Dalla biopsia liquida al paziente: il valore prognostico

Che cosa abbiamo imparato nei tumori solidi?

Dr. Fortunato Orazio

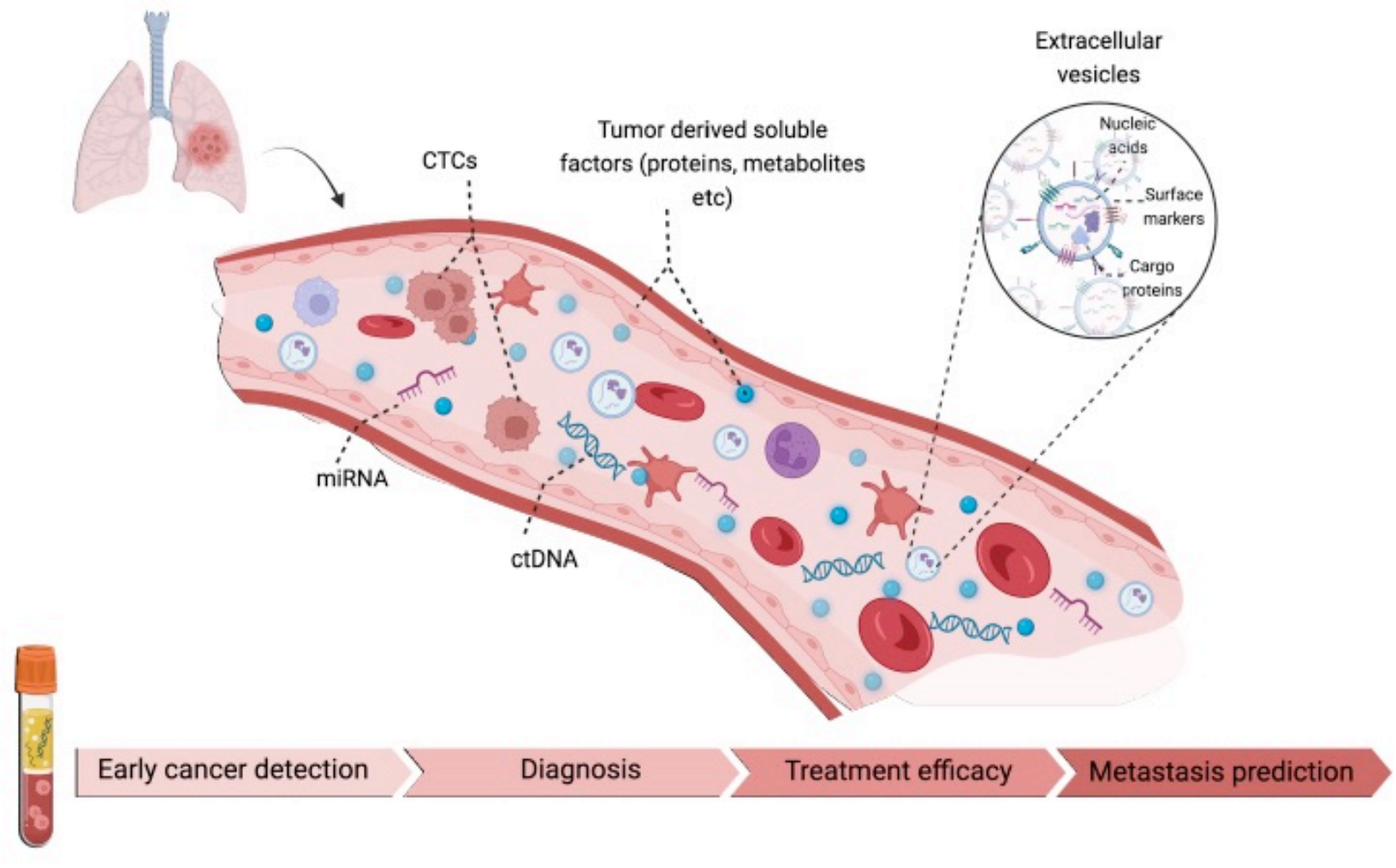
Unità di Epigenomica e biomarcatori dei Tumori Solidi

Fondazione IRCCS Istituto Nazionale dei Tumori di Milano

Disclosures

No disclosures

La biopsia liquida nei tumori solidi



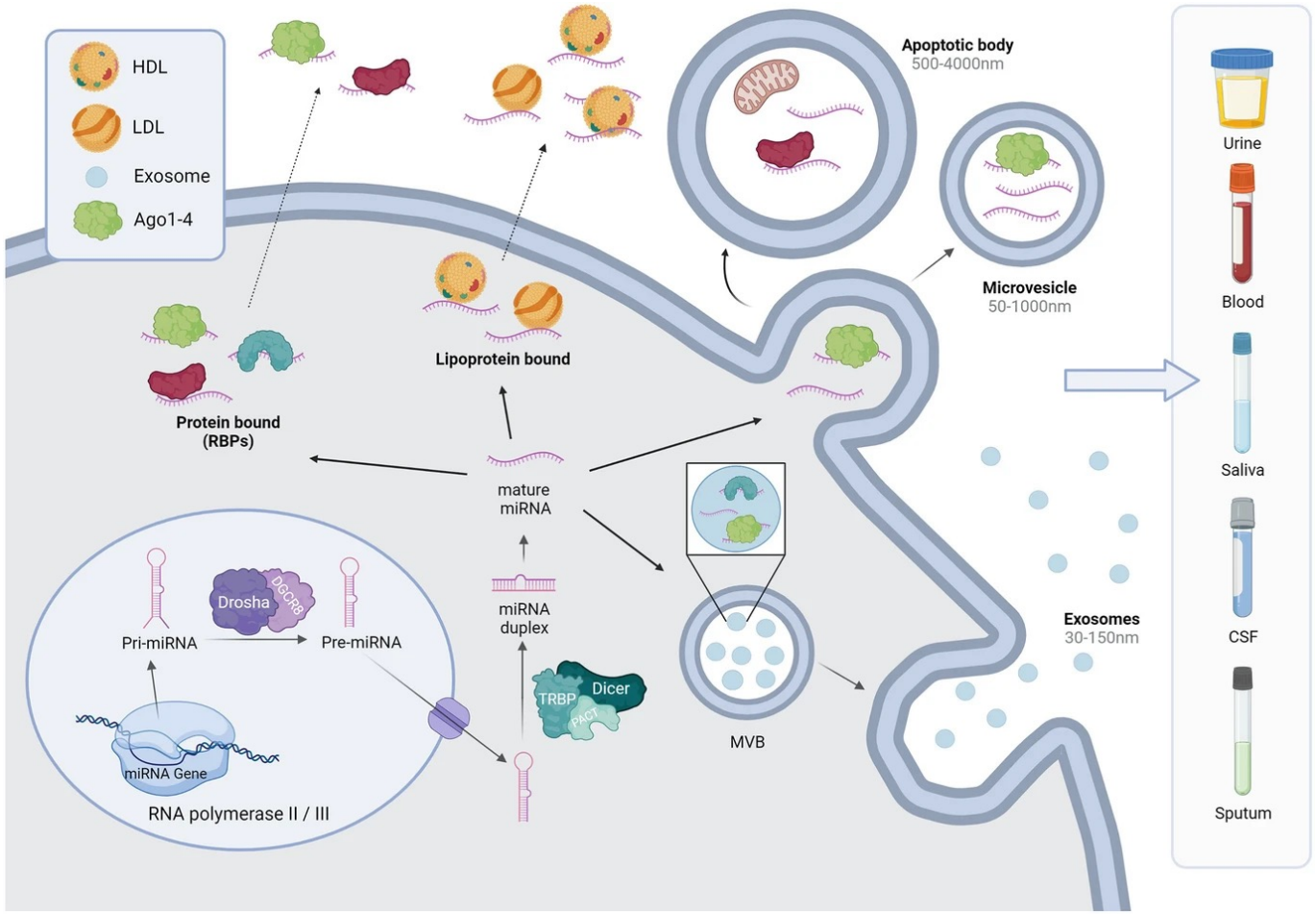
Vantaggi:

