

**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?**

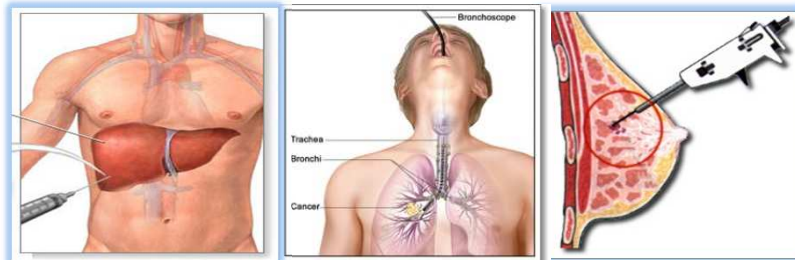
**Prof. Domenico Russo**

Unit of Blood Diseases and Bone Marrow Transplantation  
Cell therapies and Research in Hematology Program  
Department of Clinical and Experimental Sciences  
University of Brescia  
ASST Spedali Civili of Brescia



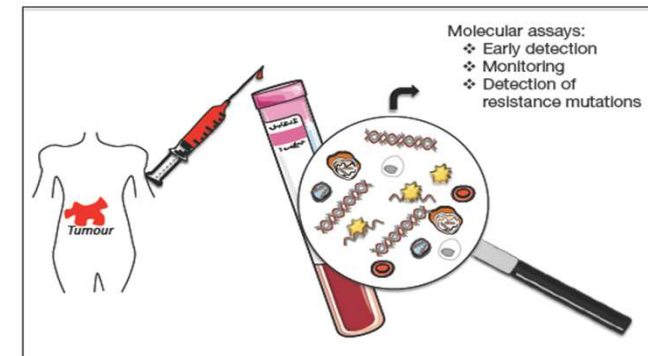
# CONVENTIONAL VS LIQUID BIOPSY

## *Conventional Biopsy*



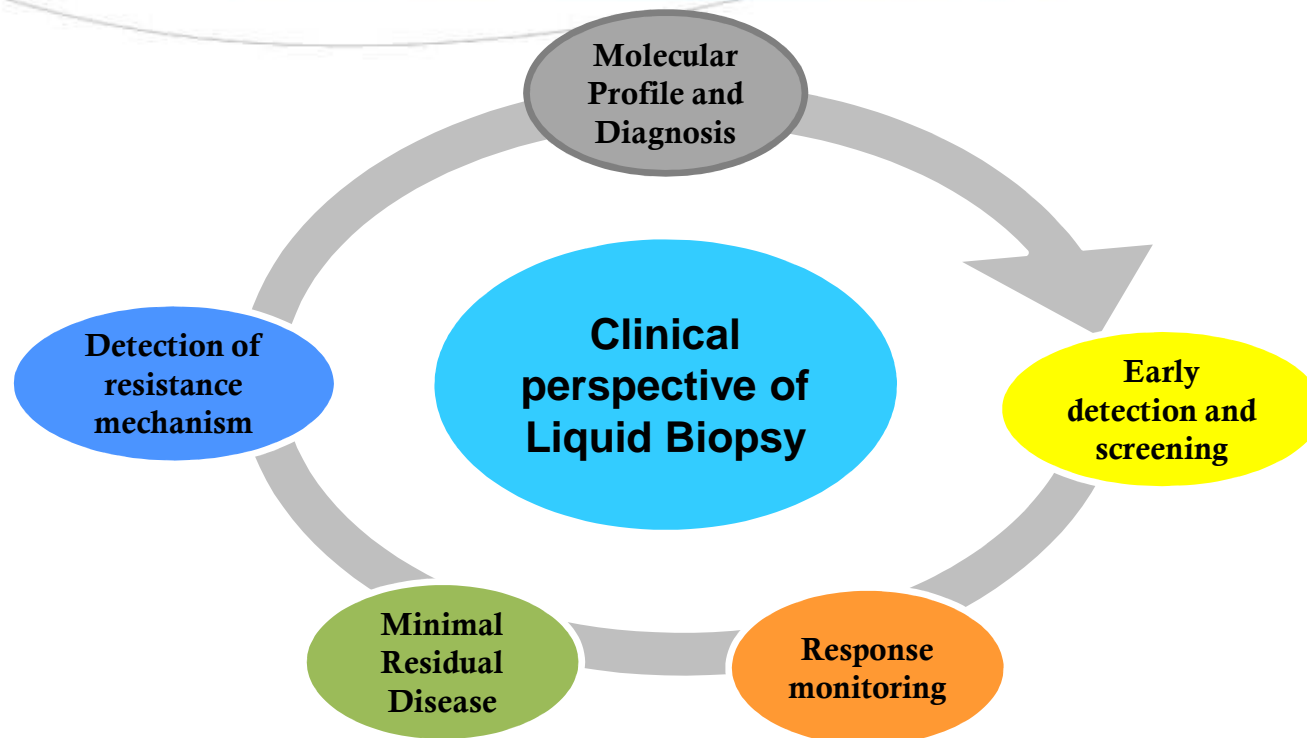
- Invasive, painful
- Expensive & time consuming
- Re-biopsy often not possible or accepted
- Not suitable for cancer monitoring
- Does not address tumor heterogeneity

