

Dottorato di Ricerca in
Intelligenza Artificiale in Medicina e Innovazione nella Ricerca
clinica e metodologica

Liquid Biopsy from bench to clinic: are we at the turning point?

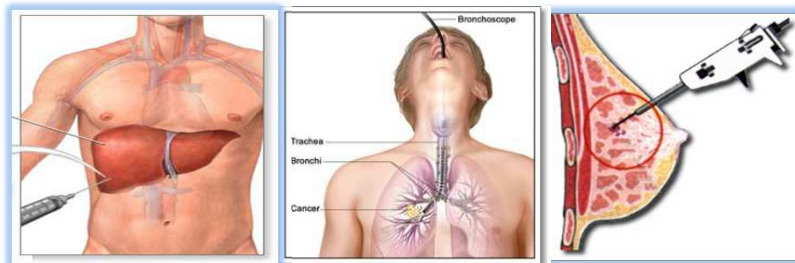
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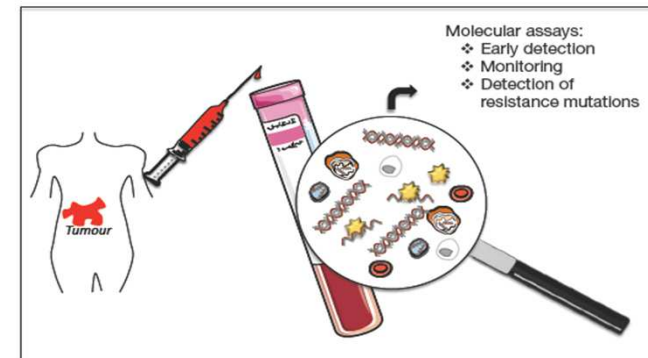
CONVENTIONAL VS LIQUID BIOPSY

Conventional Biopsy



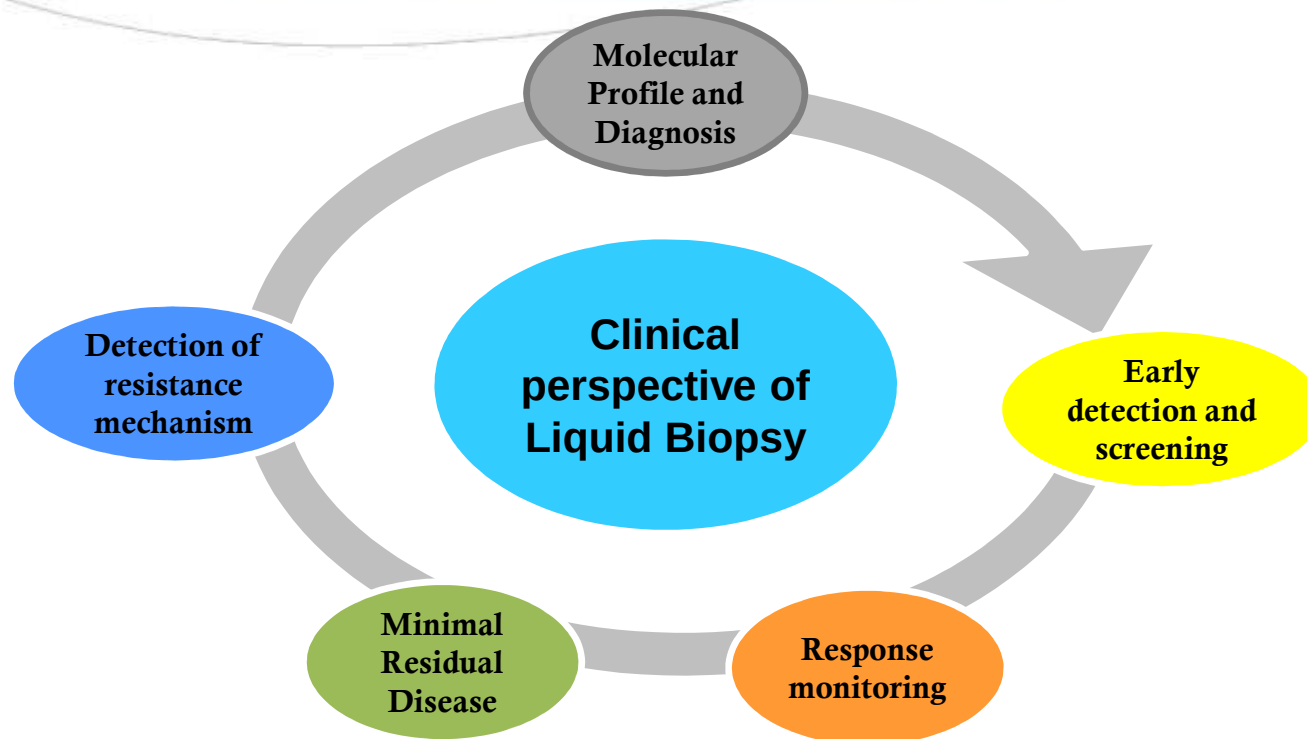
- Invasive, painful
- Expensive & time consuming
- Re-biopsy often not possible or accepted
- Not suitable for cancer monitoring
- Does not address tumor heterogeneity