1.Non invasiva
2.Ripetibilità
3. Accessibilità

Svantaggi:

1. Sensibilità e specificità
2.Limitazioni tecniche
3.Mancanza di standardizzazione
4.Possibili costi elevati

Ruolo dei microRNAs come biomarcatore prognostico



Suárez B, Advances in Experimental Medicine and Biology 2022

miR-34a

Tumori: Mammella, polmone, colon, fegato, prostata.

Ruolo: miR-34a circolante è **sottosoppresso** nei tumori

miR-21

Tumori: Mammella, polmone, colon, fegato, pancreas.

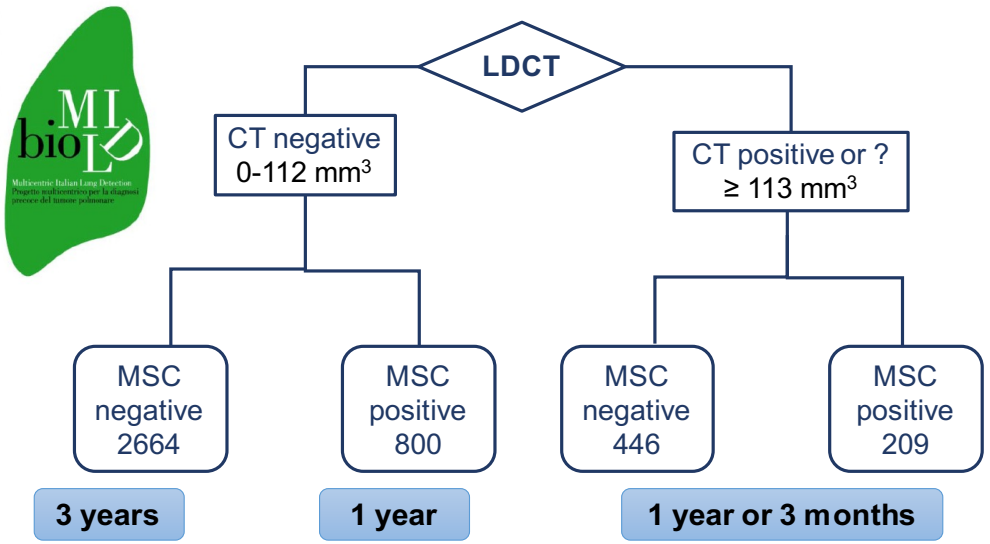
Ruolo: miR-21 **sovraespresso** nei tumori.

miR-155

Tumori: Mammella, polmone, colon, prostata.

Ruolo: miR-155 circolante è un marcatore di **prognosi negativa** nei tumori solidi.

Ruolo dei microRNAs come biomarcatore prognostico

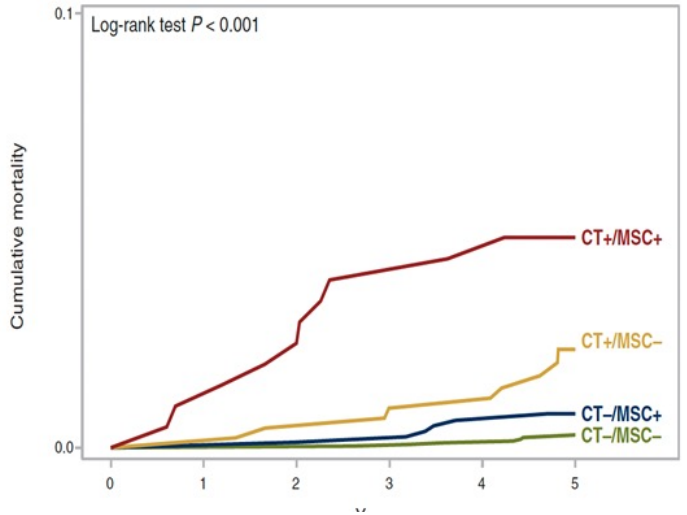


24 miRNAs plasmatici

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, mir-92a.

Rapporti tra i 24 microRNAs

MSC	Risk level
Positive	High
	Intermediate
Negative	Low



Pastorino U. et al., Annals of Oncology 2022

LDCT e il profilo miRNAs (MSC) definisce il rischio di sviluppare un tumore al polmone

La mortalità a 5 anni è 3 volte Maggiore negli individui MSC+

Ruolo dei microRNAs come biomarcatore prognostico

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

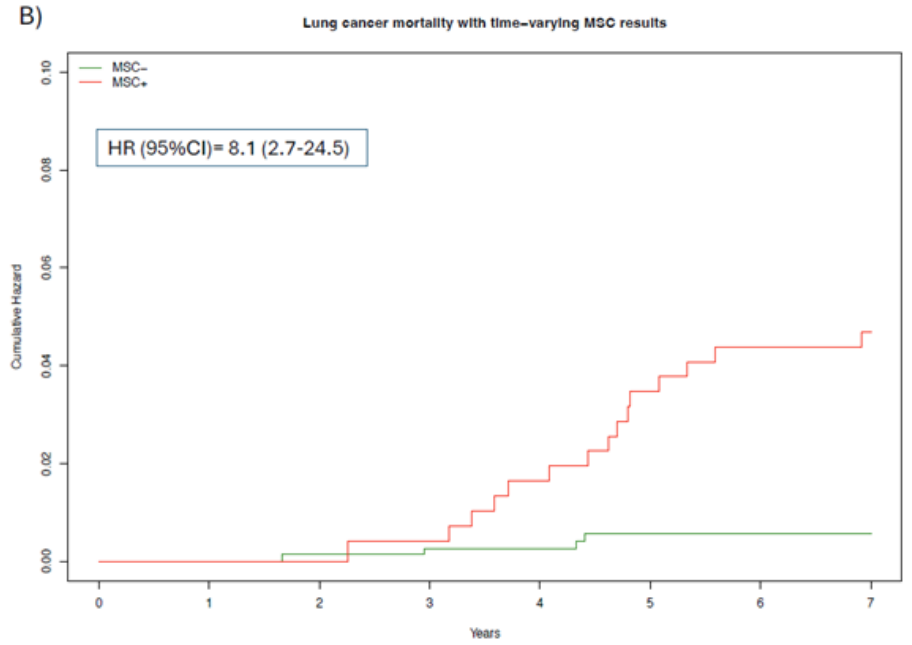
Mattia Boeri,[a] Federica Sabia,[b] Roberta E. Ledda,[b,c] Maurizio Balbi,[b,d] Paola Suatoni,[b] Miriam Segale,[a] Anna Zanghì,[a] Anna Cantarutti,[e] Luigi Rolli,[b] Camilla Valsecchi,[b] Giovanni Corrao,[e] Alfonso Marchianò,[f] Ugo Pastorino,[b,g] and Gabriella Sozzi[a,g,*]