## *Liquid Biopsy*

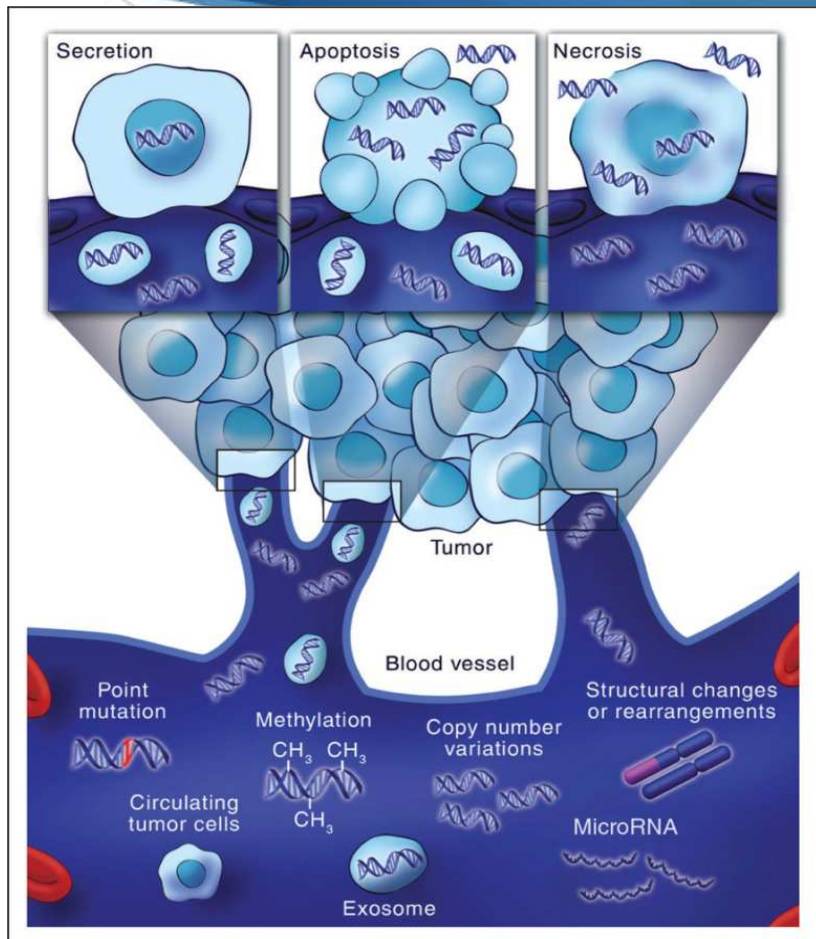


- Minimally invasive, no risk for patients
- Cheap and quick
- Re-biopsy is not a problem
- Suitable for cancer monitoring
- Addresses tumor heterogeneity