Liquid Biopsy

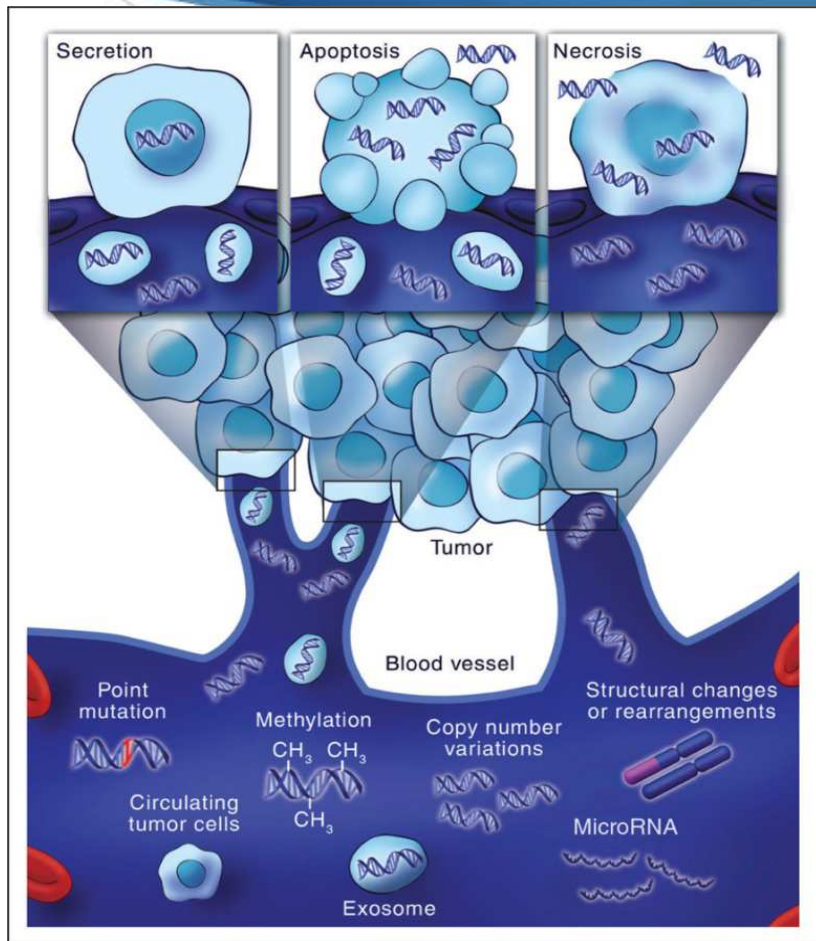


- Minimally invasive, no risk for patients
- Cheap and quick
- Re-biopsy is not a problem
- Suitable for cancer monitoring
- Addresses tumor heterogeneity

Clinical Applications for Liquid Biopsy



BIOMARKERS SOURCES



Circulating tumor cells (CTCs)

Cancer cells released by the primary tumor in circulation to form metastases at peripheral sites.

Circulating cell-free nucleic acids (cfDNA/cfRNA)

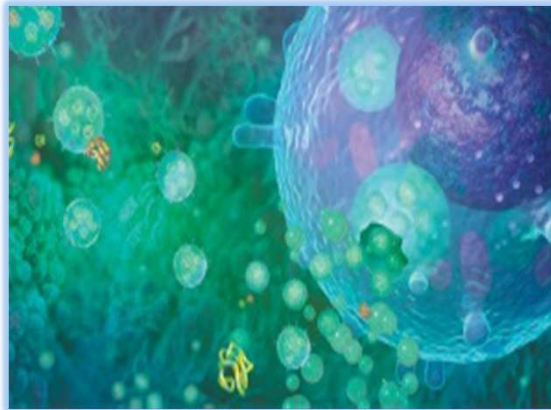
DNA but also RNA (mRNAs, microRNA and long non coding RNA). No selection available.