[a]Unit of Epigenomics & Biomarkers of Solid Tumors, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, 20133, Italy
[b]Unit of Thoracic Surgery, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, 20133, Italy
[c]Department of Medicine and Surgery (DiMeC), Section of Radiology, Unit of Surgical Sciences, University of Parma, Parma, 43121, Italy
[d]Department of Oncology, Radiology Unit, San Luigi Gonzaga Hospital, University of Turin, Orbassano, 10043, Italy
[e]Division of Biostatistics, Department of Statistics and Quantitative Methods, Epidemiology and Public Health, University of Milano-Bicocca, Milan, 20126, Italy
[f]Department of Radiology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, 20133, Italy

Summary

Background The proper management of suspicious radiologic findings is crucial to optimize the effectiveness of low-dose computed tomography (LDCT) lung cancer screening trials. In the BioMILD study, we evaluated the utility of combining a plasma 24-microRNA signature classifier (MSC) and LDCT to define the individual risk and personalize screening strategies. Here we aim to assess the utility of repeated MSC testing during annual screening rounds in 1024 participants with suspicious LDCT findings.

The Lancet Regional Health - Europe 2024;46: 101070
Published Online xxx
https://doi.org/10.1016/j.lanepe.2024.101070



MSC-	706	696	750	737	680	655	648	639
MSC+	318	326	265	270	319	326	324	321

La positività al test MSC aumenta di 8 volte il rischio di mortalità nei soggetti con CT indeterminate o positiva.

N= 1024 participants with CT ind/pos
N=1403 CT ind and 584 CT pos
N= 125 LC within 2 years (incident cancer only)

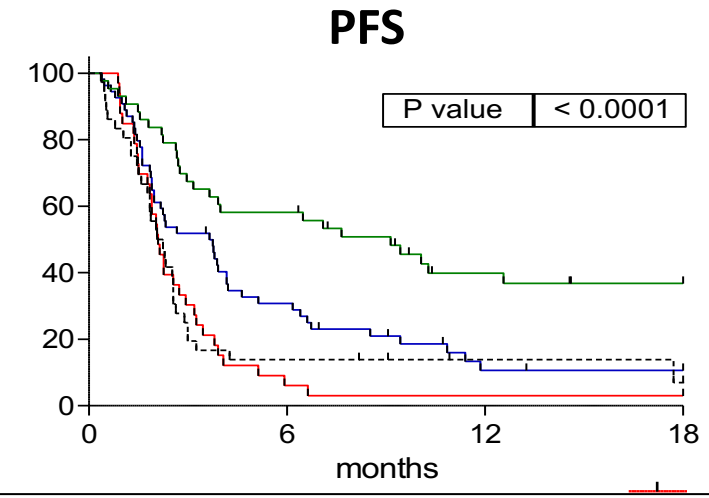
Ruolo dei microRNAs come biomarcatore prognostico (Studio Apollo)

Precision Medicine and Imaging

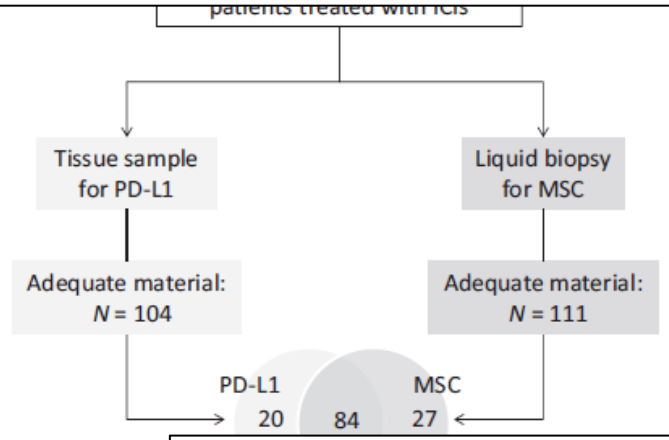
Clinical Cancer Research

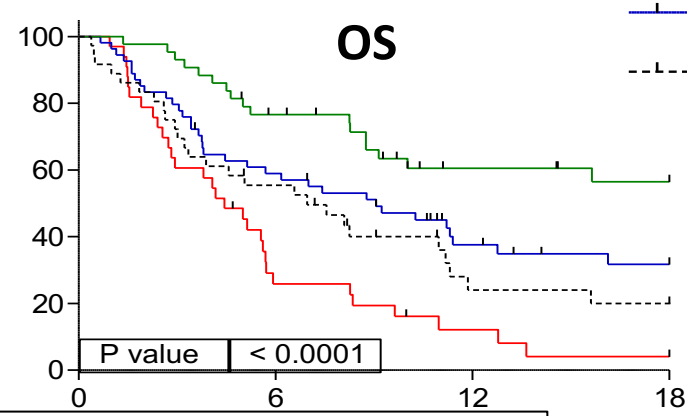
Circulating miRNAs and PD-L1 Tumor Expression Are Associated with Survival in Advanced NSCLC Patients Treated with Immunotherapy: a Prospective Study

Mattia Boeri[1], Massimo Milione[2], Claudia Proto[3], Diego Signorelli[3], Giuseppe Lo Russo[3], Carlotta Galeone[4], Carla Verri[1], Mavis Mensah[1], Giovanni Centonze[1,5], Antonia Martinetti[3], Elisa Sottotetti[3], Ugo Pastorino[6], Marina Chiara Garassino[3], and Gabriella Sozzi[1]



