APPLICATIONS OF DIFFERENT LIQUID BIOPSY APPROACHES IN HEMATOLOGICAL MALIGNANCIES

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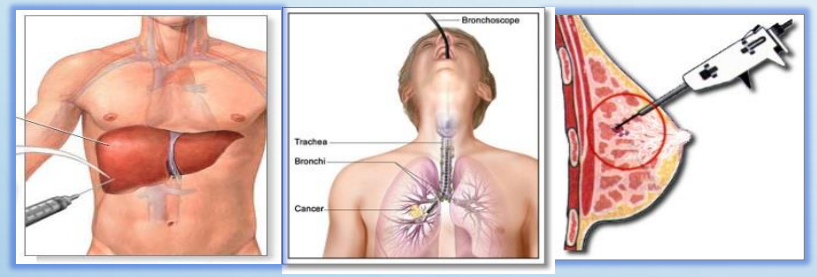
10 MAGGIO 2024

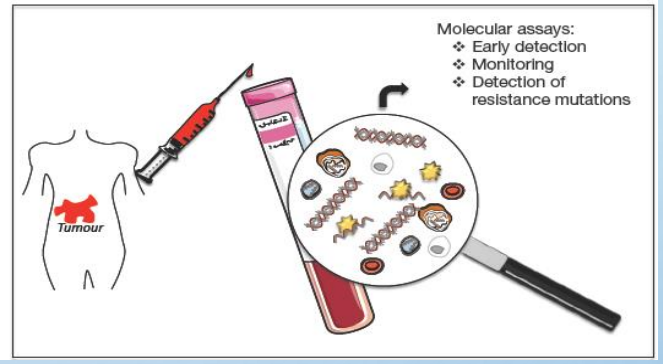
CONVENTIONAL BIOPSY VS LIQUID BIOPSY

Tumor Biopsy



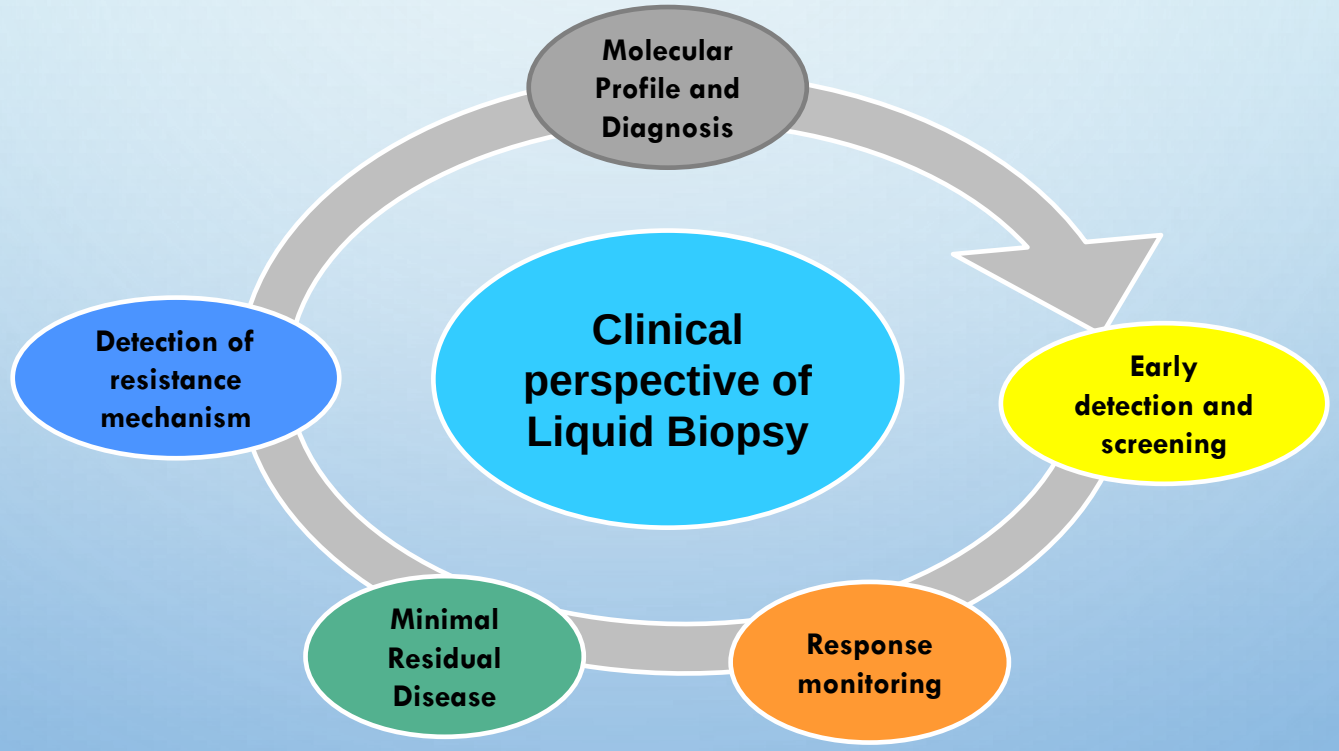
- Invasive, painful
- Expensive & time consuming
- Re-biopsy often not possible or accepted
- Not suitable for cancer monitoring
- Does not address tumor heterogeneity