# 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)**

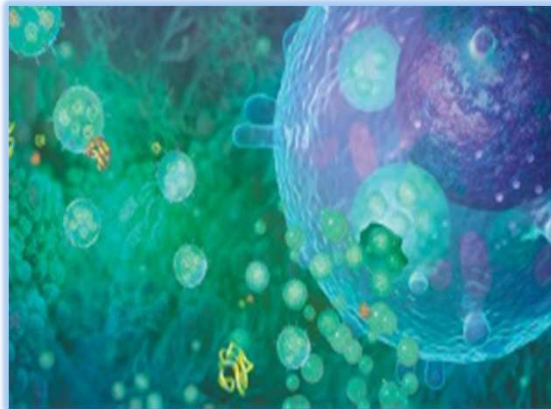
DNA but also RNA (mRNAs, microRNA and long non coding RNA). No selection available.

## **Extracellular vesicles (EVs)**

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

# EXOSOMES

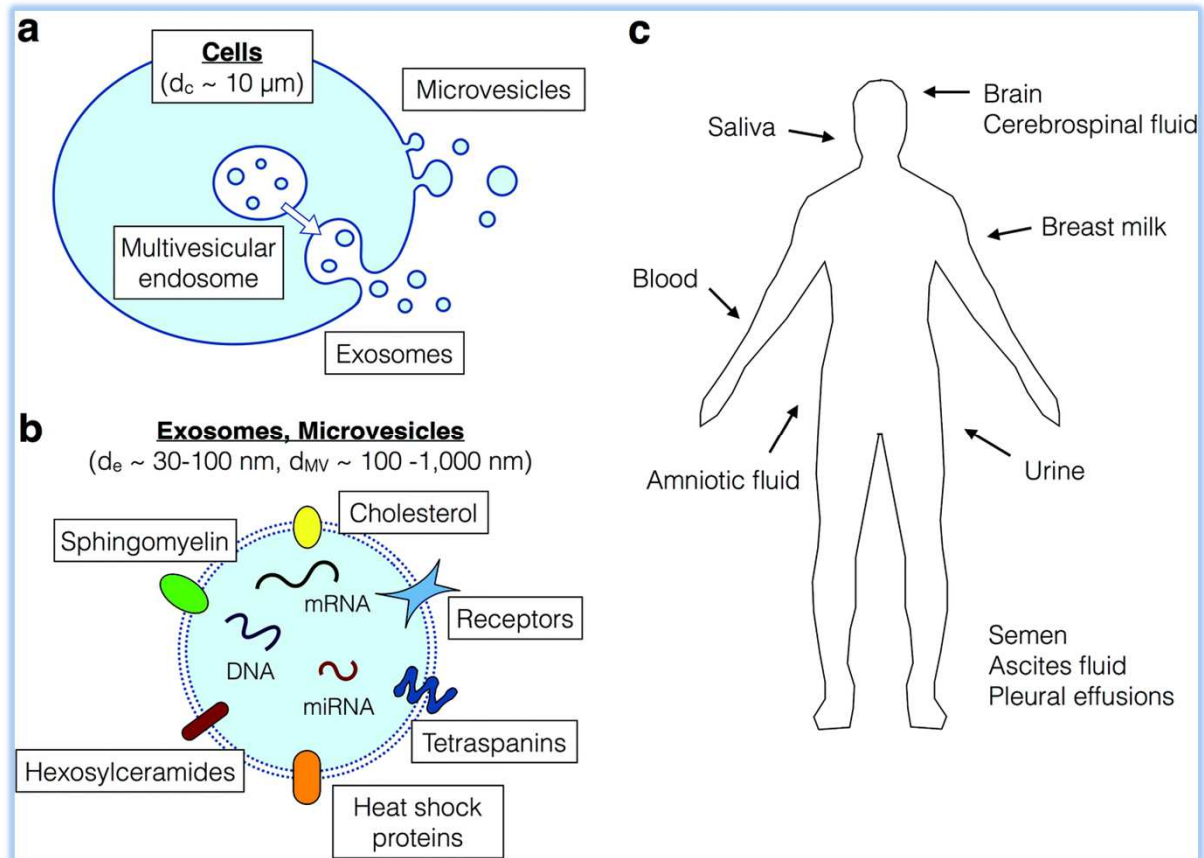
*"The exosomes, small bubbles, produced naturally by cells to 'float' various macromolecules in the body ...."*