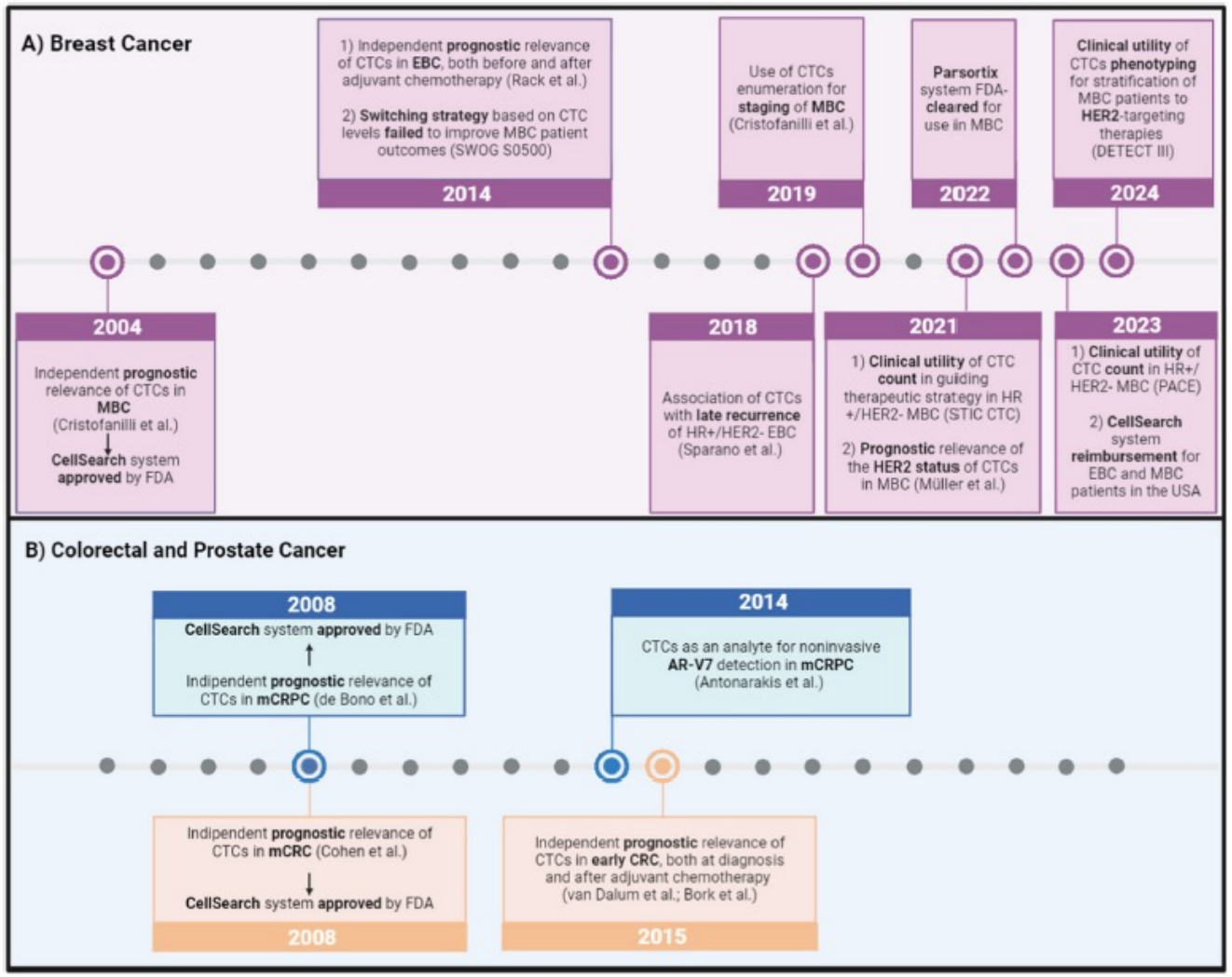
MSC è associato alla sopravvivenza di pazienti con tumore al polmone trattati con farmaci immunoterapici





E' un marcatore indipendente dall'espressione di PD-L1 e altre variabili cliniche (NLR, Fumo, Età, Sesso e Istologia)

CTC come biomarcatore prognostico nei tumori solidi



Malattia metastatica

Tumori:

Mammella (cut-off ≥ 5 CTC/7.5 mL)
Colon (cut-off ≥ 3 CTC/7.5 mL)
Prostata (cut-off ≥ 5 CTC/7.5 mL)

} Approvati da FDA

Diagnosi precoce

Sfide: Bassa sensibilità e CTC molto rare

Tumori:

Mammella (cut-off ≥ 1 CTC/7.5 mL)
Colon (presenza di CTC)

Cellule Tumorali Circolanti come biomarcatore prognostico nei tumori solidi

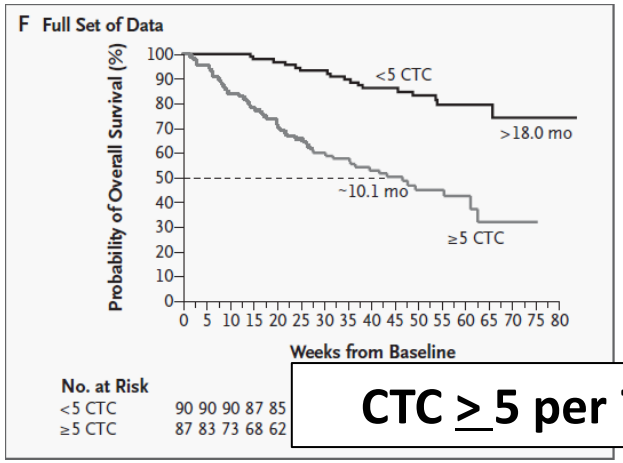
Tumore al seno

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer

Massimo Cristofanilli, M.D., G. Thomas Budd, M.D., Matthew J. Ellis, M.B., Ph.D., Alison Stopeck, M.D., Jeri Matera, B.S., R.Ph., M. Craig Miller, B.S., James M. Reuben, Ph.D., Gerald V. Doyle, D.D.S., W. Jeffrey Allard, Ph.D., Leon W.M.M. Terstappen, M.D., Ph.D., and Daniel F. Hayes, M.D.



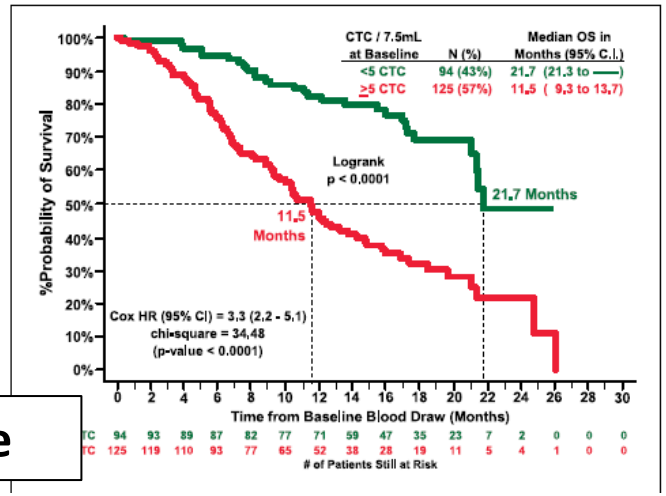
Tumore alla prostata

Cancer Therapy: Clinical

Circulating Tumor Cells Predict Survival Benefit from Treatment in Metastatic Castration-Resistant Prostate Cancer

Johann S. de Bono,[1] Howard I. Scher,[2] R. Bruce Montgomery,[3] Christopher Parker,[1] M. Craig Miller,[4] Henk Tissing,[4] Gerald V. Doyle,[4] Leon W.W.M. Terstappen,[4] Kenneth J. Pienta,[5] and Derek Raghavan[6]

Metodica: CellSearch (Espressione di Epcam)



