La biopsia liquida in ematologia

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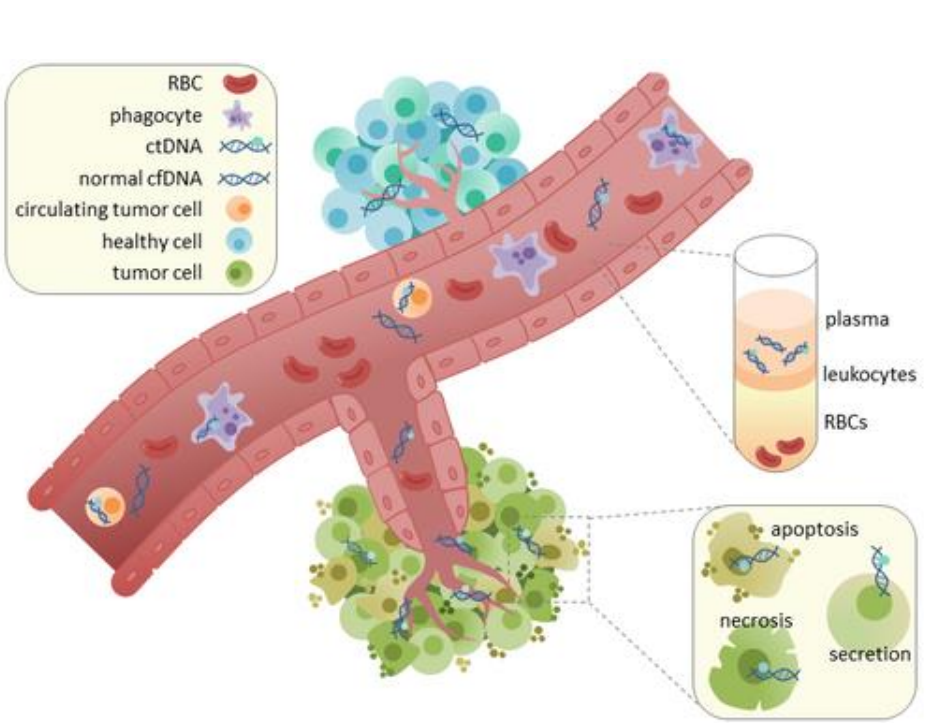
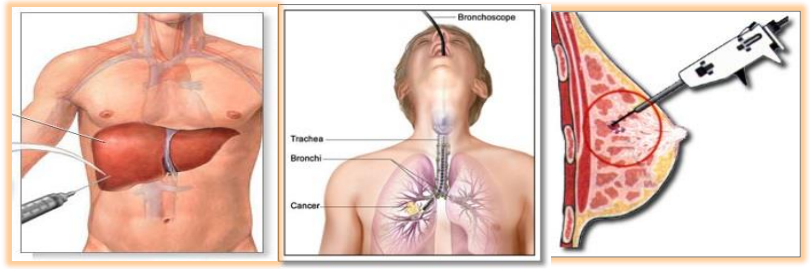
LIQUID BIOPSY



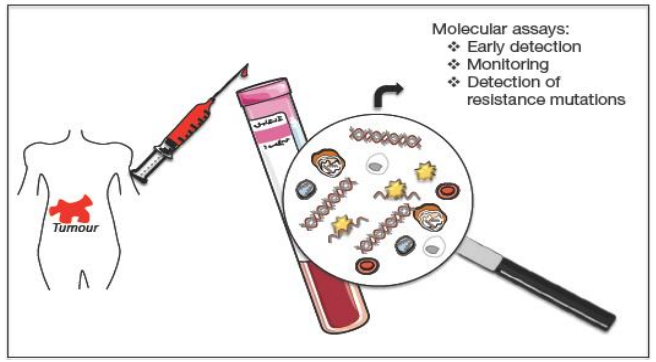
CONVENTIONAL BIOPSY VS LIQUID BIOPSY

Tumor Biopsy



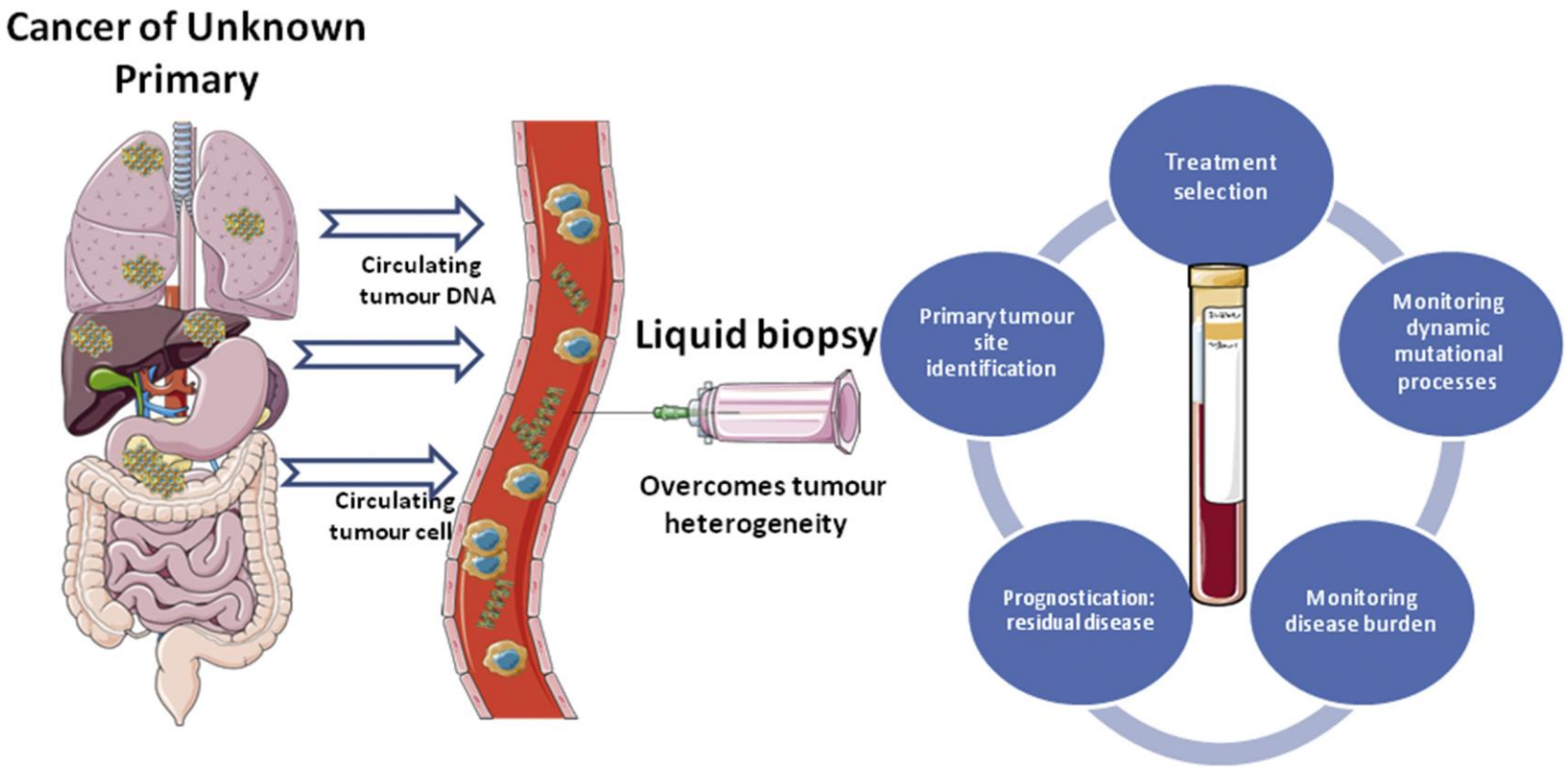
- Invasive, painful
- Expensive & time consuming
- Re-biopsy often not possible or accepted
- Not suitable for cancer monitoring
- Does not address tumor heterogeneity

