (MSC)

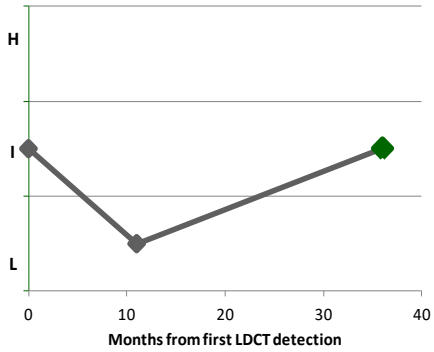
Screening Surgery Monitoring

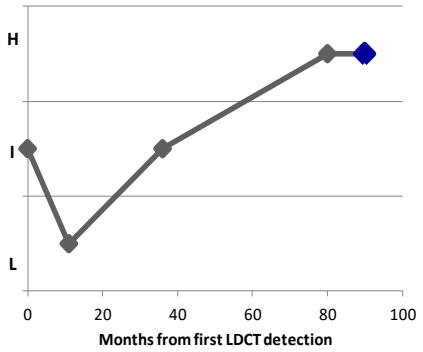
- Second primary lung cancer
- Brain metastasis from lung cancer
- Lung metastasis from breast cancer



Longitudinal plasma samples (n=100) from 31 out of 44 (70.5%) alive patients

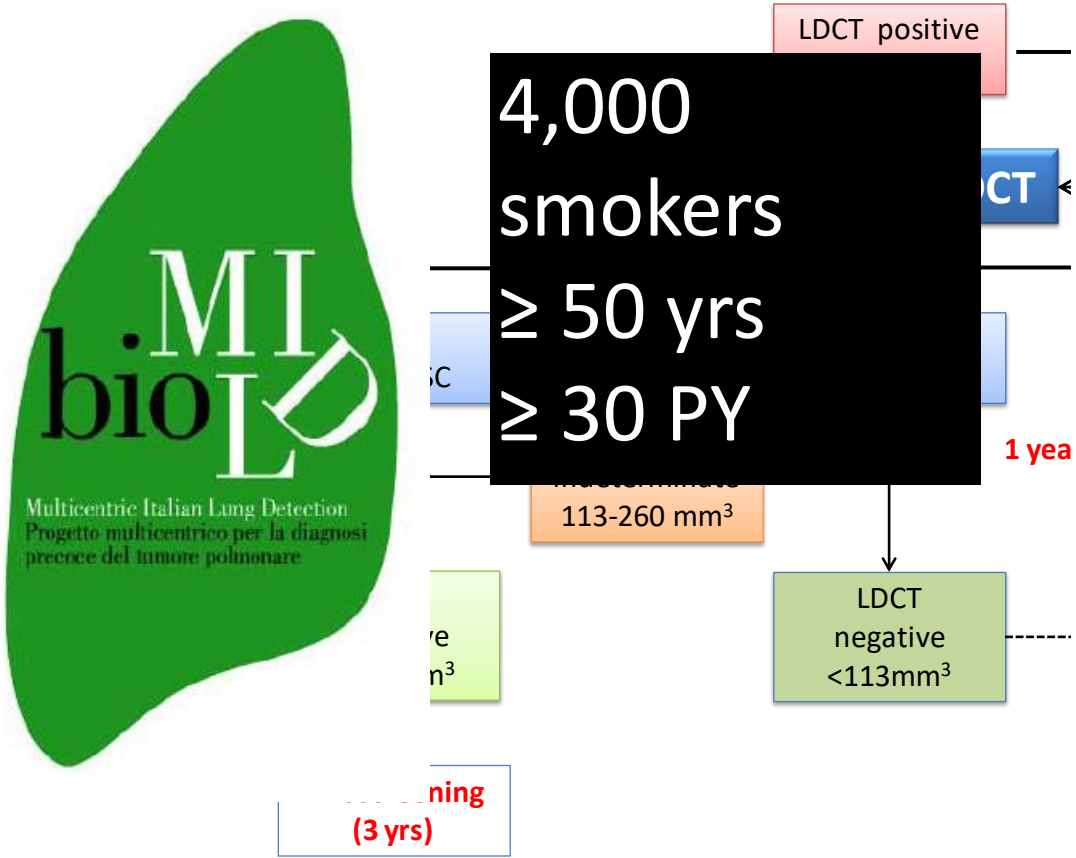
- 28 disease free patients
- 3 relapsing patients