CTC ≥ 5 per 7.5 ml/sangue sono associate a prognosi peggiore

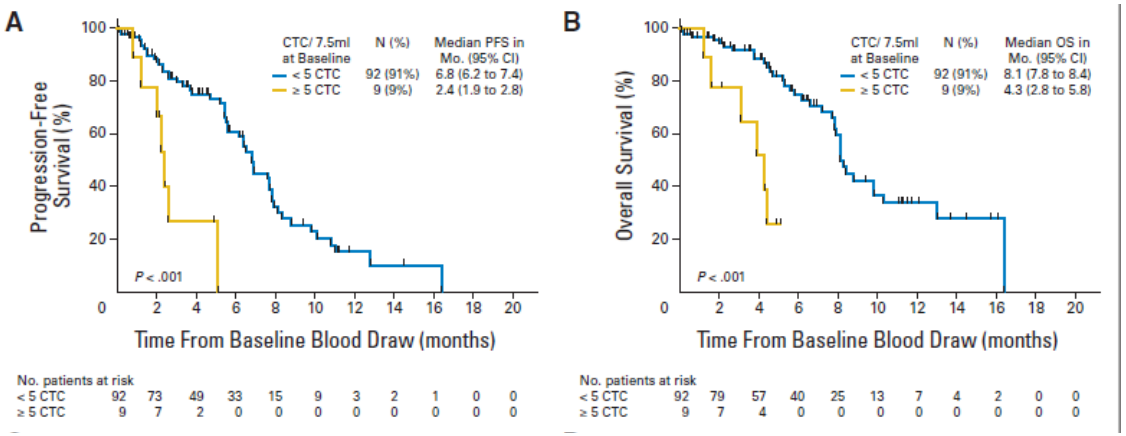
Cellule Tumorali Circolanti come biomarcatore prognostico nel tumore al polmone

VOLUME 29 · NUMBER 12 · APRIL 20 2011

JOURNAL OF CLINICAL ONCOLOGY

Evaluation and Prognostic Significance of Circulating Tumor Cells in Patients With Non–Small-Cell Lung Cancer

Matthew G. Krebs, Robert Sloane, Lynsey Priest, Lee Lancashire, Jian-Mei Hou, Alastair Greystoke, Tim H. Ward, Roberta Ferraldeschi, Andrew Hughes, Glen Clack, Malcolm Ranson, Caroline Dive, and Fiona H. Blackhall



CTC ≥ 5 per 7.5 ml/sangue sono associate a prognosi peggiore

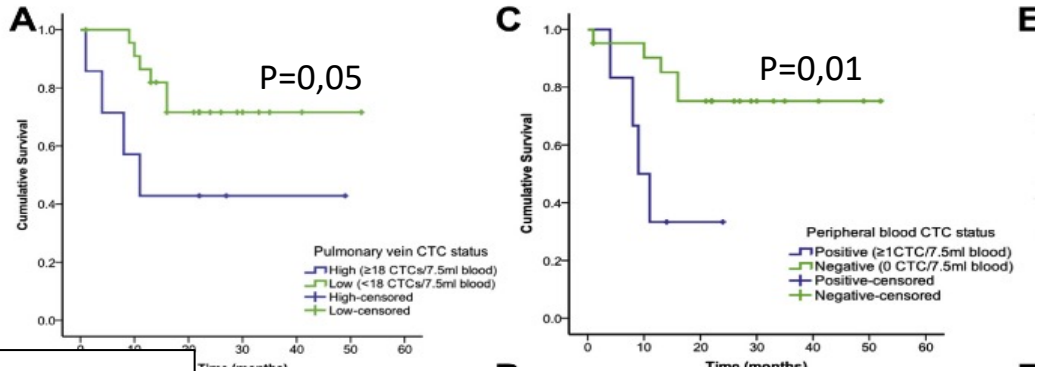
BRIEF REPORT

IASLC

CrossMark

Circulating Tumor Cells Detected in the Tumor-Draining Pulmonary Vein Are Associated with Disease Recurrence after Surgical Resection of NSCLC

Phil A. J. Crosbie, MBChB, MRCP, PhD,[a,b,c,*] Rajesh Shah, M.B.B.S., MS,[a,c,d] Piotr Krysiak,[a,d] Cong Zhou, BSc, PhD,[e] Karen Morris, BSc (Hons), PhD,[b,c] Jonathan Tugwood, BSc (Hons), PhD,[b,c] Richard Booton, MBChB, PhD,[a,c] Fiona Blackhall, BSc (Hons), MBChB (Hons), PhD,[c,e,f] Caroline Dive, BPharm, PhD[b,c]



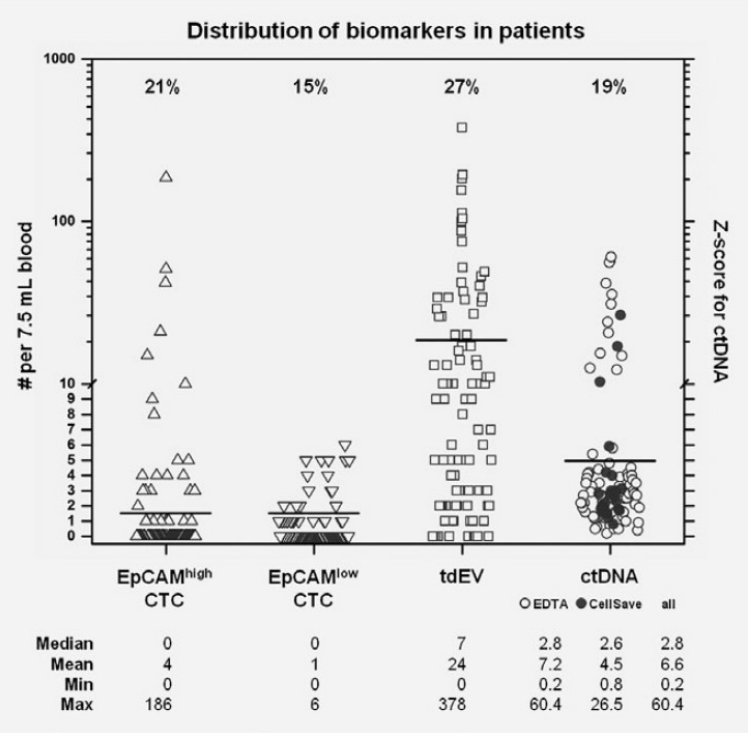
CTCs (≥1 CTC per 7.5 mL/sangue) sono maggiormente presenti nella vena polmonare