Liquid Biopsy

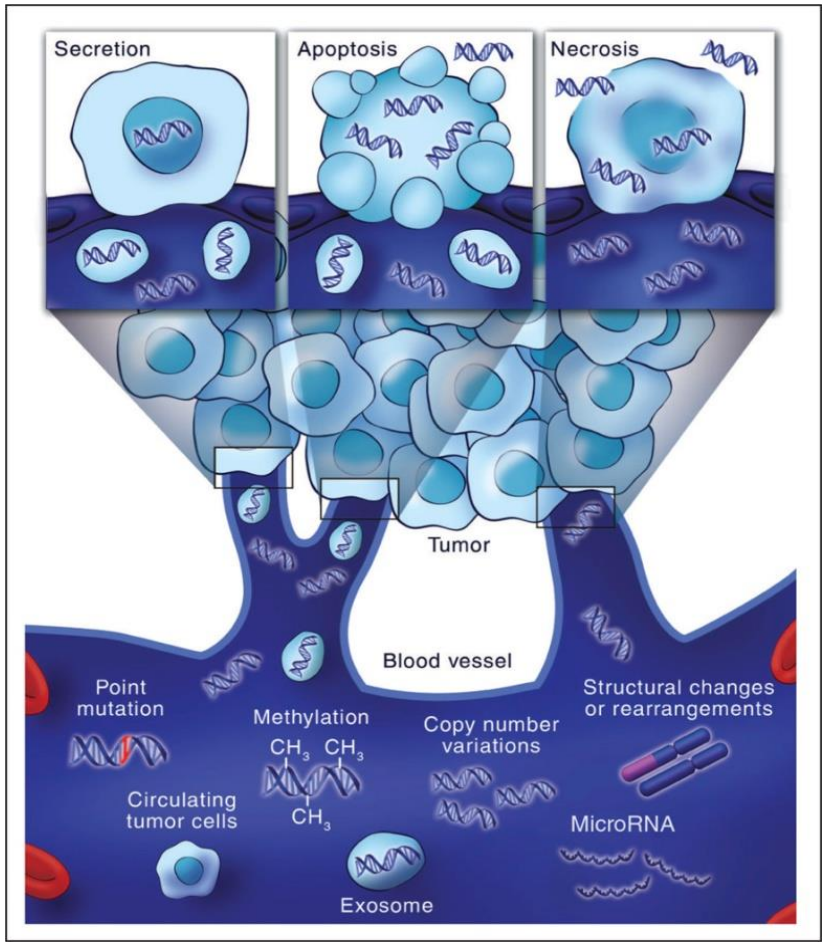


- Minimally invasive, no risk for patients
- Cheap and quick
- Re-biopsy is not a problem
- Suitable for cancer monitoring
- Addresses tumor heterogeneity

CLINICAL APPLICATIONS FOR LIQUID BIOPSY



BIOMARKERS SOURCES



Circulating cell-free nucleic acids

DNA, but also RNA (mRNAs, microRNA and long non coding RNA)

Circulating tumor cells

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

Extracellular vesicles

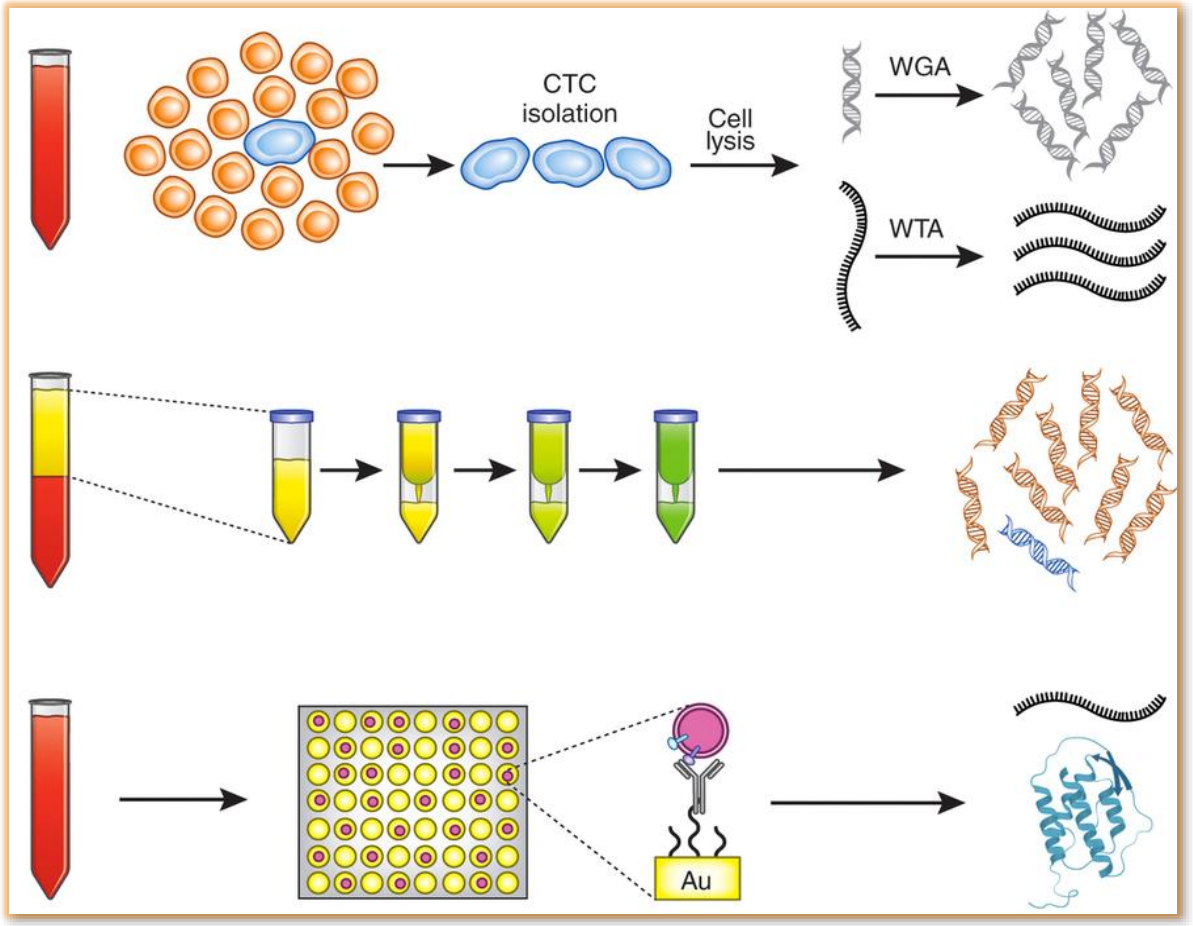
Lipid vesicles containing proteins and nucleic acids (RNAs and DNA)

BIOMARKERS SOURCES

CTC

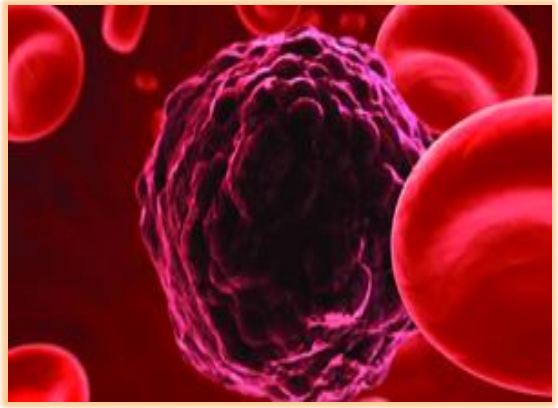
cfDNA

EVs



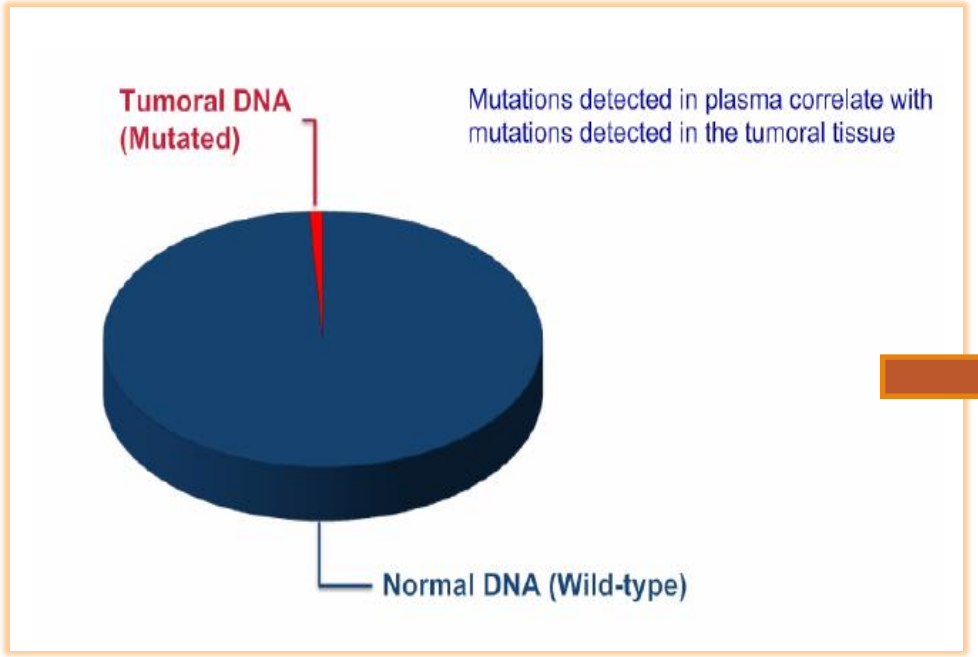
Circulating Tumor Cells (CTC)

Circulating tumor cells



- ✓ **Extremely rare (5-20 cells/ml of plasma)**
- ✓ **Low sample recovery**
- ✓ **Expensive and cumbersome analysis (up to 50 ml of plasma required)**