Extracellular vesicles (EVs)

Lipid vesicles containing proteins and nucleic acids (RNAs and DNA) from the cell of origin. Selection and enrichment strategies available.

EXOSOMES

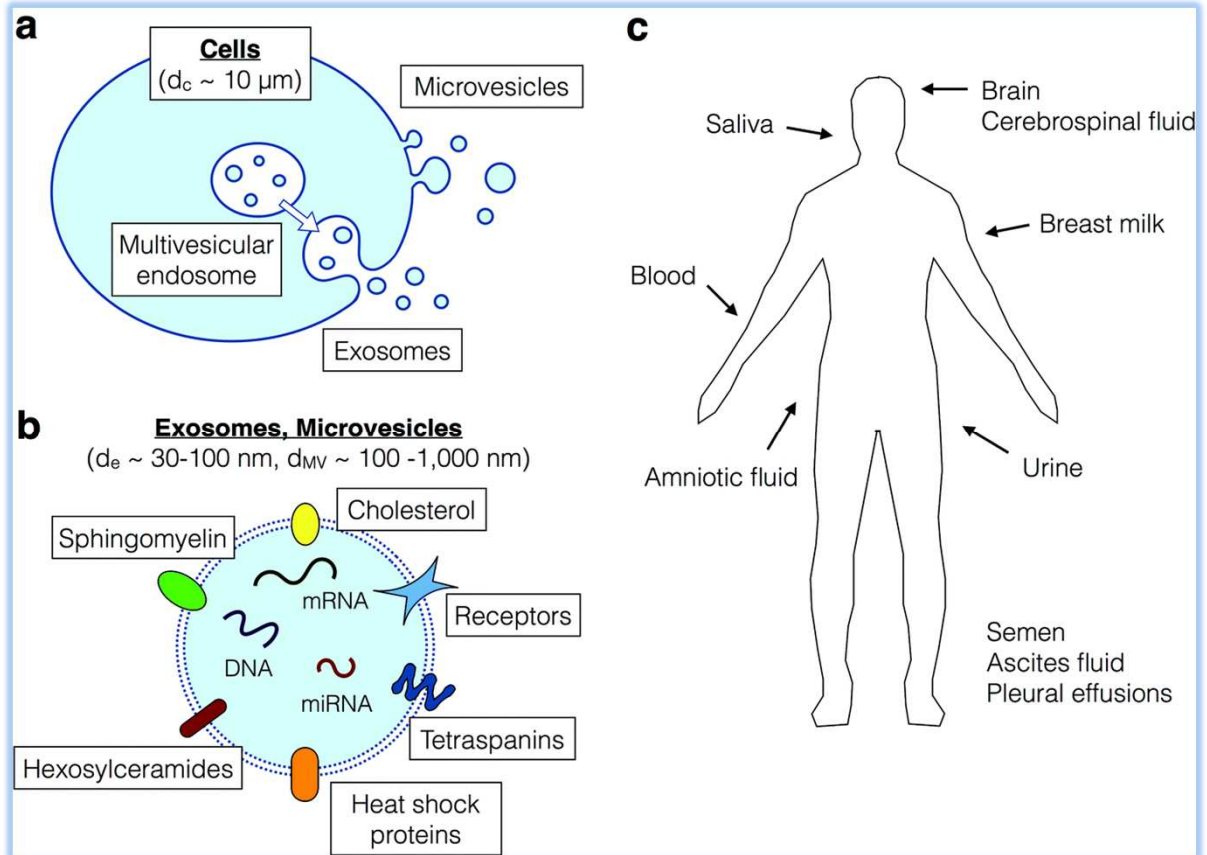
"The exosomes, small bubbles, produced naturally by cells to 'float' various macromolecules in the body"