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)

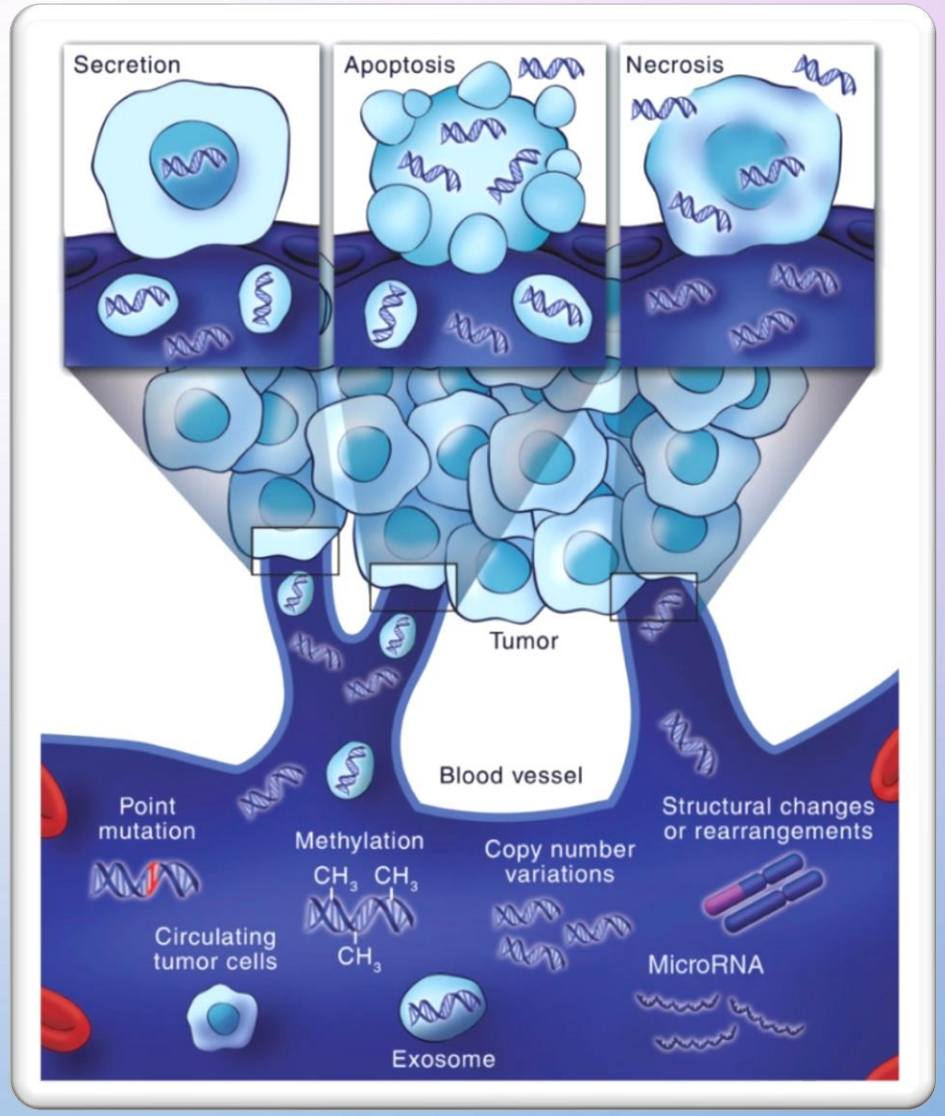
- **CIRCULATING TUMOR CELLS**

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)