Sestini et al, Oncotarget 2015

The Biomild prospective validation study



Total registered	9,236
Eligibile	5,772
Enrolled	4,301
Baseline	**4,119**
LDCT	8,000
miRNA test	**9,168**

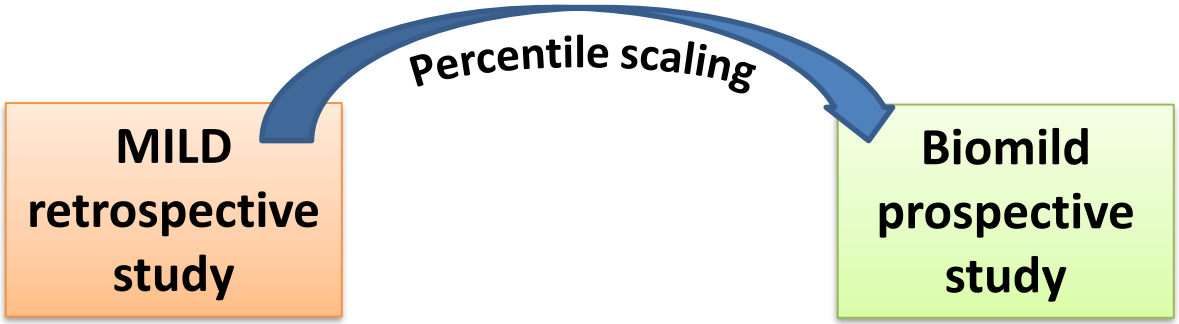
Mean age: 60 years

Males: 2,499 - Females: 1,615

Active smokers: 3258

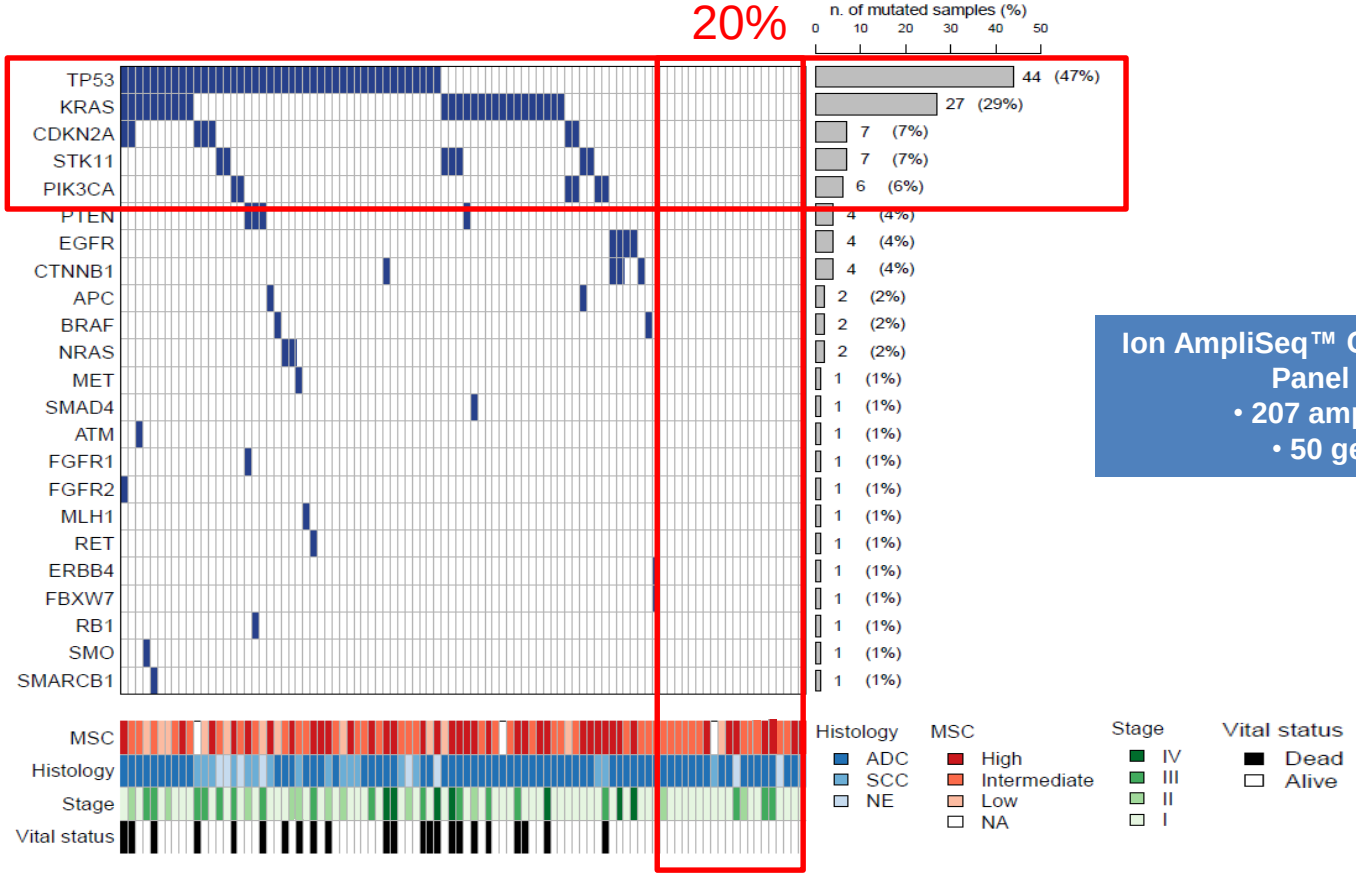
Former smokers: 856

MSC results at the BASELINE



	Expected		Observed		Delta	
MSC risk level						
High	236	6%	164	4%	-72	-2%
Intermediate	708	18%	902	23%	194	5%
Low	2986	76%	2866	73%	-120	-3%