✓ Easy to recover and analyzed from blood

✓ Heterogeneous population

✓ Carriers of different protected biomarkers from the cell of origin



# Liquid Biopsy Techniques

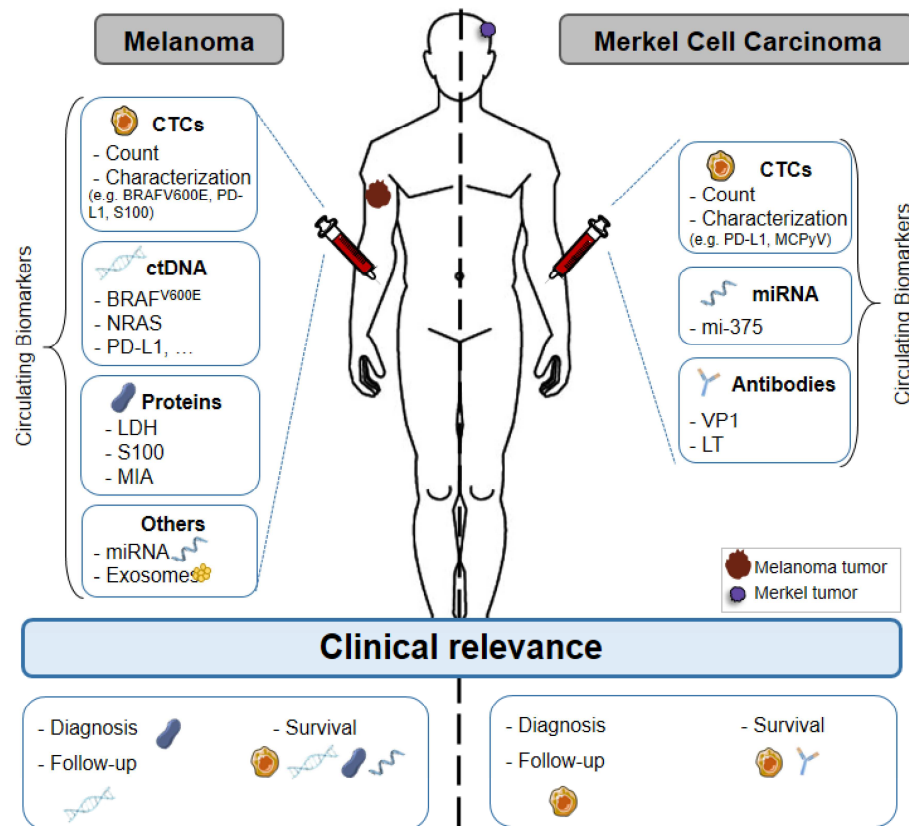
## Proteic markers

- ◆ **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**

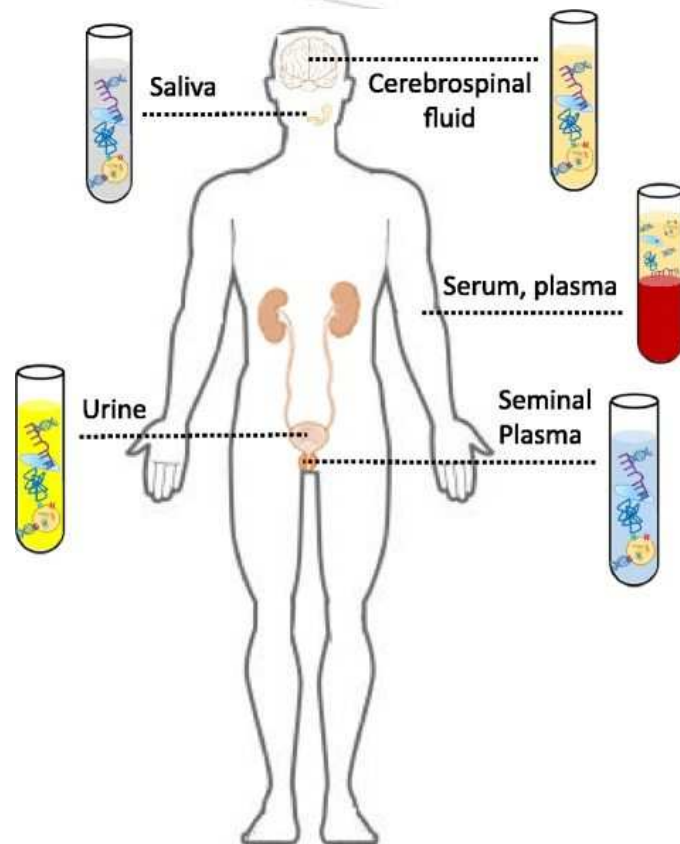
Check for updates

Davide Zocco<sup>1,2</sup>, Simona Bernardi<sup>3,5</sup>, Mauro Novelli<sup>2,3</sup>, Chiara Astrua<sup>2,3</sup>, Paolo Fava<sup>2,3</sup>, Natasa Zarovni<sup>1</sup>, Francesco M. Carpi<sup>1</sup>, Laura Bianciardi<sup>1</sup>, Ottavia Malavenda<sup>2,3</sup>, Pietro Quaglino<sup>2,3</sup>, Chiara Foroni<sup>4,5</sup>, Domenico Russo<sup>4,5</sup>, Antonio Chiesi<sup>2</sup> & Maria Teresa Fierro<sup>2,3</sup>