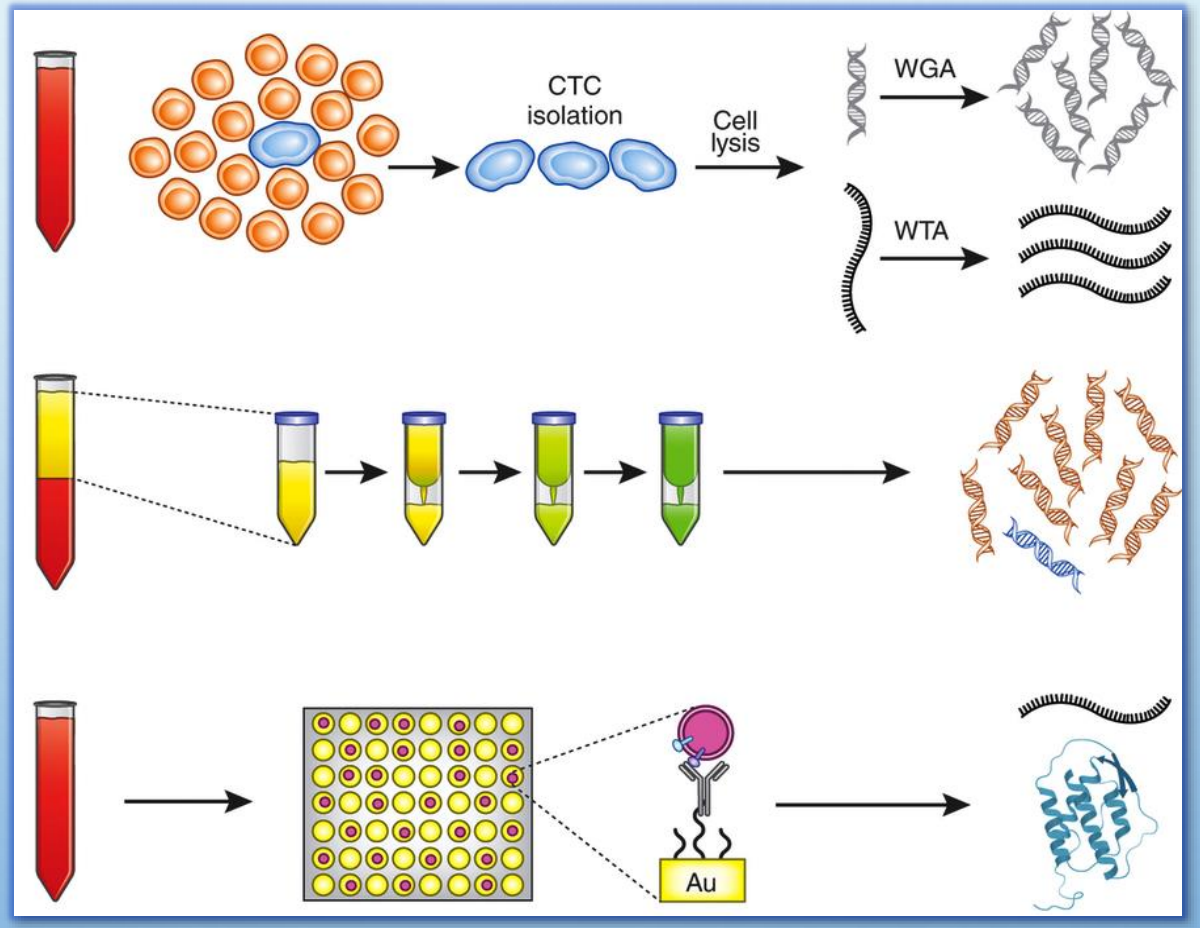


BIOMARKERS SOURCES

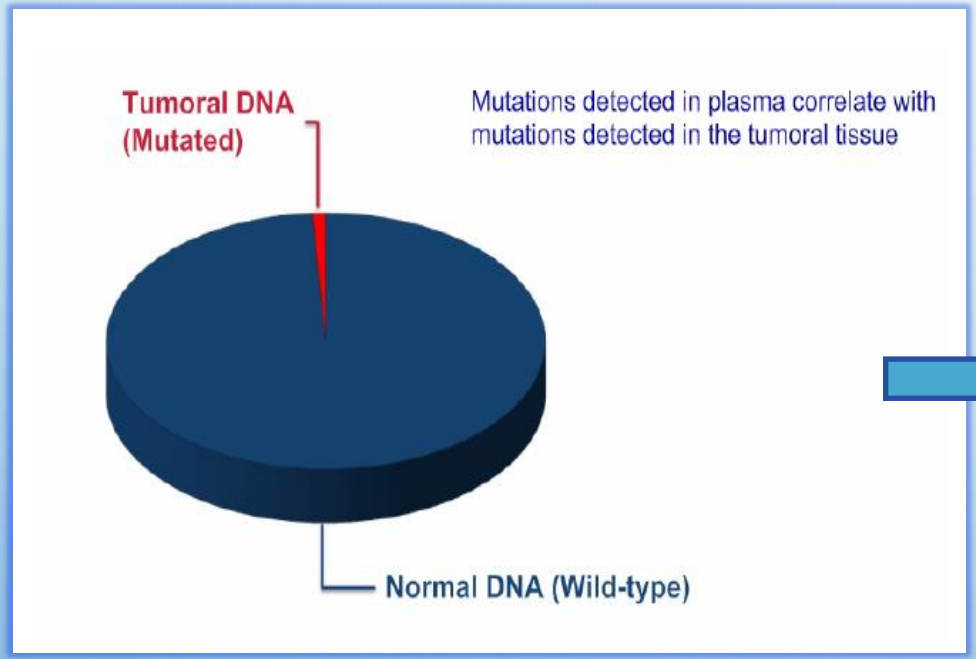
CTC

cfDNA

EVs