✓ Easy to recover and analyzed from blood

✓ Heterogeneous population

✓ Carriers of different protected biomarkers from the cell of origin



Liquid Biopsy Techniques

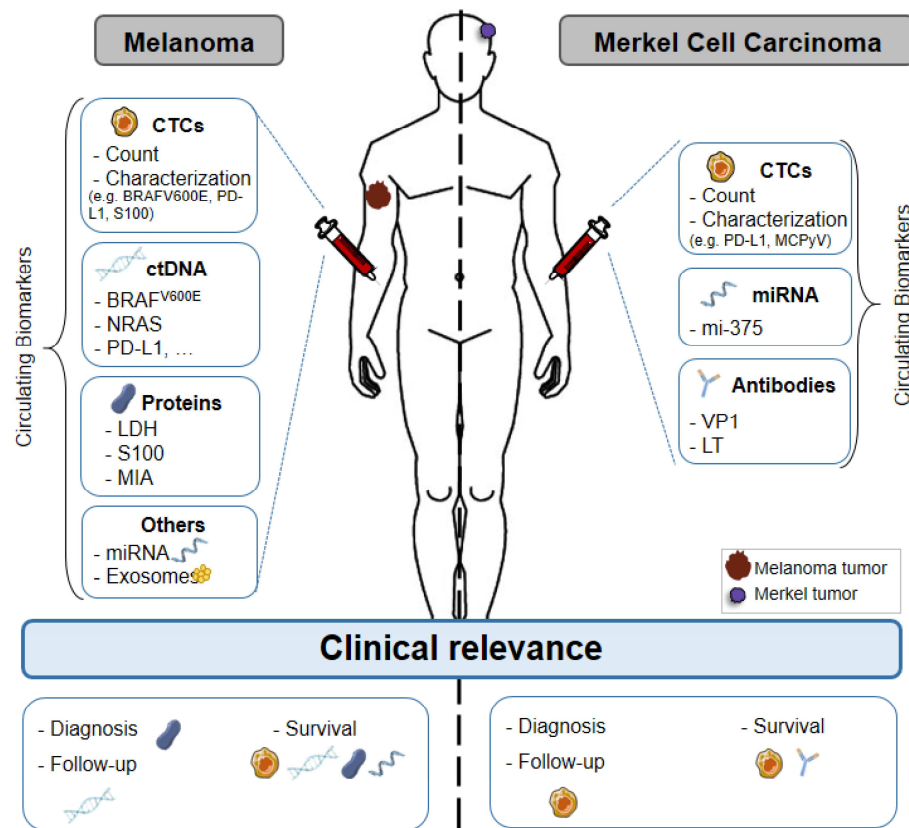
Proteic markers

- **Western Blot:** CTC and vesicular biomarkers analysis
- **microBCA:** vesicular proteic biomarkers analysis
- **Mass spectrometry/Raman Technology:** CTC and vesicular proteomic analysis

Nucleic Acids markers

- **Real-time PCR:** cfDNA analysis and vesicular biomarkers analysis
- **Next Generation Sequencing:** cfDNA analysis and vesicles cargo sequencing
- **Digital PCR:** cfDNA analysis and vesicular biomarkers analysis

LIQUID BIOPSY IN ONCOLOGY: melanoma



SCIENTIFIC REPORTS
nature research

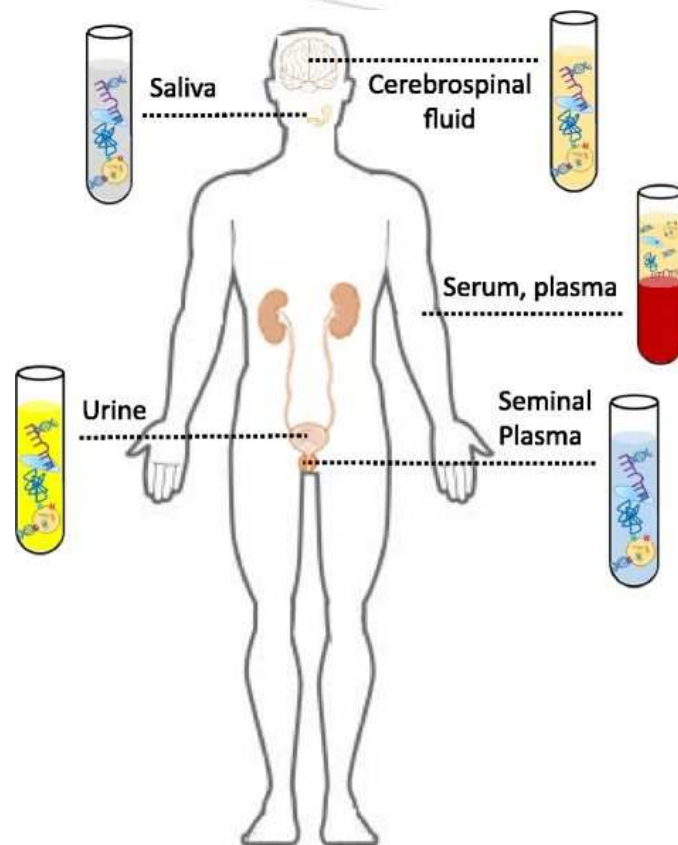
OPEN Isolation of extracellular vesicles improves the detection of mutant DNA from plasma of metastatic melanoma patients