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 <sup>1,2,\*</sup>, Natasa Zarovni <sup>3,\*</sup>, Laura Bianciardi <sup>3</sup>, Simona Bernardi <sup>1,4</sup>, Luca Triggiani <sup>5</sup>, Davide Zocco <sup>3</sup>, Marta Venturella <sup>3</sup>, Antonio Chiesi <sup>3</sup>, Francesca Valcamonico <sup>2</sup> and Alfredo Berruti <sup>2</sup>

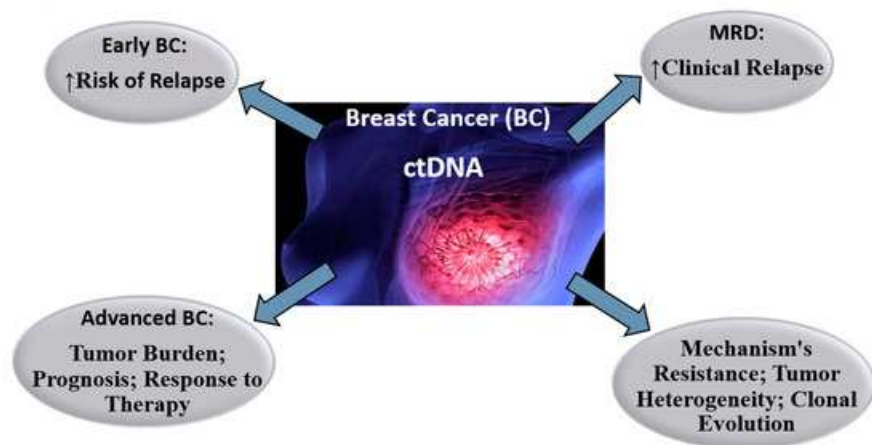


- Tumor-derived exosomes may serve as carrier of multiple castration-resistance markers related with AR alteration (mutations and/or deletion).
- AR alterations may be easily detected by dPCR.
- This approach allows early detection of relapse.