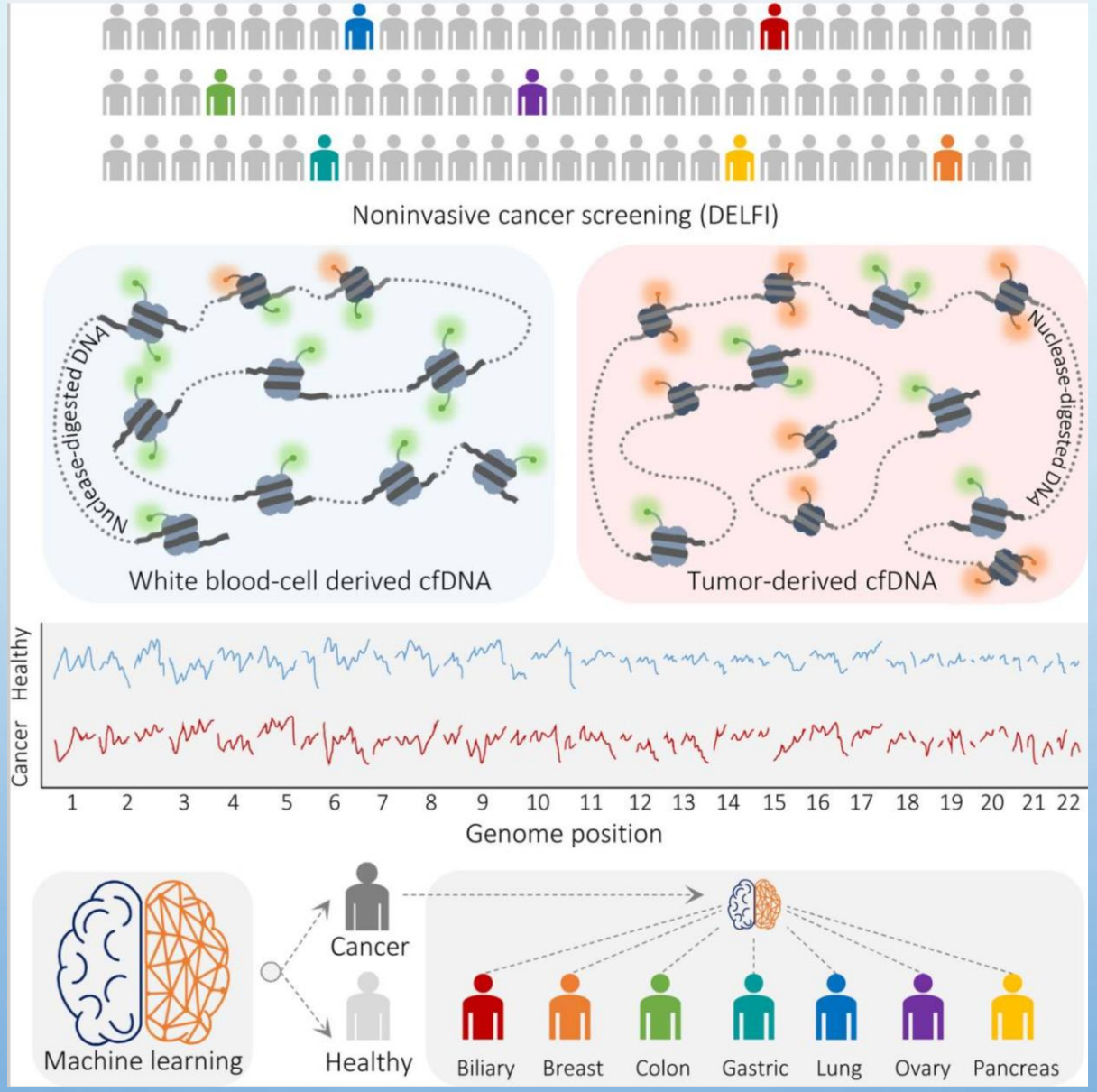
ctDNA



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