The MSC
Correlative studies

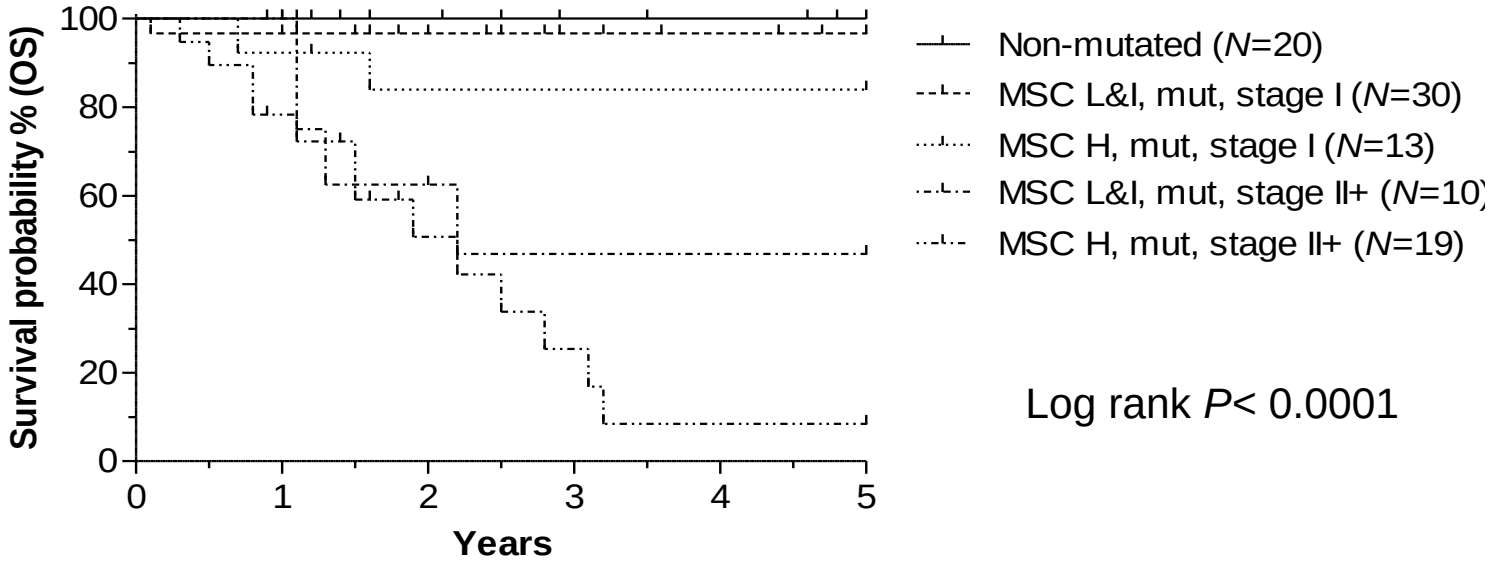


Ion AmpliSeq™ Cancer Hotspot Panel v.2 :
- **207 amplicons**
- **50 genes**

Verri at al., JTO 2017