Combinazione di marcatori circolanti

IJC
International Journal of Cancer

Single tube liquid biopsy for advanced non-small cell lung cancer

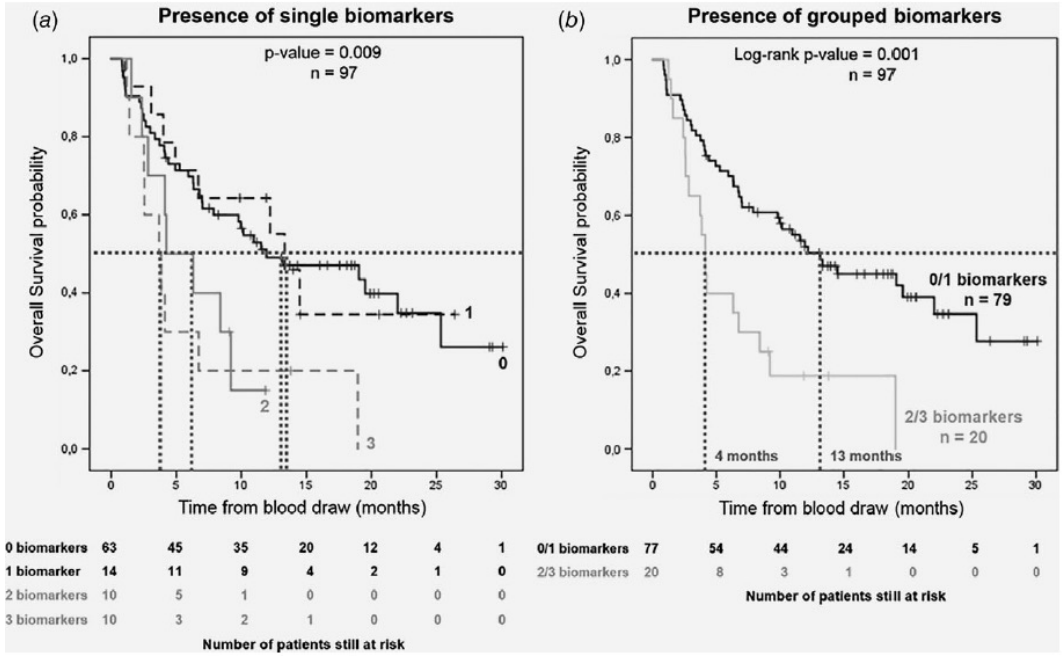
Sanne de Wit[1], Elisabetta Rossi[2,3], Sabrina Weber[4], Menno Tamminga[5], Mariangela Manicone[3], Joost F. Swennenhuis[1], Catharina G.M. Groothuis-Oudshoorn[6], Riccardo Vidotto[3], Antonella Facchinetti[2,3], Leonie L. Zeune[1,7], Ed Schuuring[5], Rita Zamarchi[3], T. Jeroen N. Hiltermann[3], Michael R. Speicher[4], Ellen Heitzer[4,8], Leon W.M.M. Terstappen[1] and Harry J.M. Groen[5]



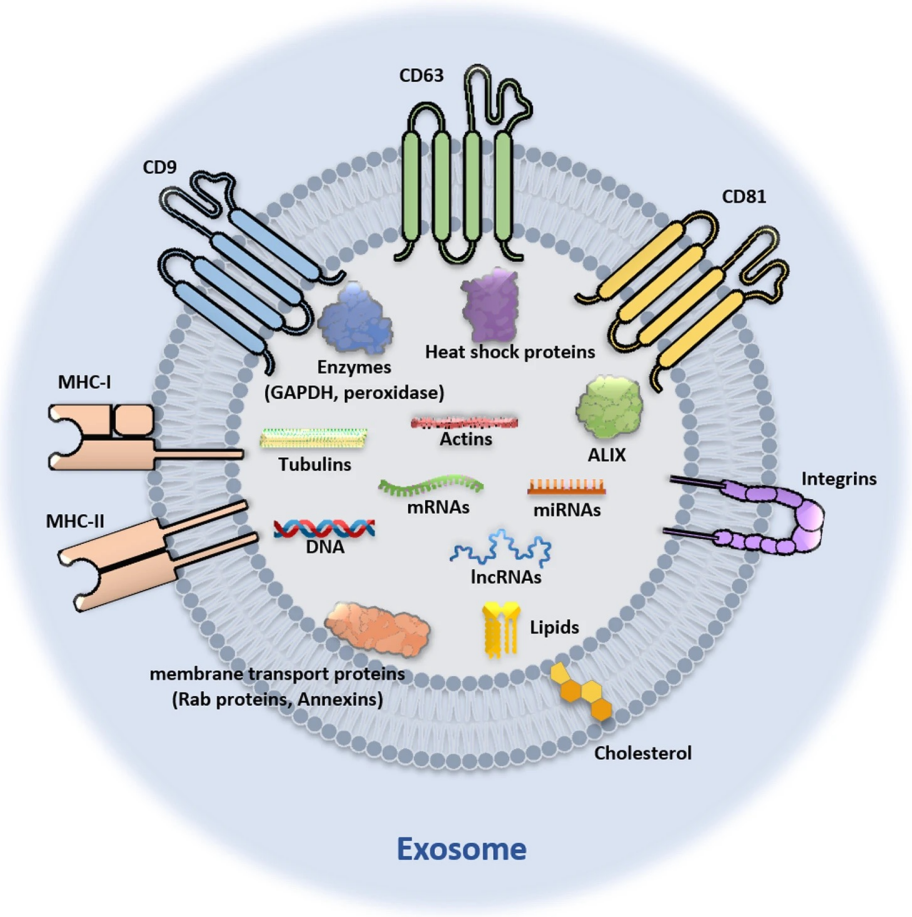
4 biomarcatori:

- EpCAMhigh CTC (CellSearch)
- EpCAMlow CTC (by filtration)
- Tumor-derived Evs (cut-off > 18)
- CtDNA

La presenza di 2 o più biomarcatori è associato a prognosi peggiore



Le vescicole extracellulari



Liu C, CDD 2024

- Contengono biomolecole (DNA, RNA, proteine e lipidi) che riflettono direttamente lo stato fisiologico;
- Sono stabili nel flusso sanguigno e in altri fluidi biologici
- Il contenuto è protetto dal doppio strato fosfolipidico;
- Sono presentii in numerosi fluidi biologici come il sangue, l'urina, il liquido cerebrospinale, o la saliva.

Marcatori prognostici:

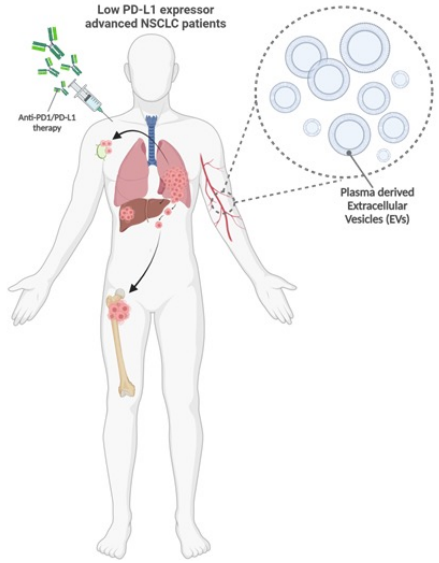
- Livelli di **TUBB3 mRNA** sono associate a peggior prognosi nel tumore alla prostate metastatica
- Otto proteine (**CA15-3, CA125, CEA, HER2, EGFR, PSMA, EpCAM, and VEGF**) costituiscono un marcatore prognostico nel tumore alla mammella metastico