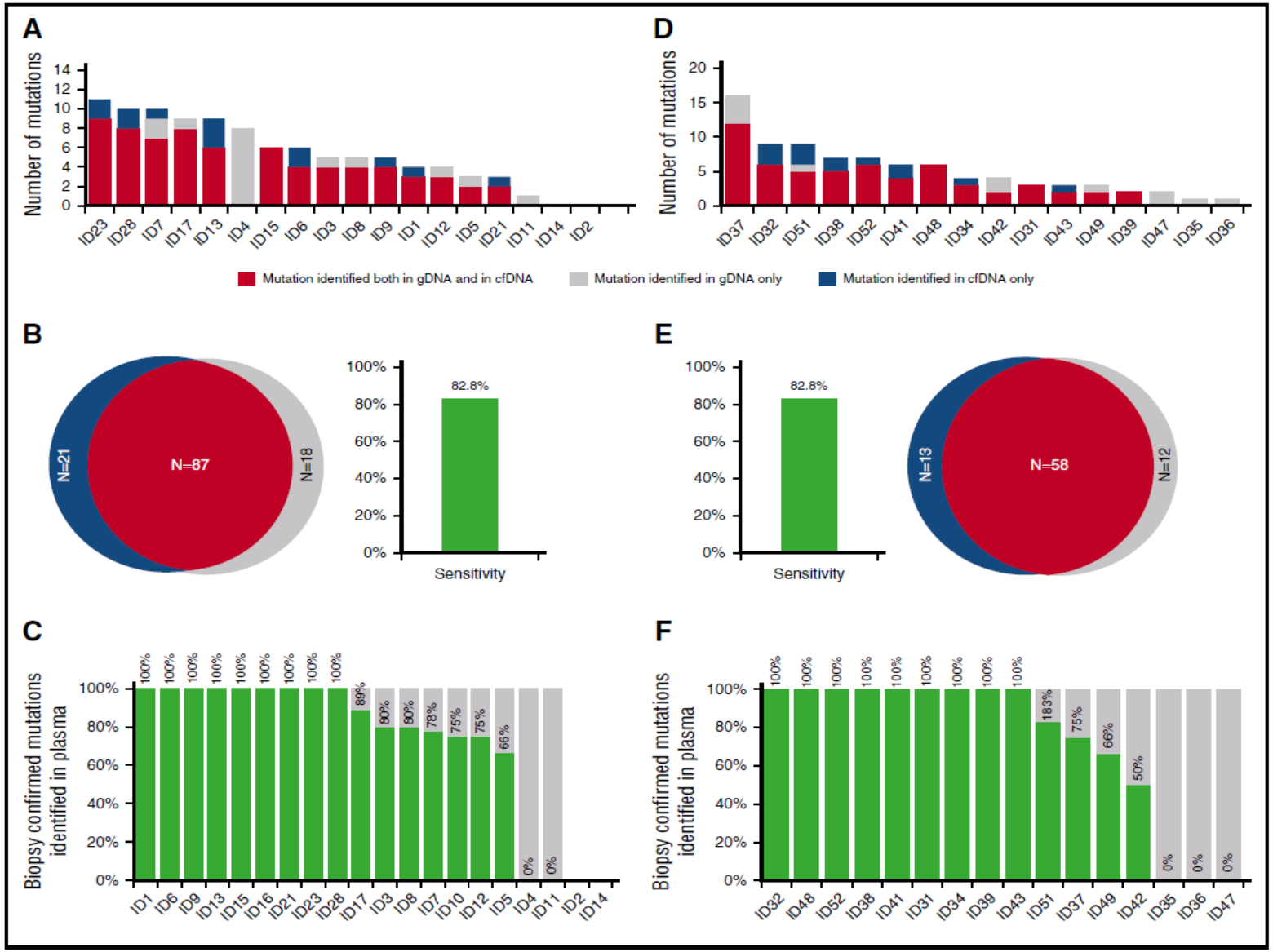
ctDNA



cfDNA is the vast majority (95% to 99%) of the total DNA extracted from blood

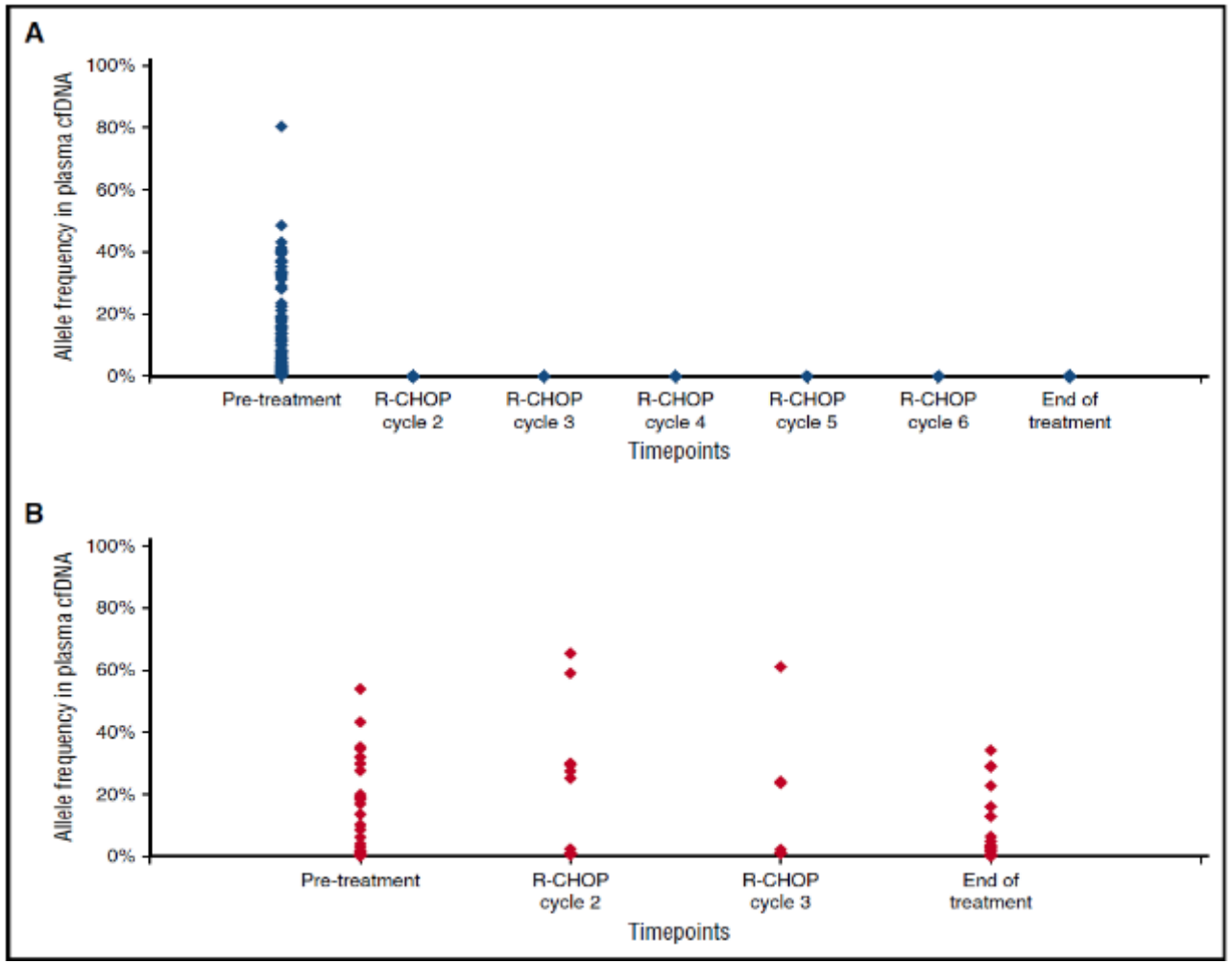
ctDNA CANNOT BE SELECTIVELY ISOLATED

La Biopsia liquida nei linfomi



Rossi D., et al, Blood 2017

La Biopsia liquida nei linfomi: il monitoraggio



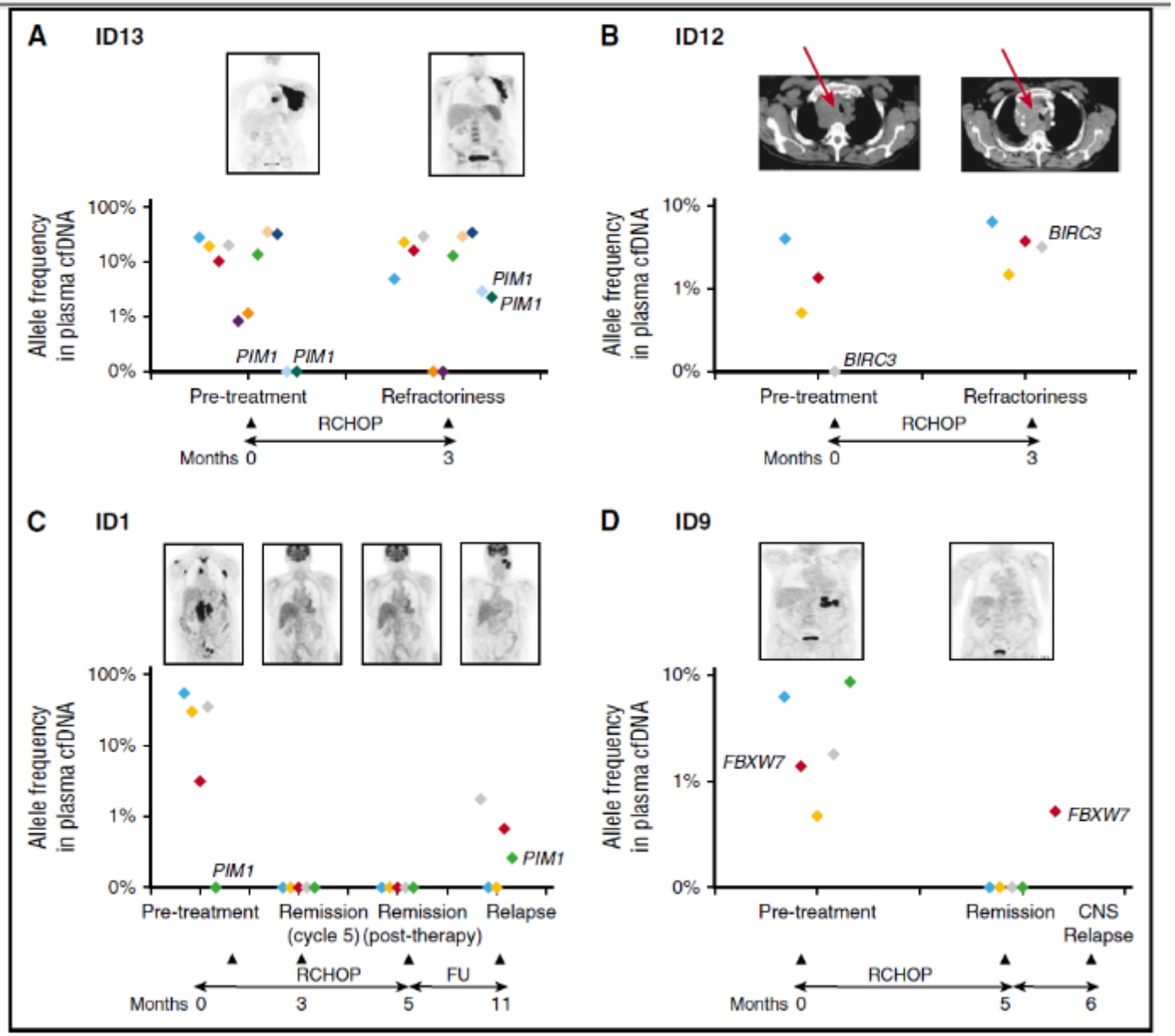
GOOD RESPONDERS

REFRACTORY PATIENTS

Rossi D., et al,
Blood 2017

La Biopsia liquida nei linfomi