Davide Zocco^{1,2,3}, Simona Bernardi^{4,5}, Mauro Novelli^{2,3}, Chiara Astrua^{2,3}, Paolo Fava^{2,3}, Natasa Zarovni¹, Francesco M. Carpi¹, Laura Bianciardi¹, Ottavia Malavenda^{2,3}, Pietro Quaglino^{2,3}, Chiara Foroni^{4,5}, Domenico Russo^{4,5}, Antonio Chiesi¹ & Maria Teresa Fierro^{2,3}



The combination of tumor-derived exosomes enrichment and dPCR allows sensitivity increasement in the detection of BRAF V617E mutation

LIQUID BIOPSY IN ONCOLOGY: prostate cancer



biomedicines



Article

When Less Is More: Specific Capture and Analysis of Tumor Exosomes in Plasma Increases the Sensitivity of Liquid Biopsy for Comprehensive Detection of Multiple Androgen Receptor Phenotypes in Advanced Prostate Cancer Patients

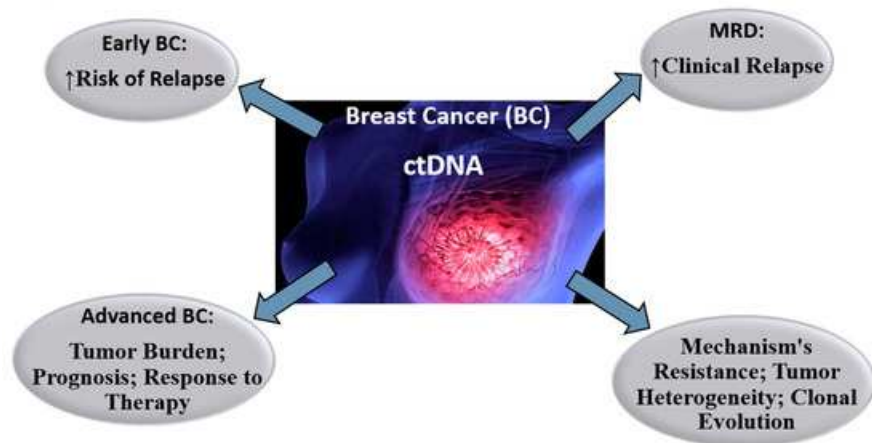
Chiara Foroni ^{1,2,*}, Natasa Zarovni ^{3,*}, Laura Bianciardi ³, Simona Bernardi ^{1,4}, Luca Triggiani ⁵, Davide Zocco ³, Marta Venturella ³, Antonio Chiesi ³, Francesca Valcamonico ² and Alfredo Berruti ²



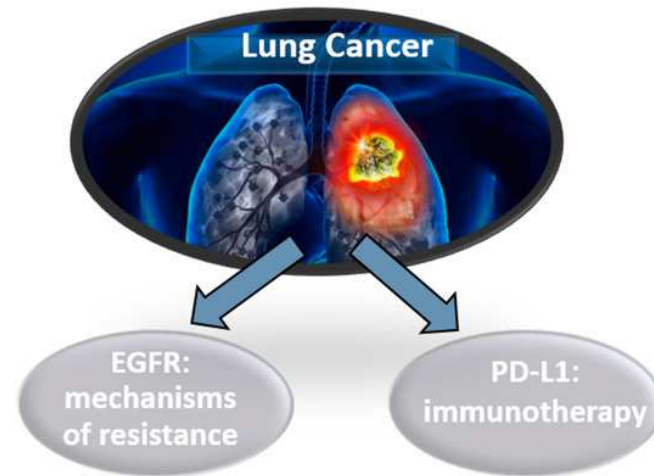
- Tumor-derived exosomes may serve as carrier of multiple castration-resistance markers related with AR alteration (mutations and/or deletion).
- AR alterations may be easily detected by dPCR.
- This approach allows early detection of relapse.