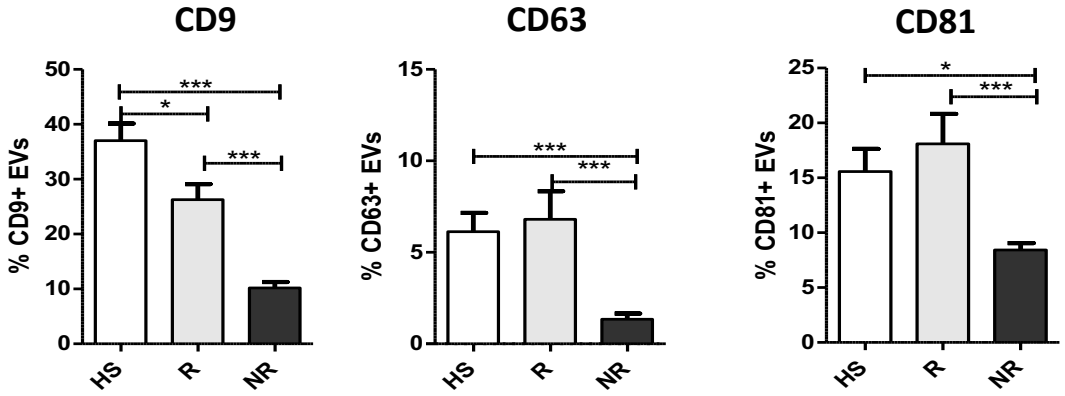
CD81+ EVs sono associate ad una miglior prognosi

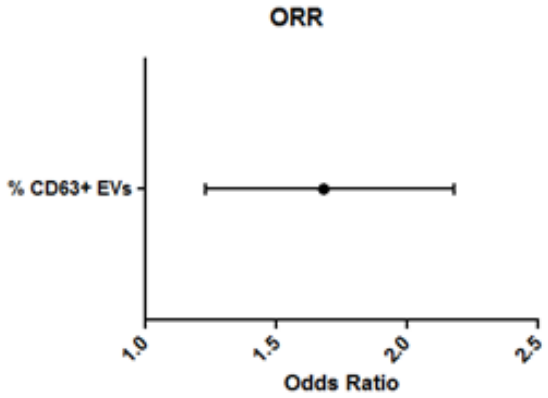
Circulating CD81-expressing extracellular vesicles as biomarkers of response for immune-checkpoint inhibitors in advanced NSCLC

Diego Signorelli[1†‡], Patrizia Ghidotti[2‡], Claudia Proto[1], Marta Brambilla[1], Alessandro De Toma[1], Roberto Ferrara[1], Giulia Galli[1†], Monica Ganzinelli[1], Giuseppe Lorusso[1], Arsela Prelaj[1], Mario Occhipinti[1], Giuseppe Viscardi[1†], Valentina Capizzuto[3], Francesca Pontis[2], Ilaria Petraroia[2], Anna Maria Ferretti[3], Mario Paolo Colombo[4], Valter Torri[5], Gabriella Sozzi[2], Marina Chiara Garassino[1], Elena Jachetti[4§] and Orazio Fortunato[2*§]

[1]Thoracic Oncology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,
[2]Tumor Genomics Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,
[3]Sezione Via G. Fantoli 16/15, Istituto di Scienze e Tecnologie Chimiche-CNR, Milan, Italy,
[4]Molecular Immunology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,
[5]Oncology Department, Istituto 'Mario Negri' – IRCCS, Milan, Italy

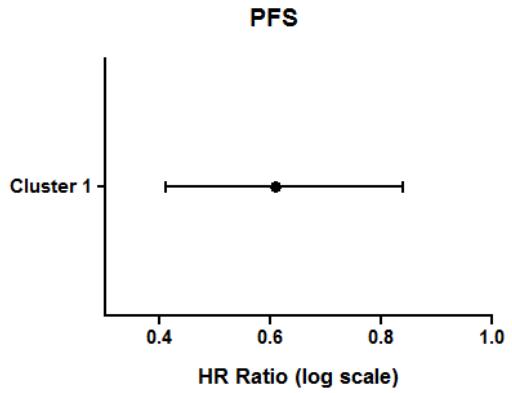


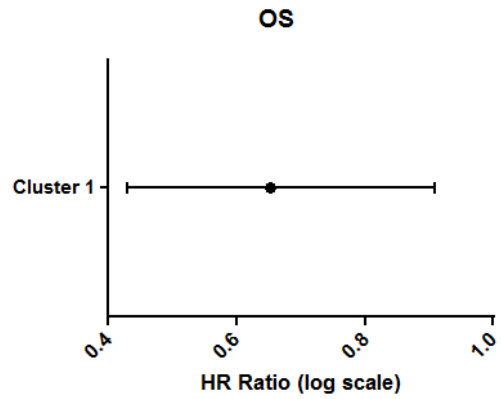




Cluster 1

% of CD9-EVs,
% of CD81-EVs,
% of CD63-EVs,
LDH, NLR, Stadio IV

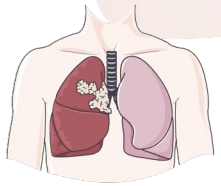




mir-320 nelle EVs correla con una prognosi peggiore

Cohort selection:

20 Lung cancer **E**arly **S**tage patients **A**live after 5 years (**ESA**)
vs
20 Lung cancer **E**arly **S**tage patients **D**ied after 2 years (**ESD**)

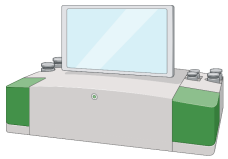


Control cohort:

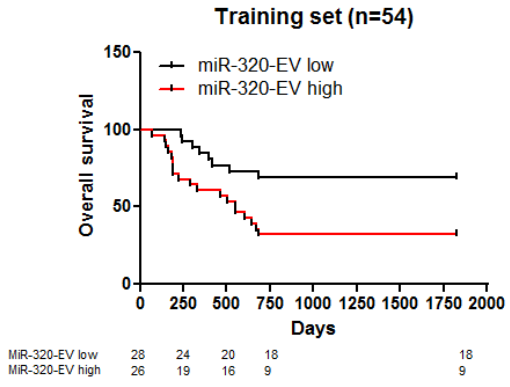
*Heavy smokers individuals (> 30 pack year) (**HS**)*