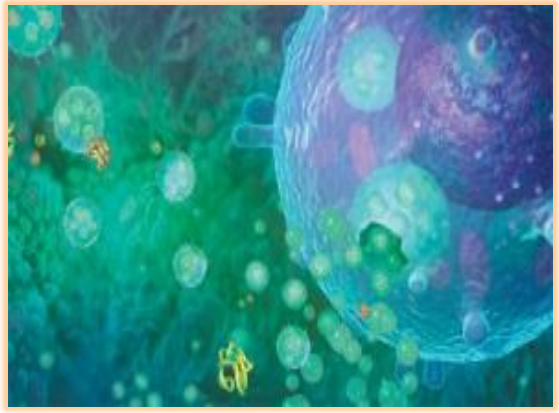
CLONAL EVOLUTION MONITORING



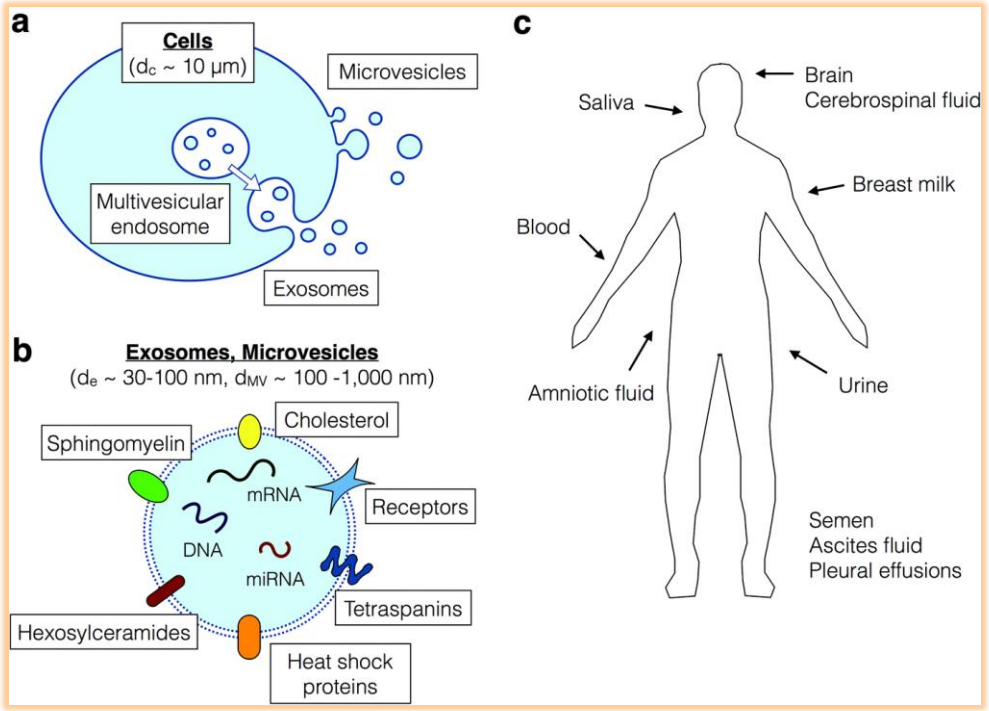
Rossi D., et al, Blood 2017

Extracellular Vesicles (EVs)

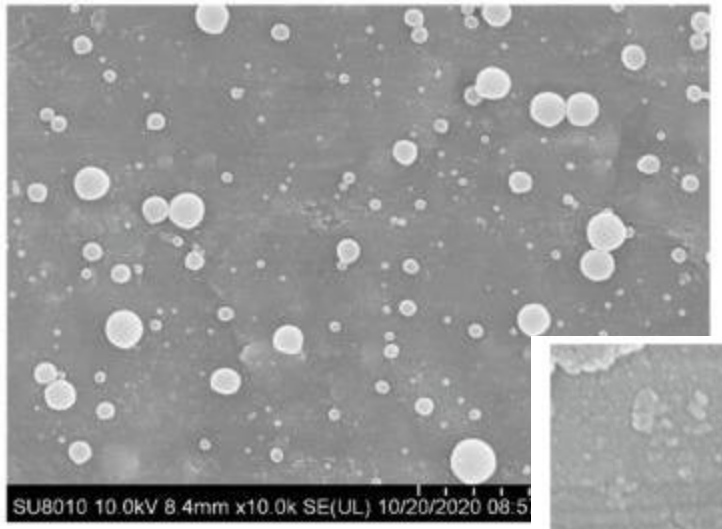
EVs

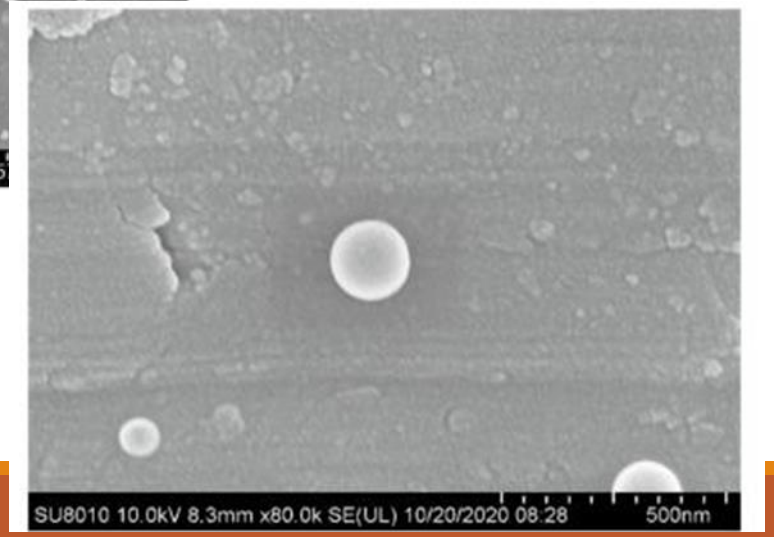


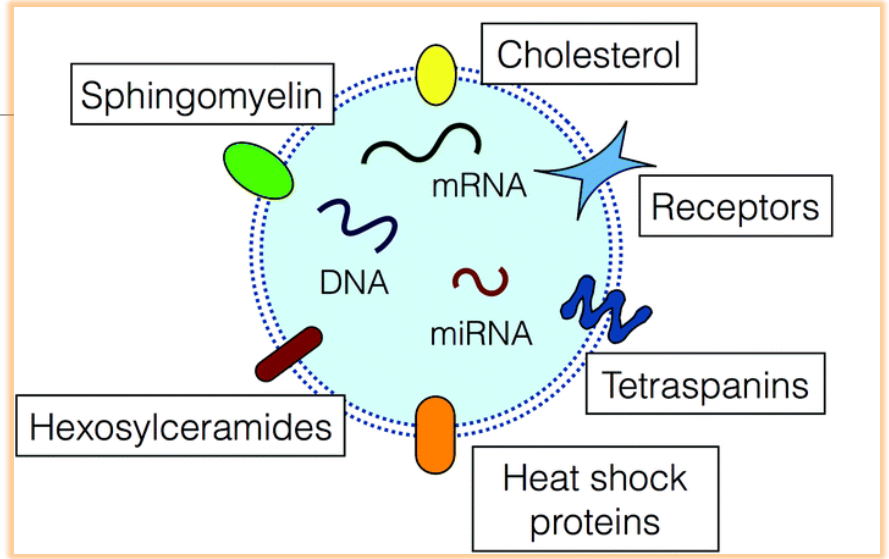
- ✓ Total Evs are 10^{7-8}/ml, tumor-Evs estimated 10^{2-3}/ml
- ✓ Easy to recover and analyzed from blood
- ✓ Heterogeneous population