The MSC
Correlative studies

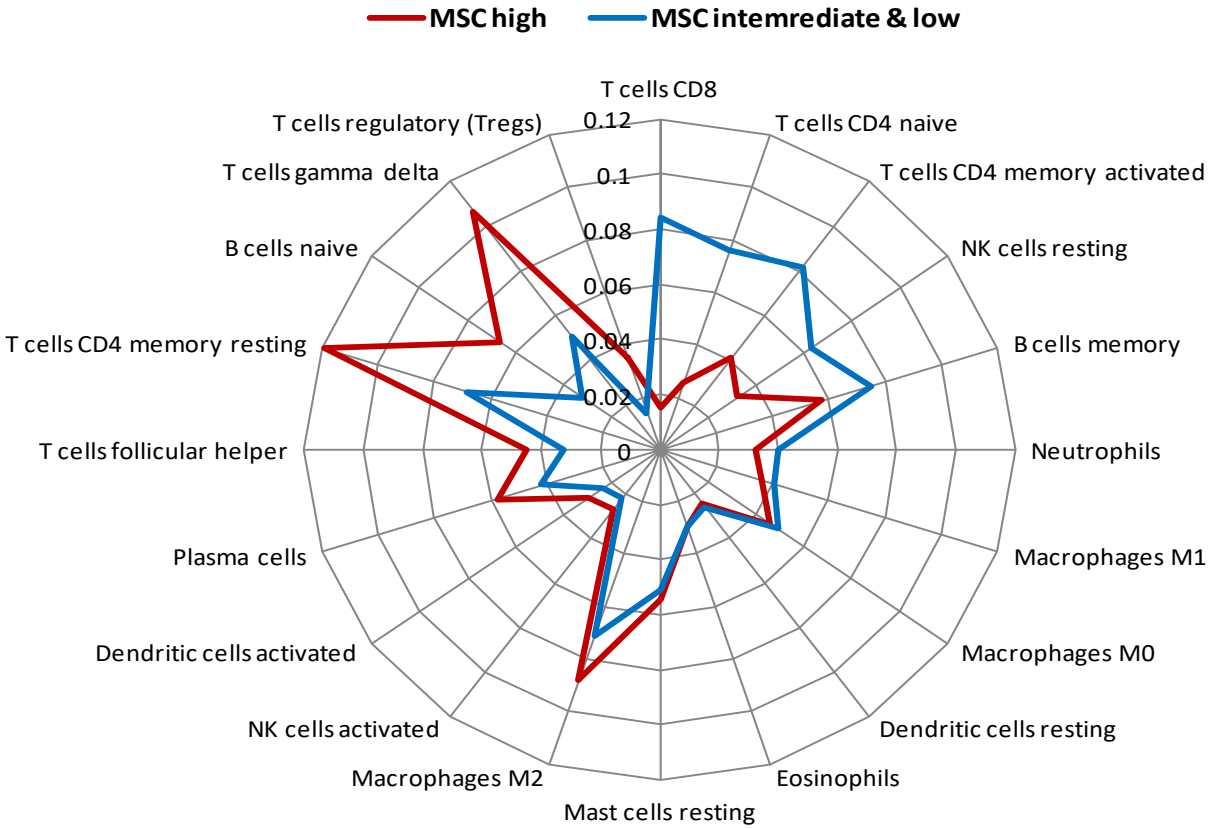
Mutational status alone and in combination with MSC and tumor stage is associated with prognosis