LIQUID BIOPSY IN ONCOLOGY: other examples

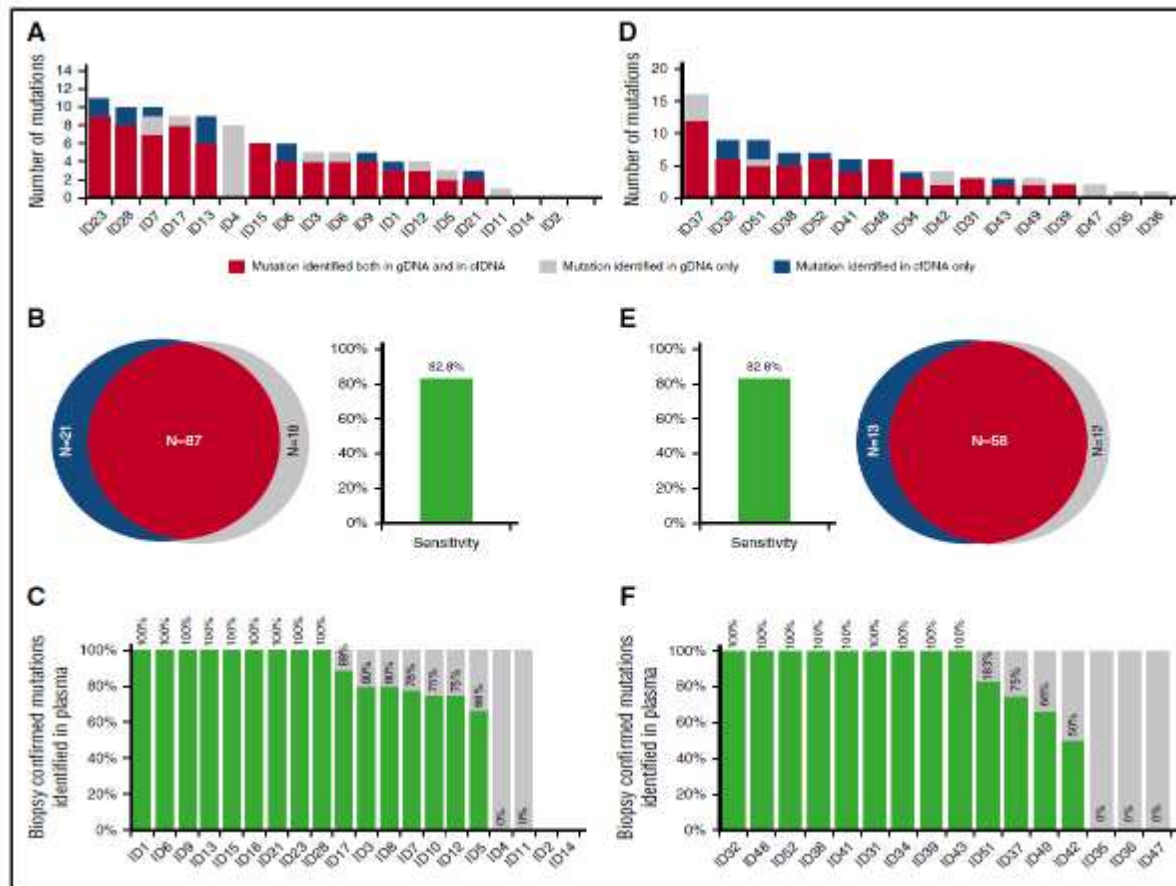
Breast Cancer



Lung Cancer

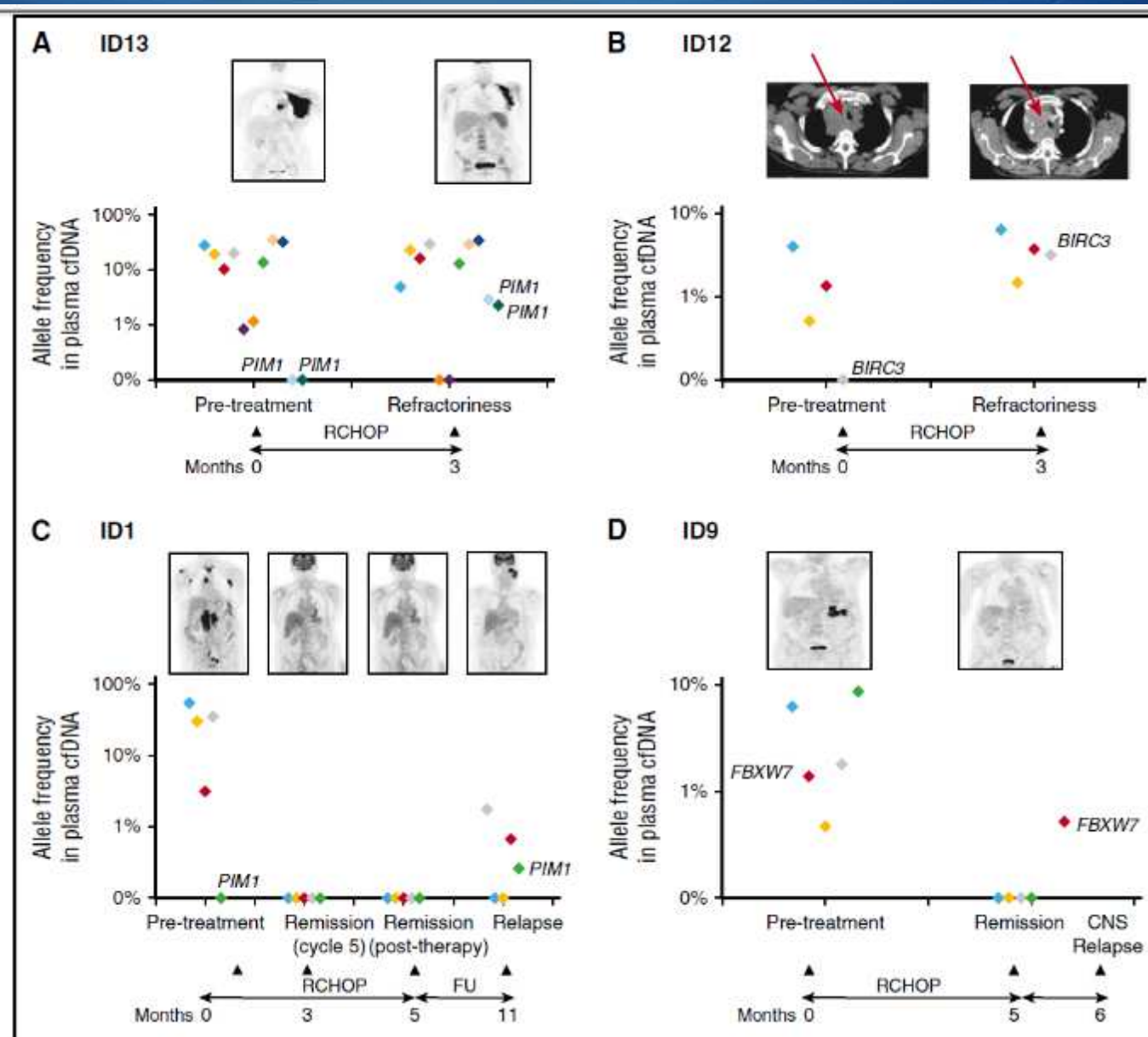


LIQUID BIOPSY IN HEMATOLOGY: Lymphomas



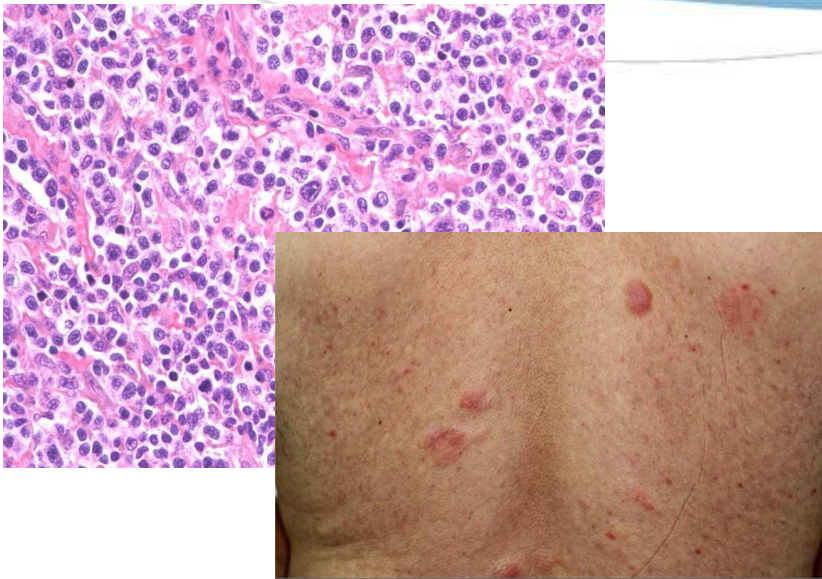
LB IN LYMPHOMA: ctDNA

CLONAL EVOLUTION MONITORING



Rossi D., et al, Blood
2017

LB IN RARE LYMPHOMA: PTCL



PTCL

- Relatively rare
- Clinically Aggressive
- Extremely heterogeneous lesions
- Highly challenge for molecular analysis
- No specific molecular markers identified



LB may be the key to uncover the molecular landscape underlying PTCL and help the clinicians in improving the prognostication and the monitoring of this rare neoplasia.