EVs


SU8010 10.0kV 8.4mm x10.0k SE(UL) 10/20/2020 08:5

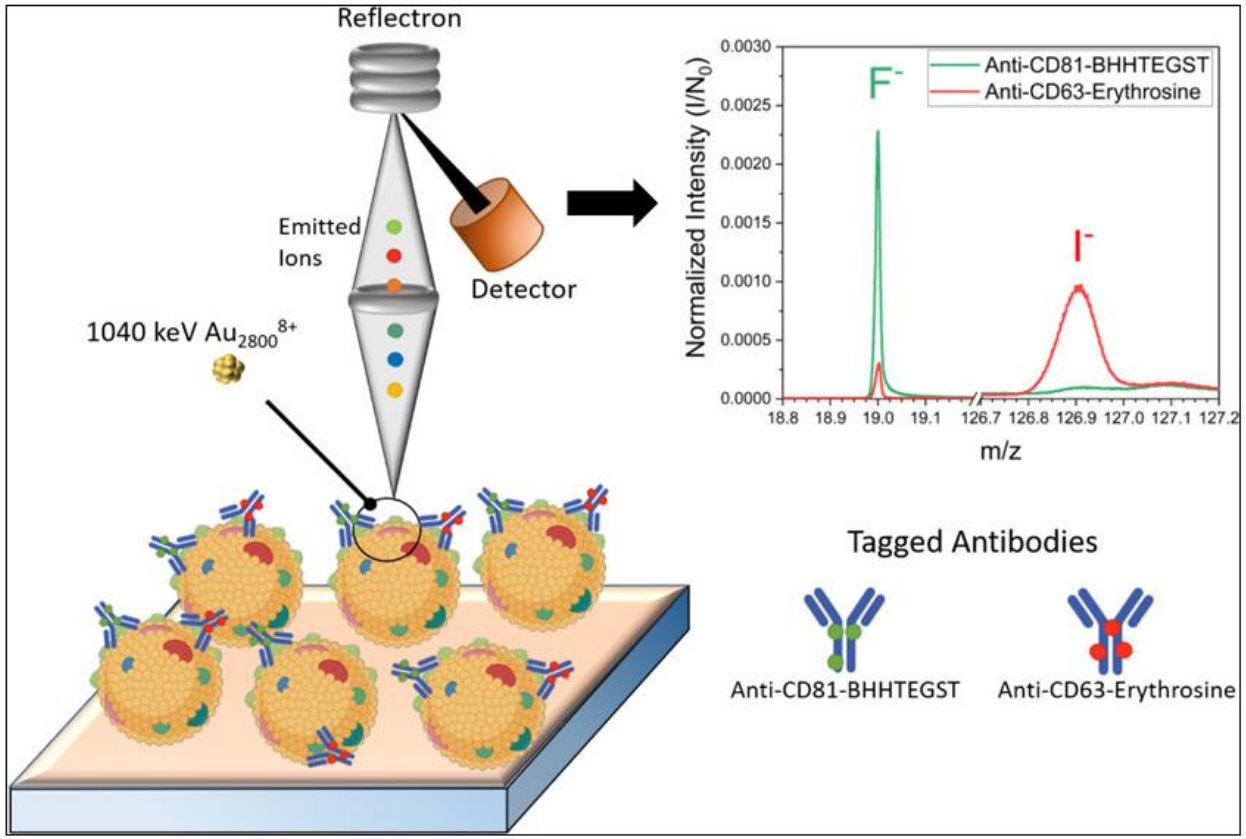

SU8010 10.0kV 8.3mm x80.0k SE(UL) 10/20/2020 08:28
500nm


Cholesterol
Sphingomyelin
mRNA
Receptors
DNA
miRNA
Tetraspanins
Hexosylceramides
Heat shock proteins

TECHNIQUES FOR LIQUID BIOPSY BIOMARKERS ANALYSIS

Proteic markers

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



TECHNIQUES FOR LIQUID BIOPSY BIOMARKERS ANALYSIS

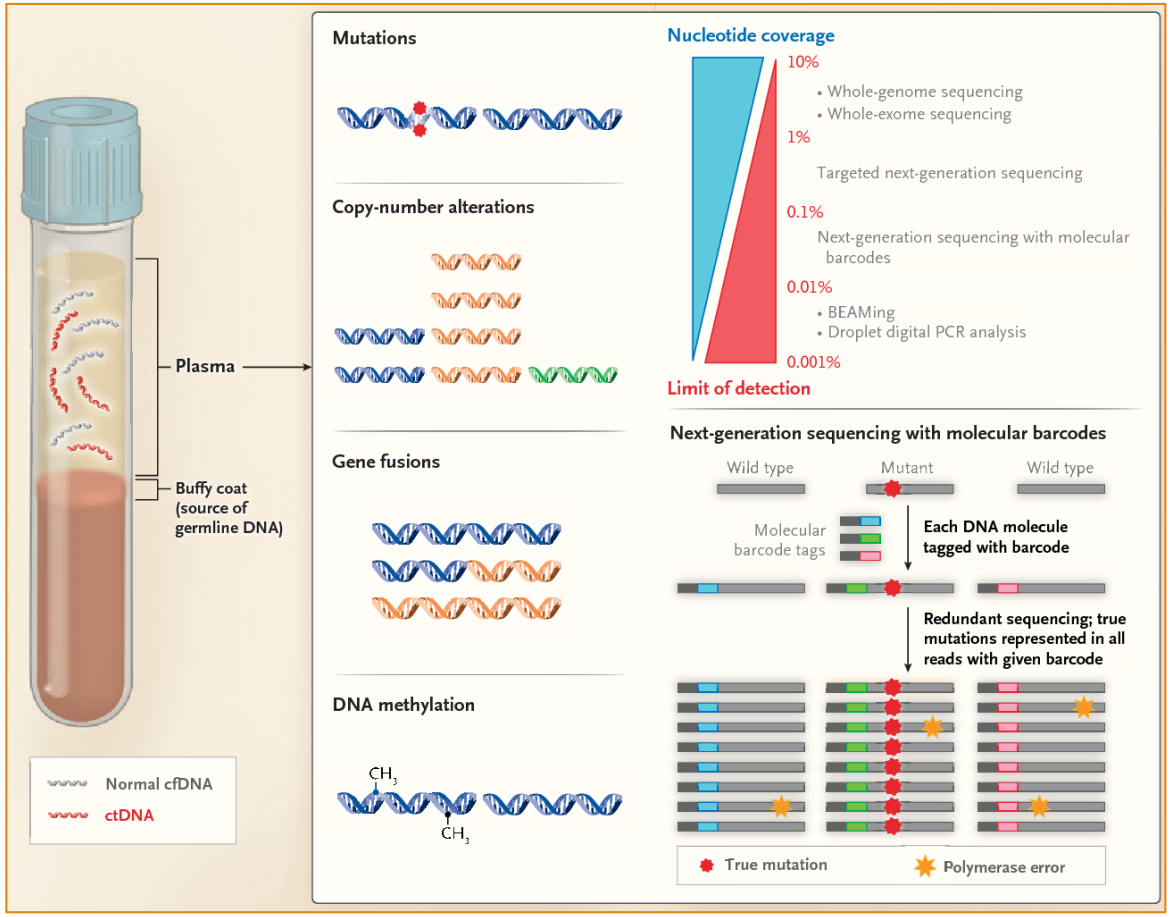
Nucleic Acids markers

Real-time PCR: cfDNA analisys and vesicular biomarkers analysis

Next Generation Sequencing: cfDNA analysis and vesicles cargo sequencing

Digital PCR: cfDNA analysis and vesicular biomarkers analysis

Microarrays: cfDNA analysis and vesicular biomarkers analysis



Gli esosomi nella biopsia liquida dei linfomi

Research Paper

mRNA in exosomas as a liquid biopsy in non-Hodgkin Lymphoma: a multicentric study by the Spanish Lymphoma Oncology Group

Mariano Provencio[1,*], Marta Rodríguez[1], Blanca Cantos[1], Pilar Sabín[2], Cristina Quero[3], Francisco R. García-Arroyo[4], Antonio Rueda[5], Constanza Maximiano[1], Delvys Rodríguez-Abreu[6], Antonio Sánchez[1], Javier Silva[1] and Vanesa García[1,7,*], on behalf of GOTEL (Spanish Lymphoma Oncology Group)

60 DLBCL
38 FL
68 Healthy Controls

→

Exosomes fraction isolation from plasma for mRNA evaluation.