Log rank $P < 0.0001$

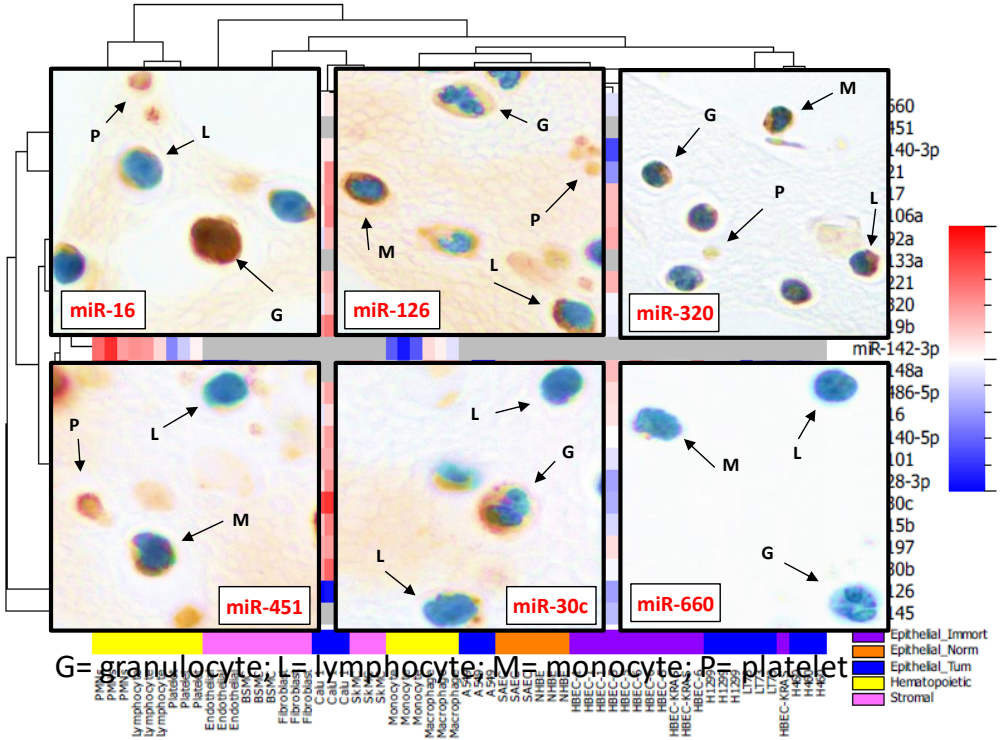
Verri at al., JTO 2017

The MSC
Correlative studies

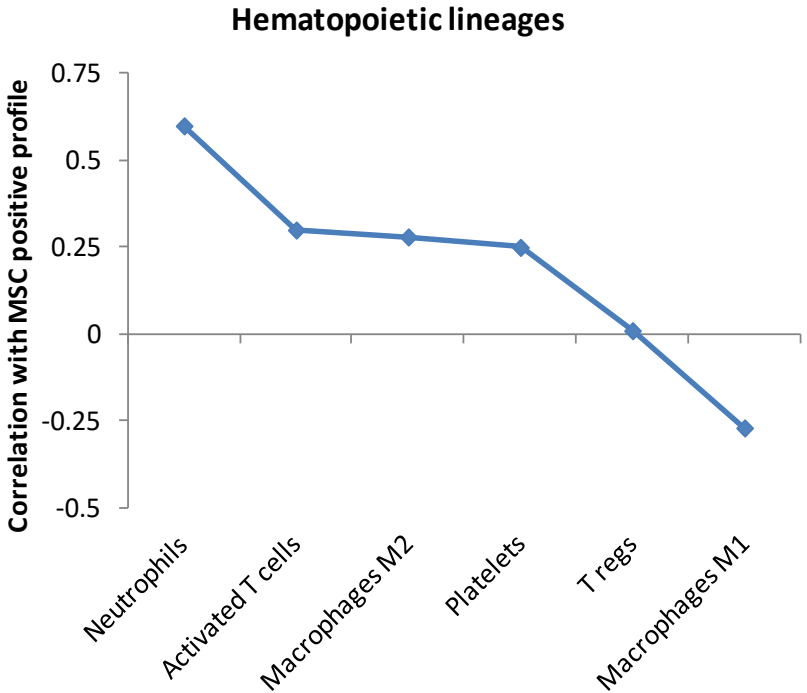


MSC high risk level reflects a tumor "cold" immunophenotype

The MSC
Correlative studies



G= granulocyte; L= lymphocyte; M= monocyte; P= platelet



Fortunato et al., JCI 2018

TAKE HOME MESSAGES

Circulating miRNA are very handful and useful biomarkers reflecting the interaction between the tumor and the host

Circulating biomarkers should be identified and validated in proper settings

Analytical and pre-analytical condition must be standardize as much as possible

TUMOR GENOMICS: Luca Roz, Orazio Fortunato, Massimo Moro, Cristina Borzi, Francesca Andriani, Carla Verri, Davide Conte, Mavis Mensah

THORACIC SURGERY: Paola Suatoni, Stefano Sestini, Ugo Pastorino and the MILD-bioMILD supporting staff

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Fondazione Pezcoller

Ministero della Salute

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