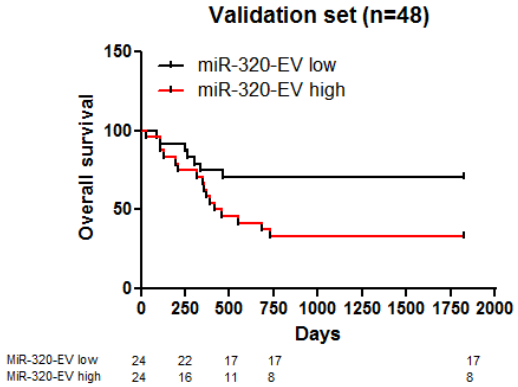
Blood collection at surgery

dPCR

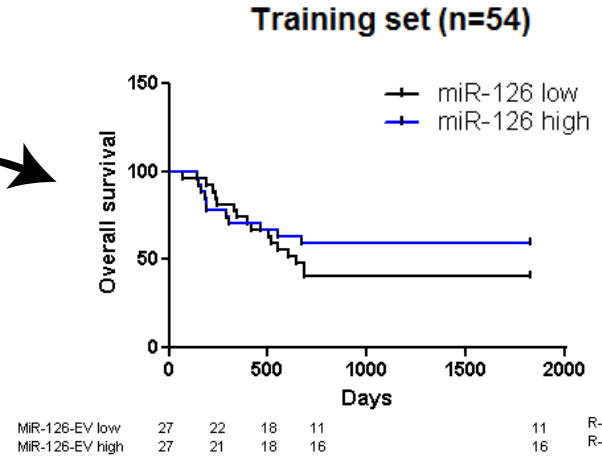


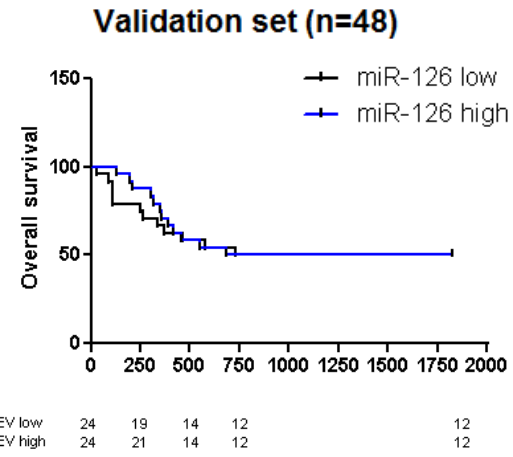
miR-320 EVs





miR-126 EVs

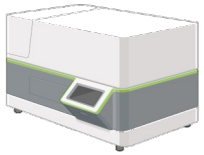




Pontis F, JECC 2021

mir-29a e C4a nelle EVs correlano con una prognosi peggiore

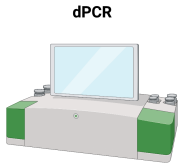
Nanostring



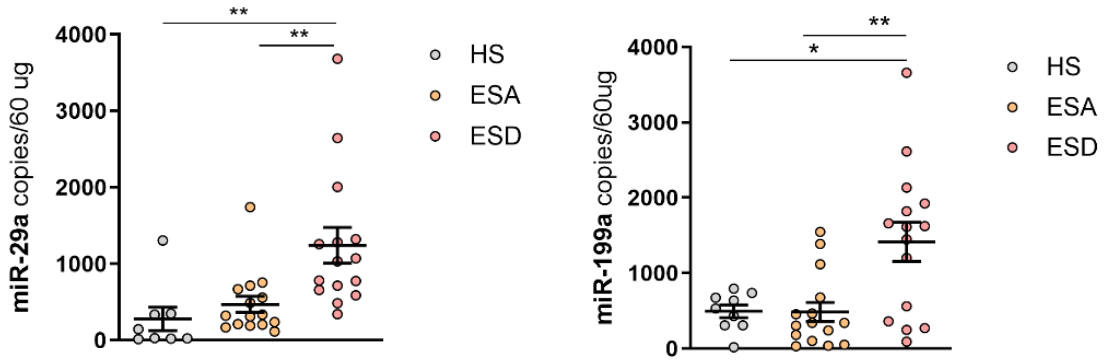
90 miRNAs detected in plasma EVs

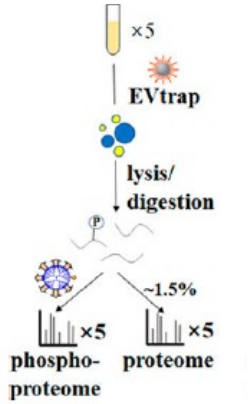
4 Differentially expressed miRNAs

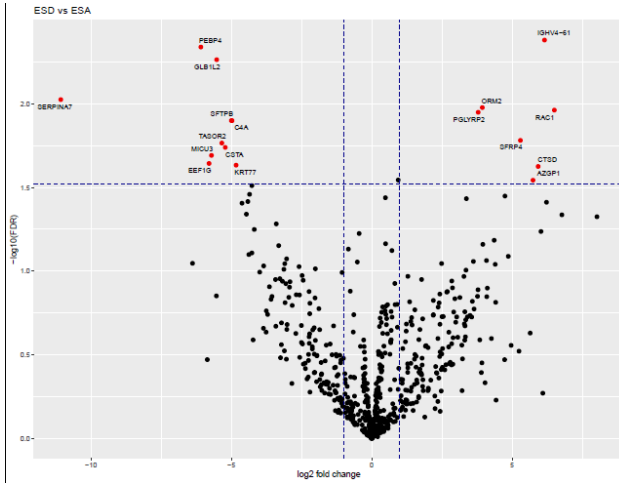
n	UniqueID	P-value
1	miR-1307-3p	0.0057
2	miR-451a	0.0171
3	miR-29a-3p	0.0177
4	miR-199a-3p	0.0258
5	miR-548ah-5p	0.0700
6	miR-514b-5p	0.0854
7	miR-608	0.0869
8	miR-802	0.0893



Validation







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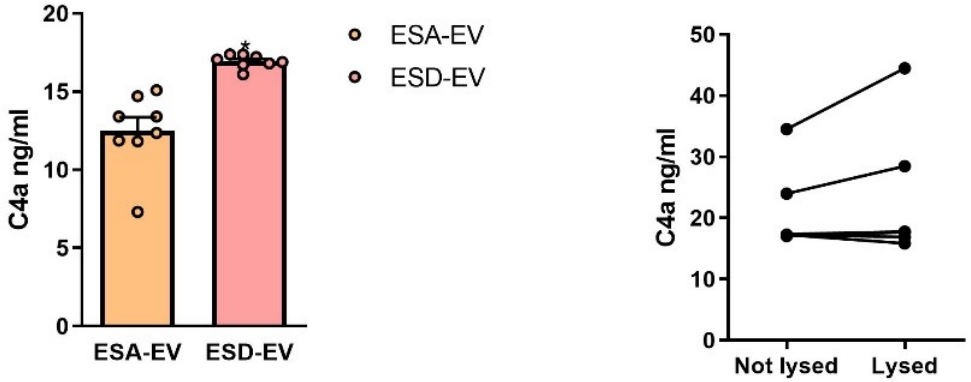

UNPUBLISHED

Sfide future

- **Sensibilità e Specificità**

Rilevamento di biomarcatori tumorali a bassa concentrazione
Difficoltà nell'identificare segni precoci o recidive in fase iniziale

- **Validazione Clinica**

Necessità di ampi studi clinici per convalidare i biomarcatori identificati

- **Standardizzazione delle Tecniche**

Differenze tra laboratori e piattaforme tecnologiche
Necessità di protocolli unificati per garantire risultati consistenti

- **Costi**

Tecnologie avanzate costose e non sempre disponibili

- **Interpretazione dei Dati**

Complessità nell'analisi bioinformatica dei dati

Ringraziamenti

Unità di Epigenomica e Biomarcatori dei tumori solidi

Francesca Pontis
Patrizia Ghidotti
Nicole Ferrario

Giulia Bertolini
Mattia Boeri
Luca Roz

Gabriella Sozzi

Unità di Chirurgia Toracica

Paola Suatoni

Ugo Pastorino