→

Correlation between mRNA composition and:

- OS
- PFS
- Response to treatment

Gli esosomi nella biopsia liquida dei linfomi

BCL-6 and c-MYC

The presence of BCL-6 and c-MYC transcript in exosomes samples of B-cell Lymphoma patients was associated with shorter survival and there were no differences in the other mRNAs analyzed. c-MYC remain an independent prognostic variable for OS when stratified by response.

La biopsia liquida per il monitoraggio della MRD?

MINIMAL RESIDUAL DISEASE: small residual quantity of tumor cells, detectable only by advanced and sensitive techniques. The number of the tumor cells is under the limit of detection of morphologic analysis. Small residual quantity of tumor cells may be present also at Complete Remission.

Is the MRD assessment by LB possible in Lymphoma patients?

La biopsia liquida per il monitoraggio della MRD?

	Advantage	Limitation
Clinical exam only	Low cost	Clinical symptoms non-specific No opportunity for early intervention
CT scans	Readily available Interpretation standardized Identify lower-risk relapse	Radiation exposure Incidental findings Low sensitivity prior to symptoms No survival benefit[a]
FDG-PET scans	Readily available Improved sensitivity over CT scans	Radiation exposure Incidental findings High false positive rate
Circulating tumor DNA	NGS assays available Tumor-specific Non-invasive Avoid tissue biopsy Detect clonal evolution	Needs clinical validation Collection not standardized

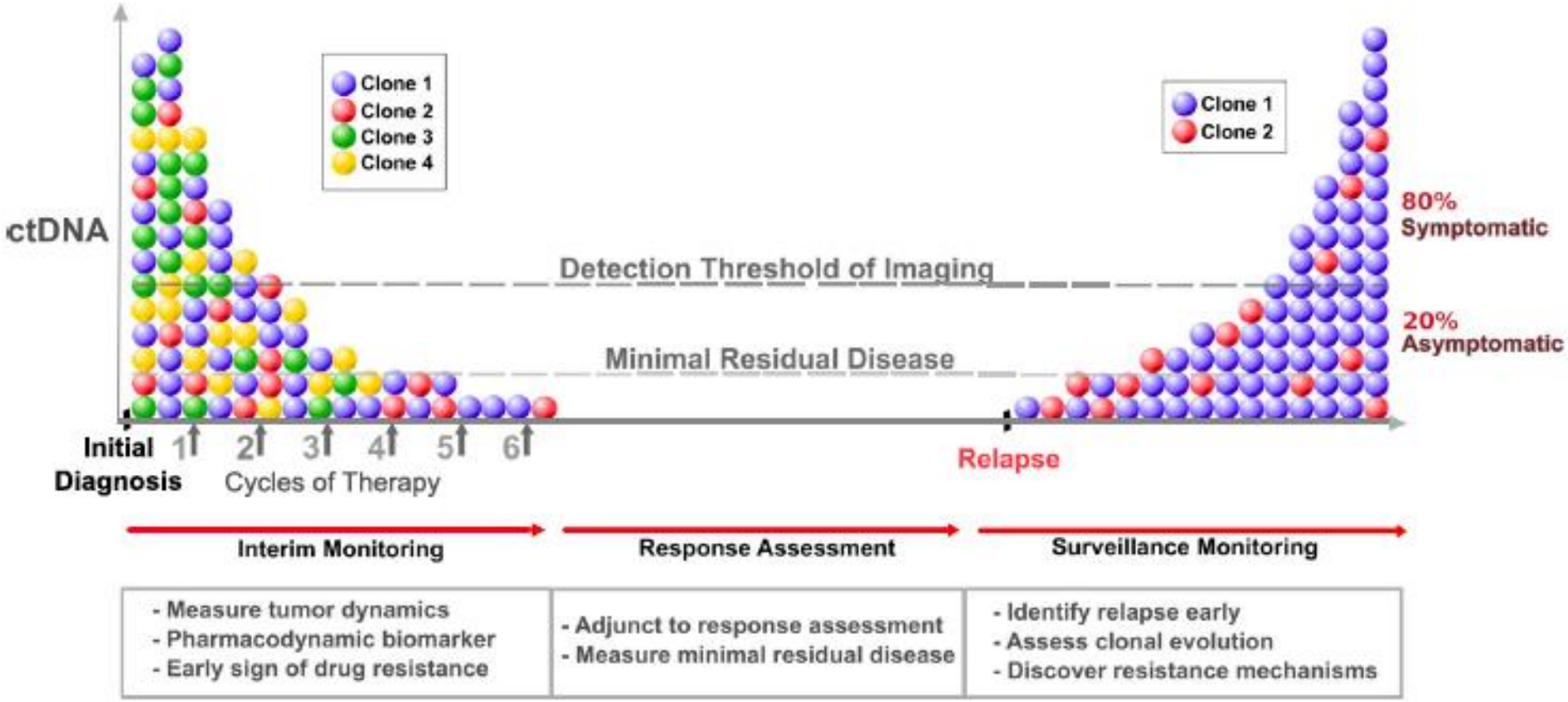
Modified by: Kwok M. et al, Curr. Treat Opinion in Oncology (2016)

La biopsia liquida per il monitoraggio della MRD?

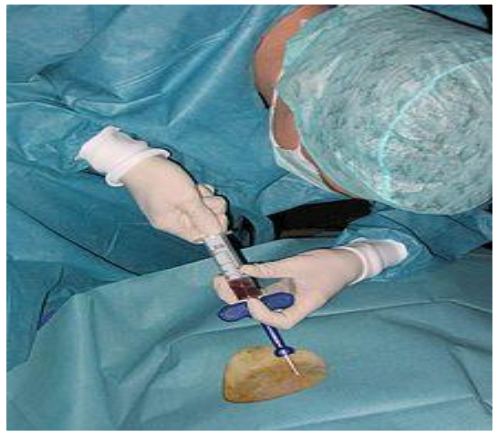
- **VDJ molecular detection seems to be the best tool to assess an MRD monitoring by LB**
- **ctDNA monitoring has proven advantages over CT scans and may enhance the ability to manage patients with potential curable lymphoma**
- **No clinical impact has been yet demonstrated, no standard exists for treating patients with "ctDNA positivity only" relapse, the optimal timing and duration of monitoring…..**

Future studies to investigate whether monitoring of ctDNA can be used to improve clinical outcomes are warranted!

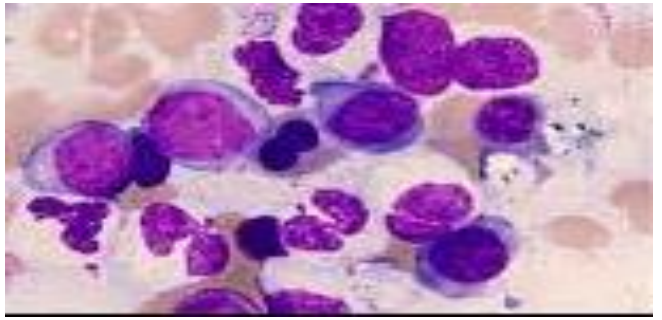
La biopsia liquida per il monitoraggio della MRD?



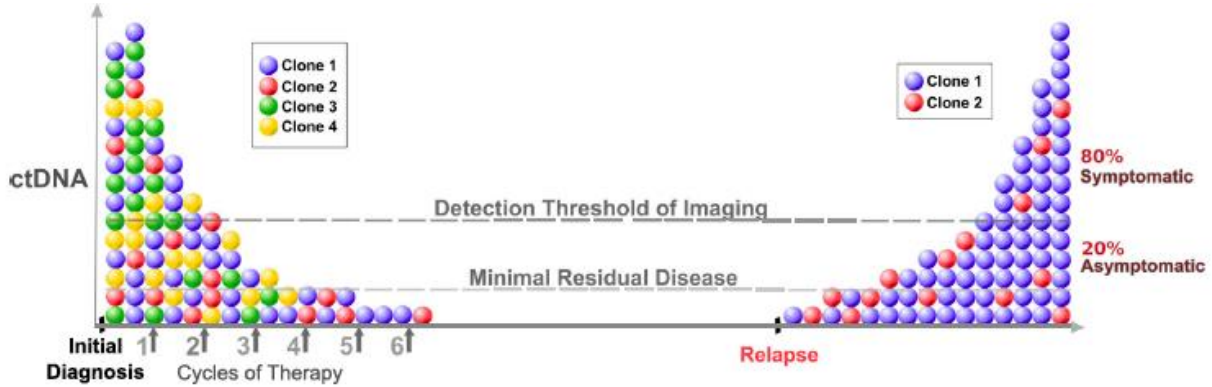
LIQUID APPROACH FOR LIQUID DISEASES???