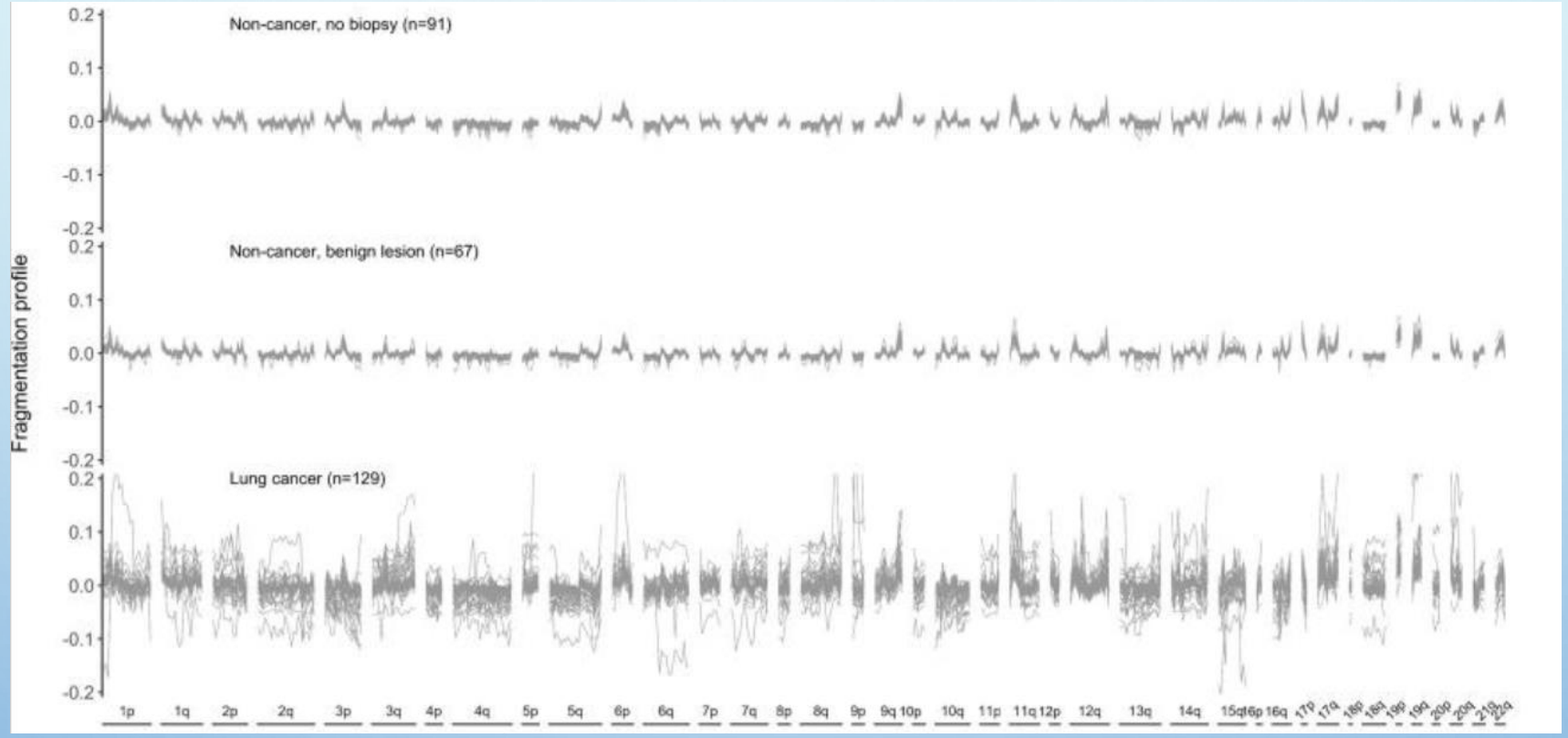
ctDNA CANNOT BE SELECTIVELY ISOLATED

The study of the FRAGMENTOMES: the hypothesis



Cristiano S. Et al, Nature, 2019

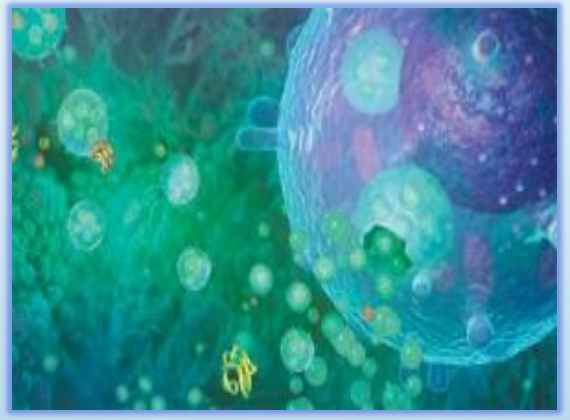
The study of the FRAGMENTOMES: the application