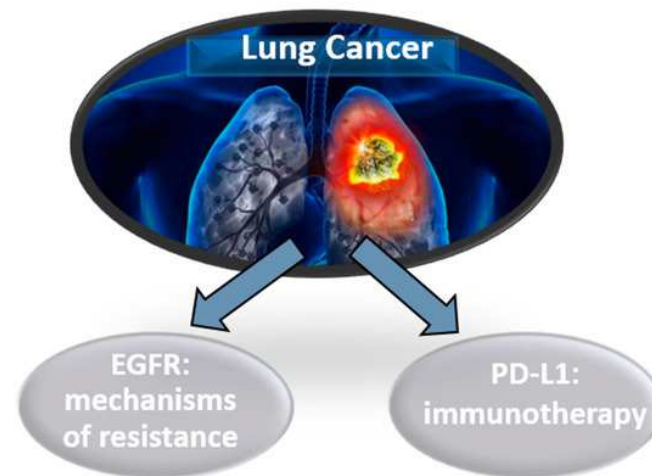


# LIQUID BIOPSY IN ONCOLOGY: other examples

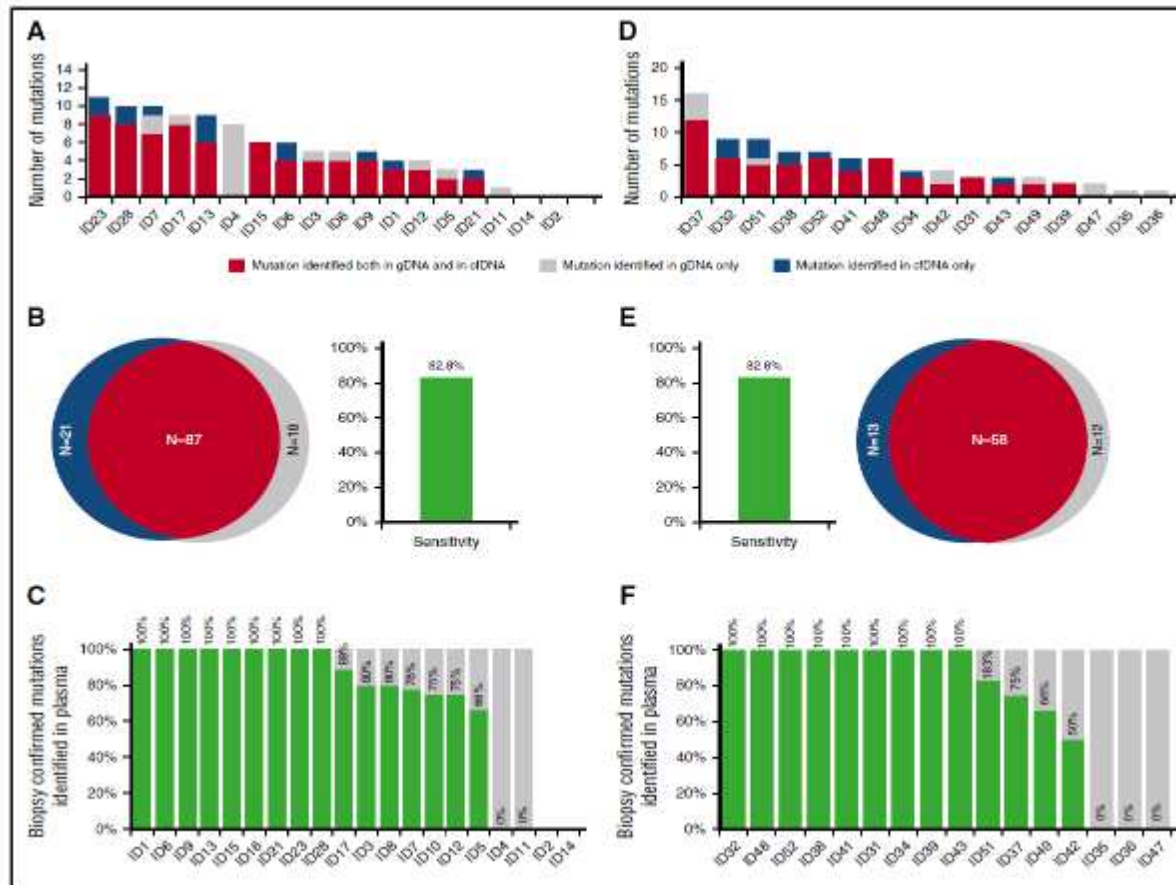
## Breast Cancer



## Lung Cancer

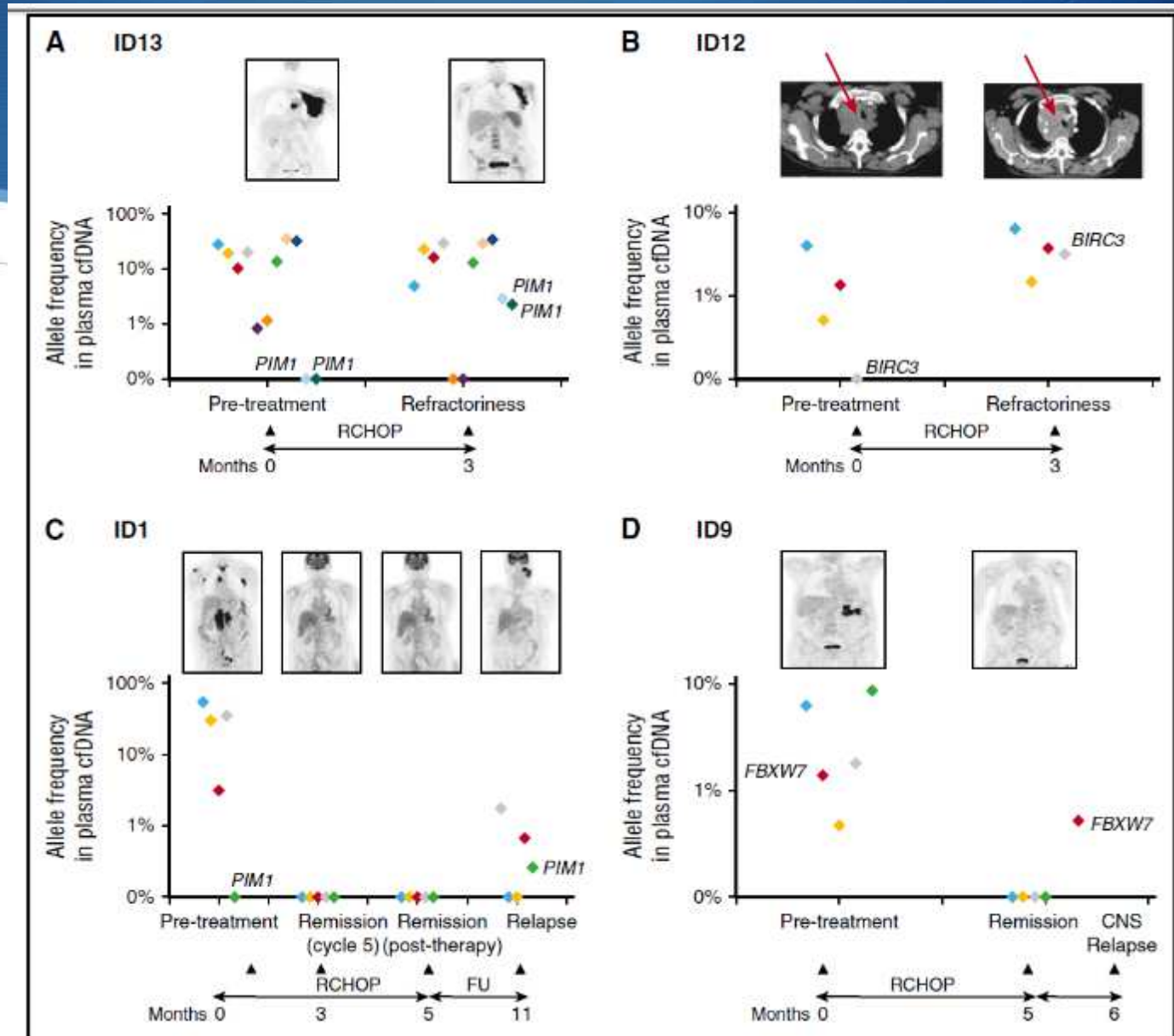


# LIQUID BIOPSY IN HEMATOLOGY: Lymphomas



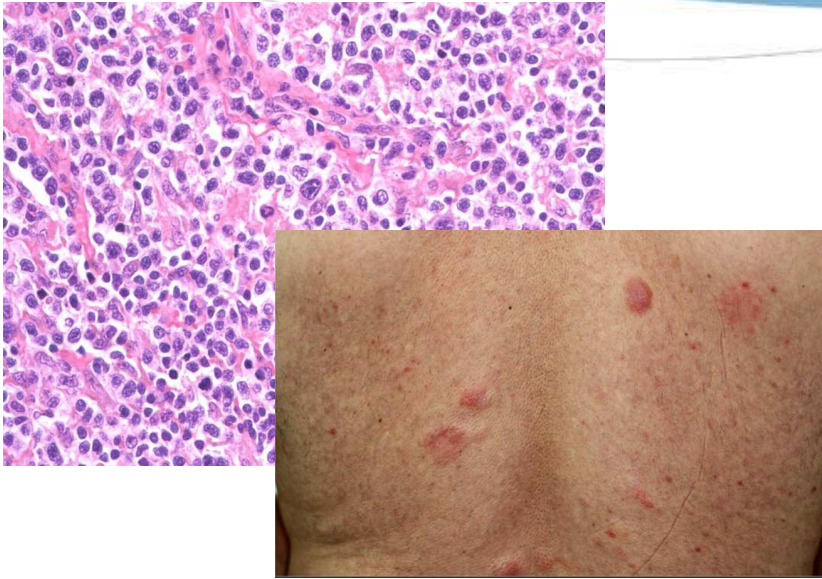
# LB IN LYMPHOMA: ctDNA

## 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<sup>1,2</sup>  · C. Zanaglio<sup>1,2</sup> · M. Farina<sup>1</sup> · N. Polverelli<sup>1</sup> · M. Malagola<sup>1</sup> · D. Russo<sup>1</sup>

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<sup>1,2\*</sup>, CHIARA FORONI<sup>1,2\*</sup>, CAMILLA ZANAGLIO<sup>1,2</sup>, FEDERICA RE<sup>1,2</sup>, NICOLA POLVERELLI<sup>1</sup>, ALESSANDRO TURRA<sup>1</sup>, ENRICO MORELLO<sup>1</sup>, MIRKO FARINA<sup>1</sup>, FEDERICA CATTINA<sup>1</sup>, LISA