Punctio sicca



Myelodysplastic syndromes



Bone marrow evaluation

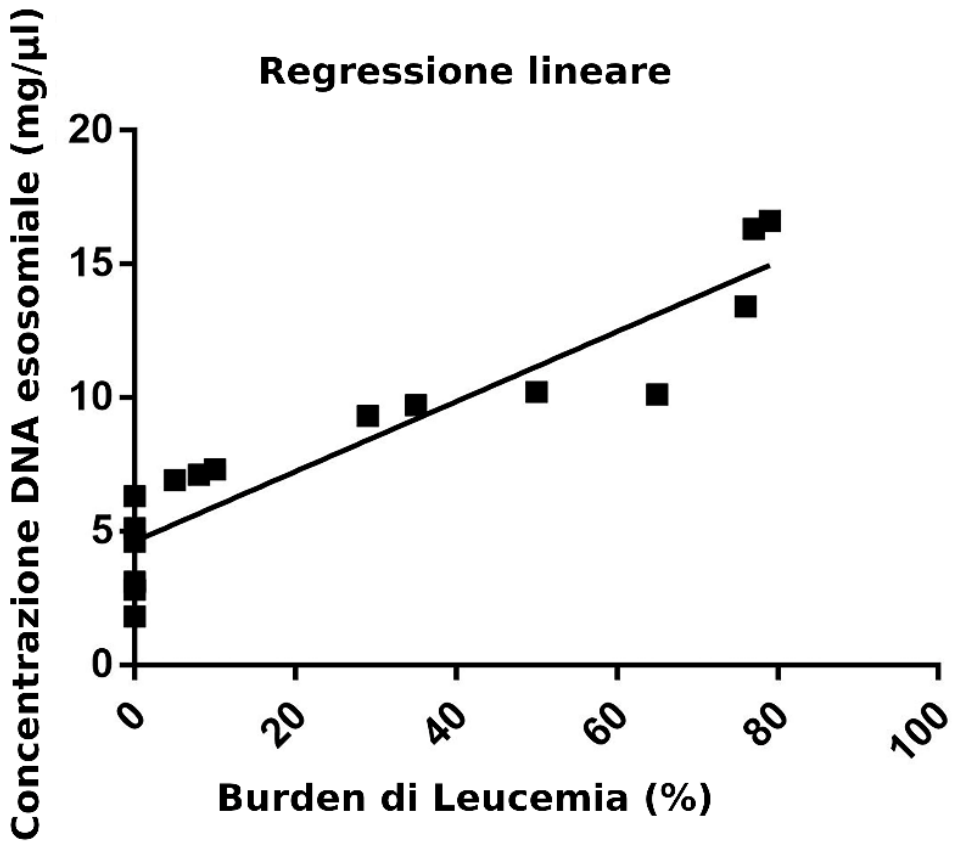
EXPERIMENTAL DESIGN

Case	*Sex*	*Age*	*Disease status at enrollment*
1	*M*	*61y*	*Relapse post-alloSCT*
2	*F*	*47y*	*Relapse*
3	*F*	*30y*	*CR post-alloSCT*
4	*M*	*71y*	*Diagnosis of AML secondary to MDS*
5	*F*	*44y*	*Relapse*
6	*F*	*64y*	*Relapse post-alloSCT*
7	*M*	*44y*	*Relapse post-alloSCT*
8	*M*	*70y*	*CR post relapse post-alloSCT*
9	*M*	*41y*	*AML MRD+ pre-alloSCT*
10	*F*	*44y*	*Pre-alloSCT*

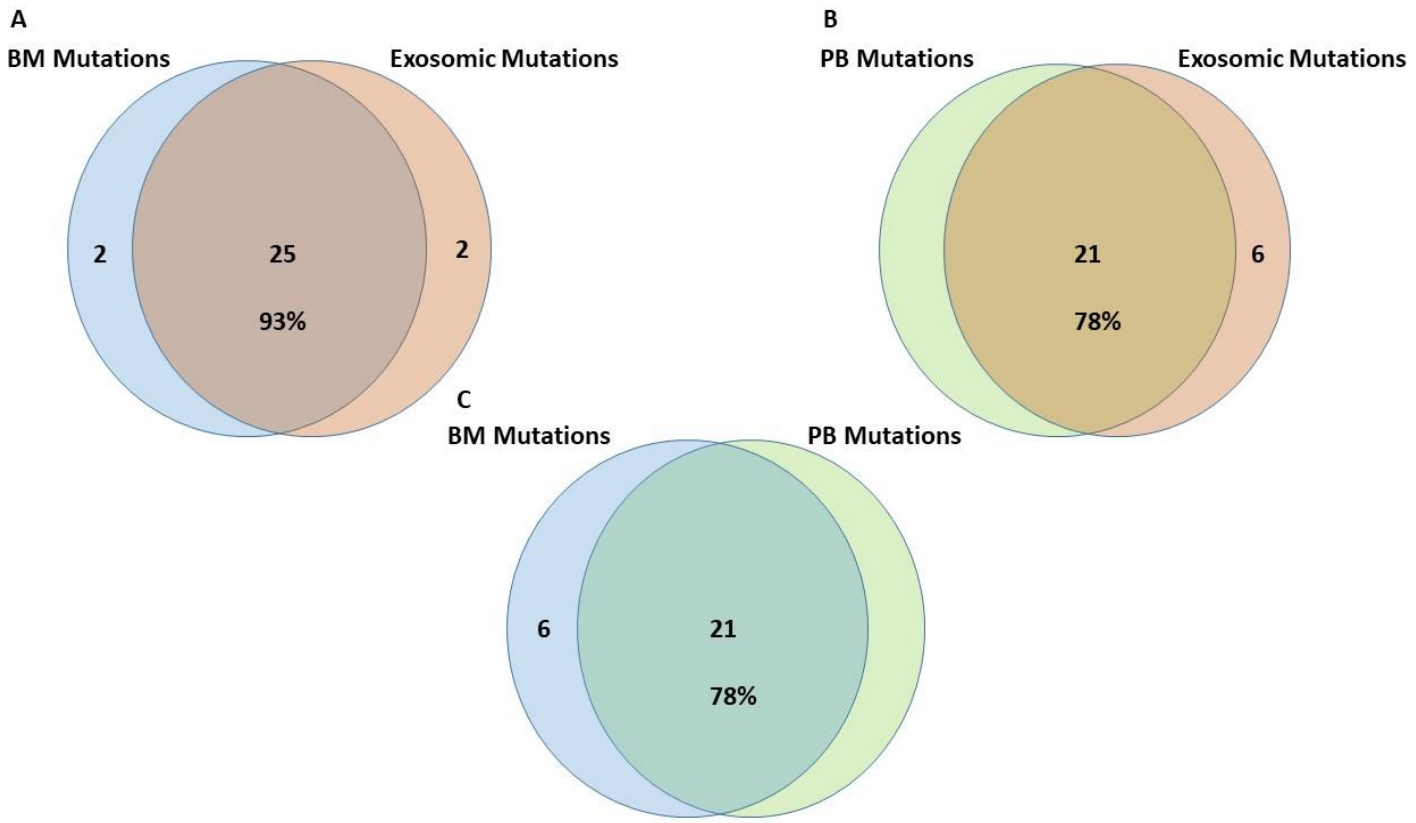
- **10 ADULT AML PATIENTS (5 MALE AND 5 FEMALE)**
- **DIFFERENT DISEASE STATUS**
- **5 FOLLOWED UP**
- **NGS ANALYSIS ON CELLULAR AND EXOSOMAL DNA USING A CUSTOM PANEL**

CORRELAZIONE TRA dsDNA ESOSOMIALE E LEUKEMIA BURDEN

(R=0,86)