Mathios D. Et al
Nature comm,
2021

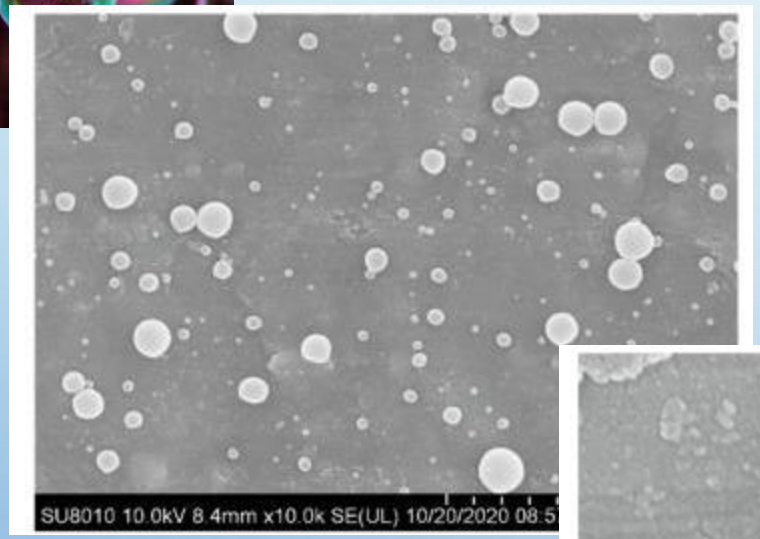
Extracellular Vesicles (EVs)

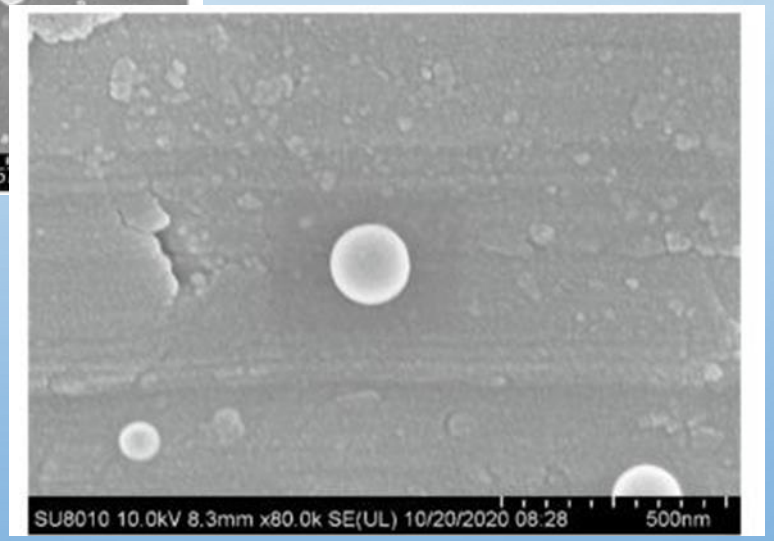
EVs

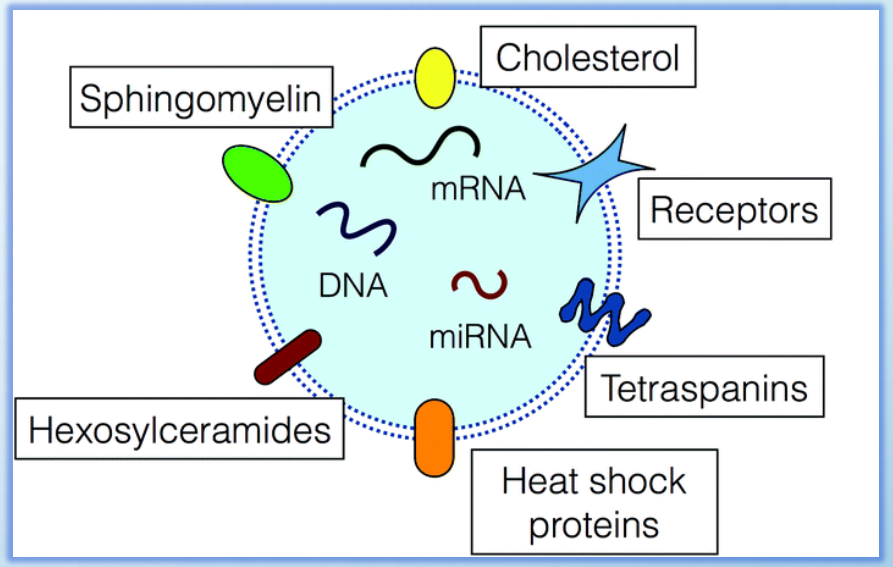


- ✓ Total EVs are 10^{7-8}/ml, tumor-Evs estimated 10^{2-3}/ml
- ✓ Easy to recover and analyzed from blood
- ✓ Heterogeneous populatiom