GANDOLFI<sup>1</sup>, TATIANA ZOLLNER<sup>1</sup>, EUGENIA ACCORSI BUTTINI<sup>1</sup>, MICHELE MALAGOLA<sup>1</sup> and DOMENICO RUSSO<sup>1</sup>

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

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Acta  
Haematologica

## Letter to the Editor

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DOI: 10.1159/000505088

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Published online: January 9, 2020

### 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, Spedali Civili di Brescia, University of Brescia, Brescia, Italy

 **biomedicines**

 MDPI

Article

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Chiara Foroni<sup>1,2,\*</sup> , Natasa Zarovni<sup>3,\*</sup> , Laura Bianciardi<sup>3</sup>, Simona Bernardi<sup>1,4</sup> , Luca Triggiani<sup>5</sup>, Davide Zocco<sup>3</sup>, Marta Venturella<sup>3</sup>, Antonio Chiesi<sup>3</sup>, Francesca Valcamonica<sup>2</sup> and Alfredo Berruti<sup>2</sup>

SCIENTIFIC  
REPORTS

nature research

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

Davide Zocco<sup>1,5</sup>, Simona Bernardi<sup>1,5</sup> , Mauro Novelli<sup>2,3</sup>, Chiara Astrua<sup>2,3</sup>, Paolo Fava<sup>2,3</sup>, Natasa Zarovni<sup>1</sup>, Francesco M. Carpi<sup>1</sup>, Laura Bianciardi<sup>1</sup>, Ottavia Malavenda<sup>2,3</sup>, Pietro Quaglino<sup>2,3</sup>, Chiara Foroni<sup>4,5</sup>, Domenico Russo<sup>4,5</sup>, Antonio Chiesi<sup>3</sup> & Maria Teresa Fierro<sup>2,3</sup>

 **biology**

Review

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

Simona Bernardi<sup>1,2,\*</sup>  and Mirko Farina<sup>1,2</sup> 