LB IN LEUKEMIA: is equally possible?

**Identification of Acute Myeloid Leukemia (AML)
molecular biomarkers in exosomes circulating in
peripheral blood of adult AML patients**



Primary aims:

Evaluation of the feasibility of a leukemia-derived exosomes enrichment combined with NGS and dPCR for the detection of known AML molecular markers for the minimal residual disease monitoring and relapse detection.

Comparison between mutations identified on exosomes and on conventional leukemic cells isolated from peripheral blood and bone marrow.

Identification of optimal timing of exosomes analysis for the most efficient monitoring.

AVAILABLE RESEARCH LINES

- Detection of BCR-ABL1 and other biomarkers in Chronic Myeloid Leukemia by leukemia-derived exosomes enrichment and dPCR
- Identification of Acute Myeloid Leukemia (AML) molecular biomarkers in exosomes circulating in peripheral blood of adult AML patients
- Analysis of small-EVs cargo in patients affected by MPNs
- Others in collaboration with other Groups

PREVIOUS PUBLICATIONS

Annals of Hematology (2021) 100:1355–1356
https://doi.org/10.1007/s00277-020-04109-z

LETTER TO THE EDITOR

dsDNA from extracellular vesicles (EVs) in adult AML

Simona Bernardi^{1,2}  · C. Zanaglio^{1,2} · M. Farina¹ · N. Polverelli¹ · M. Malagola¹ · D. Russo¹

INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE 44: 2133-2144, 2019

Feasibility of tumor-derived exosome enrichment in the onco-hematology leukemic model of chronic myeloid leukemia

SIMONA BERNARDI^{1,2*}, CHIARA FORONI^{1,2*}, CAMILLA ZANAGLIO^{1,2}, FEDERICA RE^{1,2}, NICOLA POLVERELLI¹, ALESSANDRO TURRA¹, ENRICO MORELLO¹, MIRKO FARINA¹, FEDERICA CATTINA¹, LISA GANDOLFI¹, TATIANA ZOLLNER¹, EUGENIA ACCORSI BUTTINI¹, MICHELE MALAGOLA¹ and DOMENICO RUSSO¹

¹Chair of Hematology, Unit of Blood Diseases and Stem Cell Transplantation, Department of Clinical and Experimental Sciences, University of Brescia; ²CREA Laboratory (Hematological-Research AIL Centre), ASST Spedali Civili of Brescia, I-25123 Brescia, Italy

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Letter to the Editor

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Exosomes in Chronic Myeloid Leukemia: Are We Reading a New Reliable Message?

Simona Bernardi · Michele Malagola · Nicola Polverelli
Unit of Blood Diseases and Stem Cell Transplantation, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy



biomedicines



Article

When Less Is More: Specific Capture and Analysis of Tumor Exosomes in Plasma Increases the Sensitivity of Liquid Biopsy for Comprehensive Detection of Multiple Androgen Receptor Phenotypes in Advanced Prostate Cancer Patients

Chiara Foroni^{1,2,*} , Natasa Zarovni^{3,*}, Laura Bianciardi³, Simona Bernardi^{1,4} , Luca Triggiani⁵, Davide Zocco³, Marta Venturella³, Antonio Chiesi³, Francesca Valcamonica² and Alfredo Berruti²

biology

Review

Exosomes and Extracellular Vesicles: Multiple and Complex Roles Played by These "Magic Bubbles"

Simona Bernardi^{1,2,*} , and Mirko Farina^{1,2} 

SCIENTIFIC
REPORTS

nature research



Isolation of extracellular vesicles from plasma of metastatic melanoma patients proves the detection of mutant BRAF from plasma of metastatic melanoma patients

Davide Zocco^{1,2,3}, Simona Bernardi^{1,2,3,4,5} , Mauro Novelli^{2,3}, Chiara Astrua^{2,3}, Paolo Fava^{2,3}, Natasa Zarovni¹, Francesco M. Carpi¹, Laura Bianciardi¹, Ottavia Malavenda^{2,3}, Pietro Quaglini^{2,3}, Chiara Foroni^{4,5}, Domenico Russo^{4,5}, Antonio Chiesi³ & Maria Teresa Fierro^{2,3}