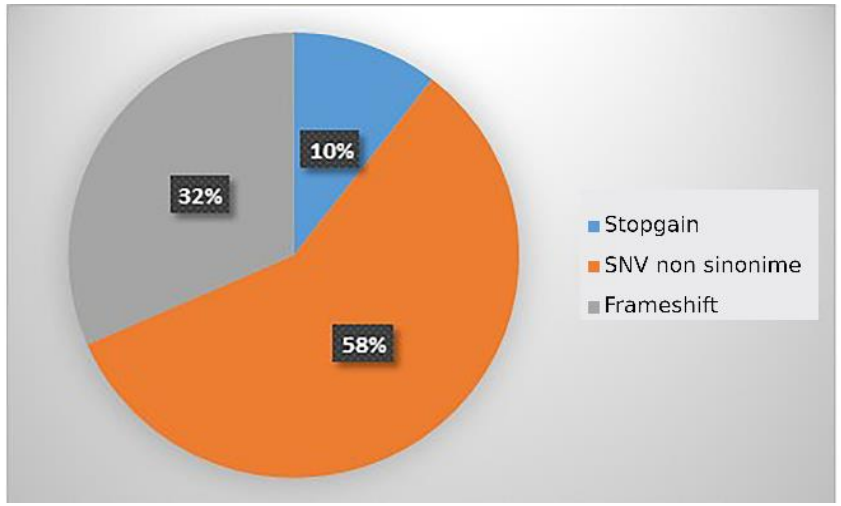
OMOLOGIA TRA LE VARIANTI RILEVATE

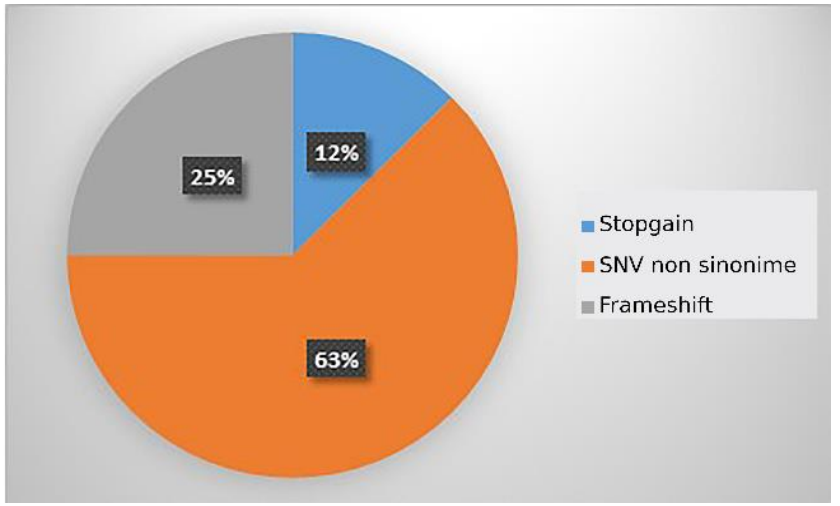


Il numero maggiore di mutazioni è stato rilevato nel dsDNA esosomiale (44 mutazioni), mentre il numero più basso in quello del dsDNA derivato da cellule del SP (30 mutazioni)

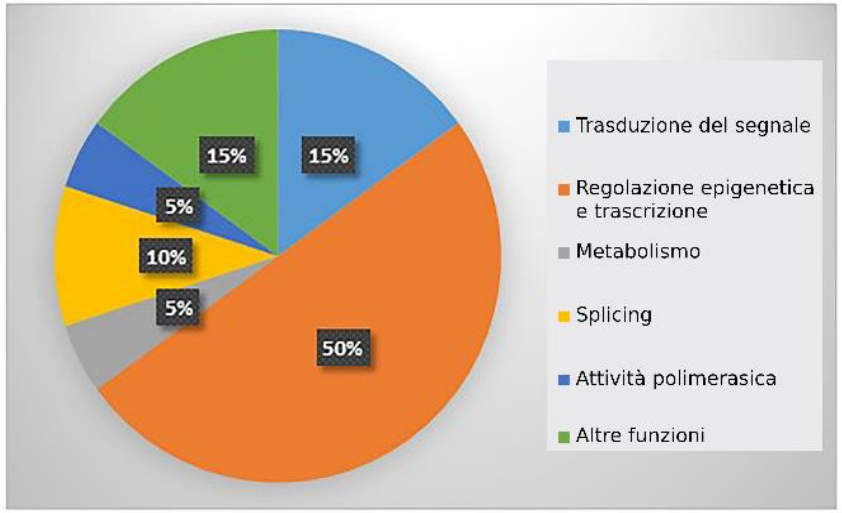
- Pari al 93% fra il dsDNA esosomiale e del MO
- Più bassa, ovvero 78%, l'omologia riscontrata fra dsDNA del SP e di origine esosomiale e fra quello del MO e del SP

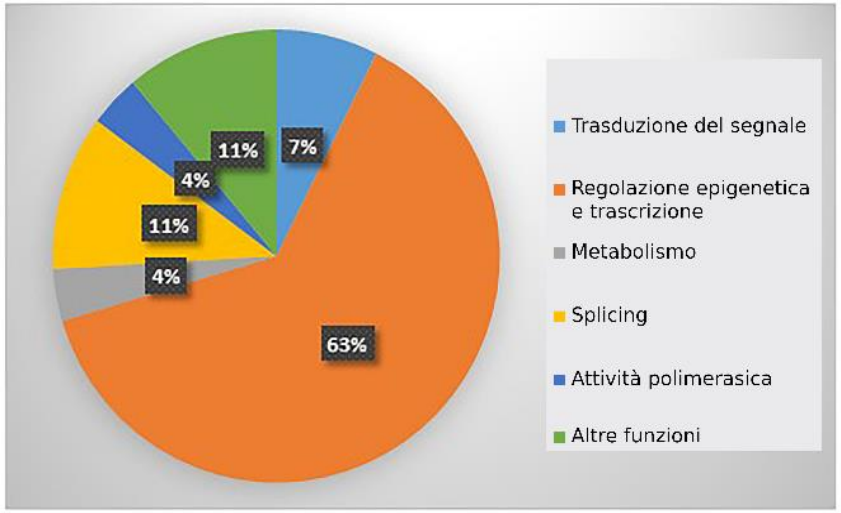
Tipologia di mutazione



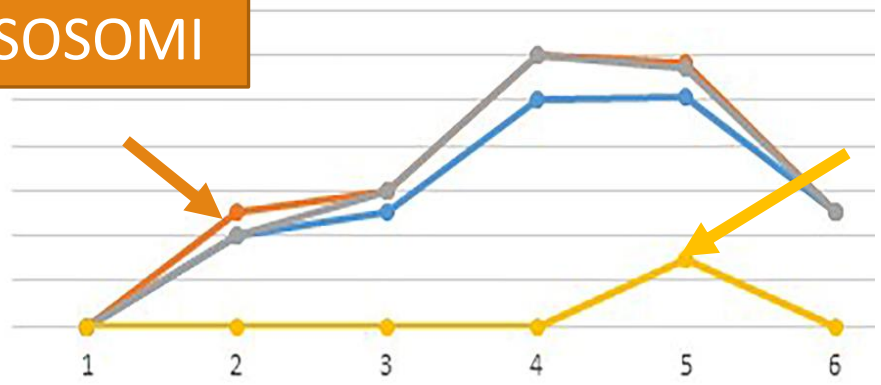


Tipo di geni mutati

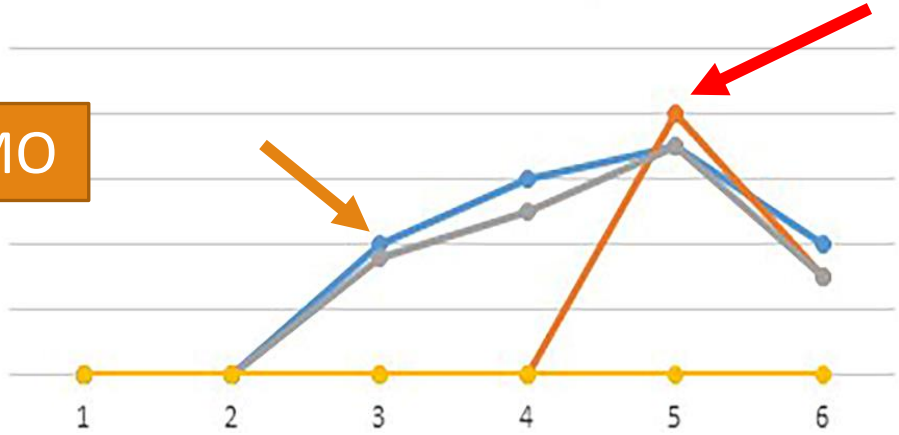




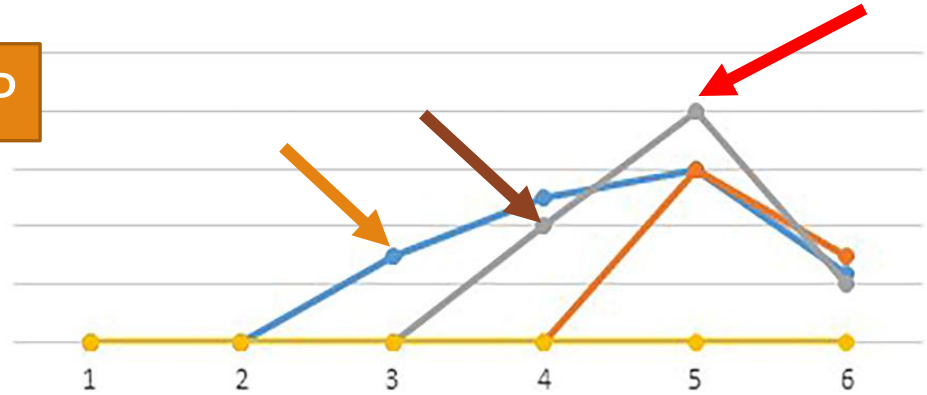
ESOSOMI



MO



SP



ANALISI DI FOLLOW UP

Tra i 10 pazienti arruolati nello studio, 5 sono stati monitorati durante il follow up.
Tra i pazienti monitorati è risultata particolarmente interessante la paziente del caso 10

TIME POINT

1	2	3	4	5	6
Pre alloSCT	RC post alloSCT 30 giorni post-alloSCT	Recidiva 7 mesi post-alloSCT	Recidiva a 10 mesi post-alloSCT	Post terapia azacitidina	Post terapia azacitidina

GENI MUTATI

ASXL1 RUNX1 TET2 IDH1

LIQUID BIOPSY IN HEMATOLOGY:

It is feasible

It is useful

It is different

It is innovative

It is reliable