EVs


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

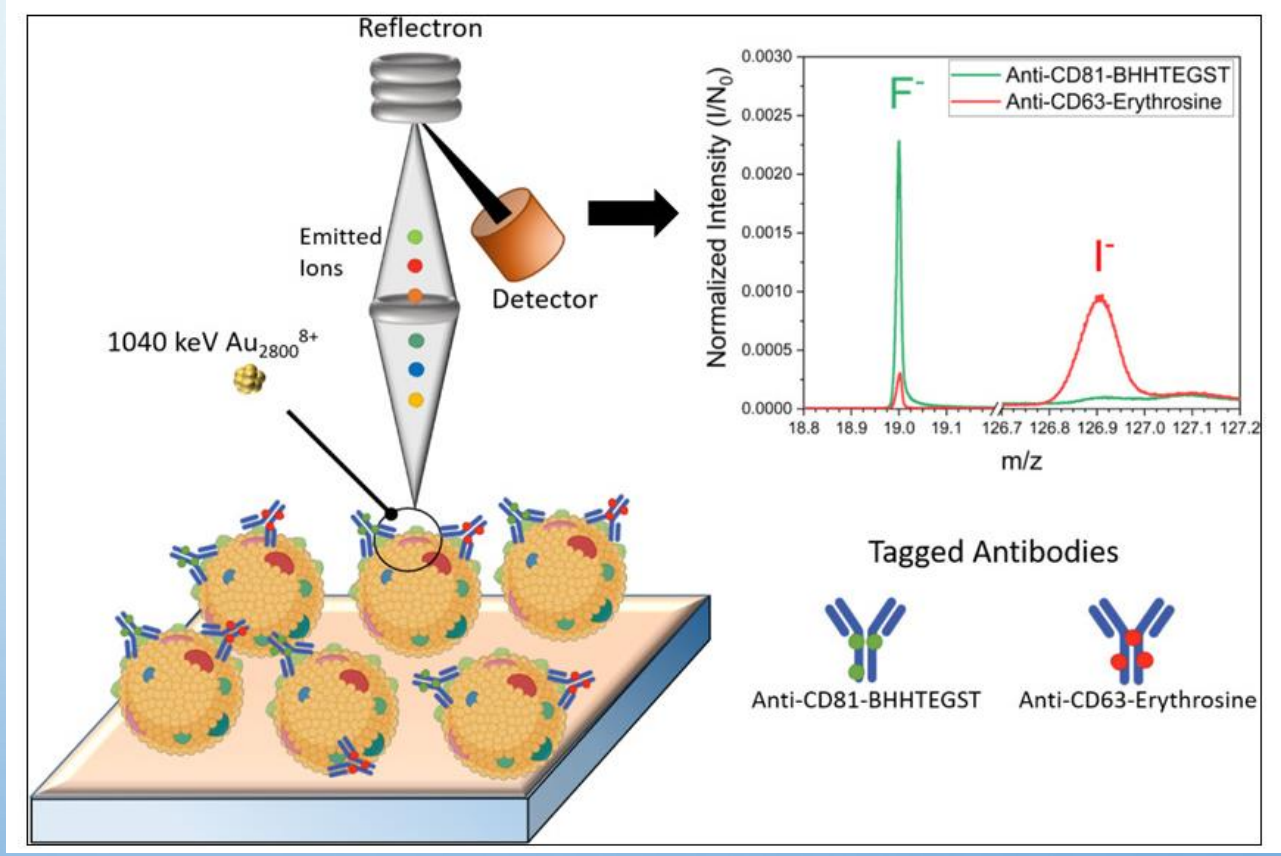

SU8010 10.0kV 8.3mm x80.0k SE(UL) 10/20/2020 08:28
500nm


Cholesterol
Sphingomyelin
mRNA
Receptors
DNA
miRNA
Tetraspanins
Hexosylceramides
Heat shock proteins

TECHNIQUES FOR LIQUID BIOPSY BIOMARKERS ANALYSIS

Proteic markers

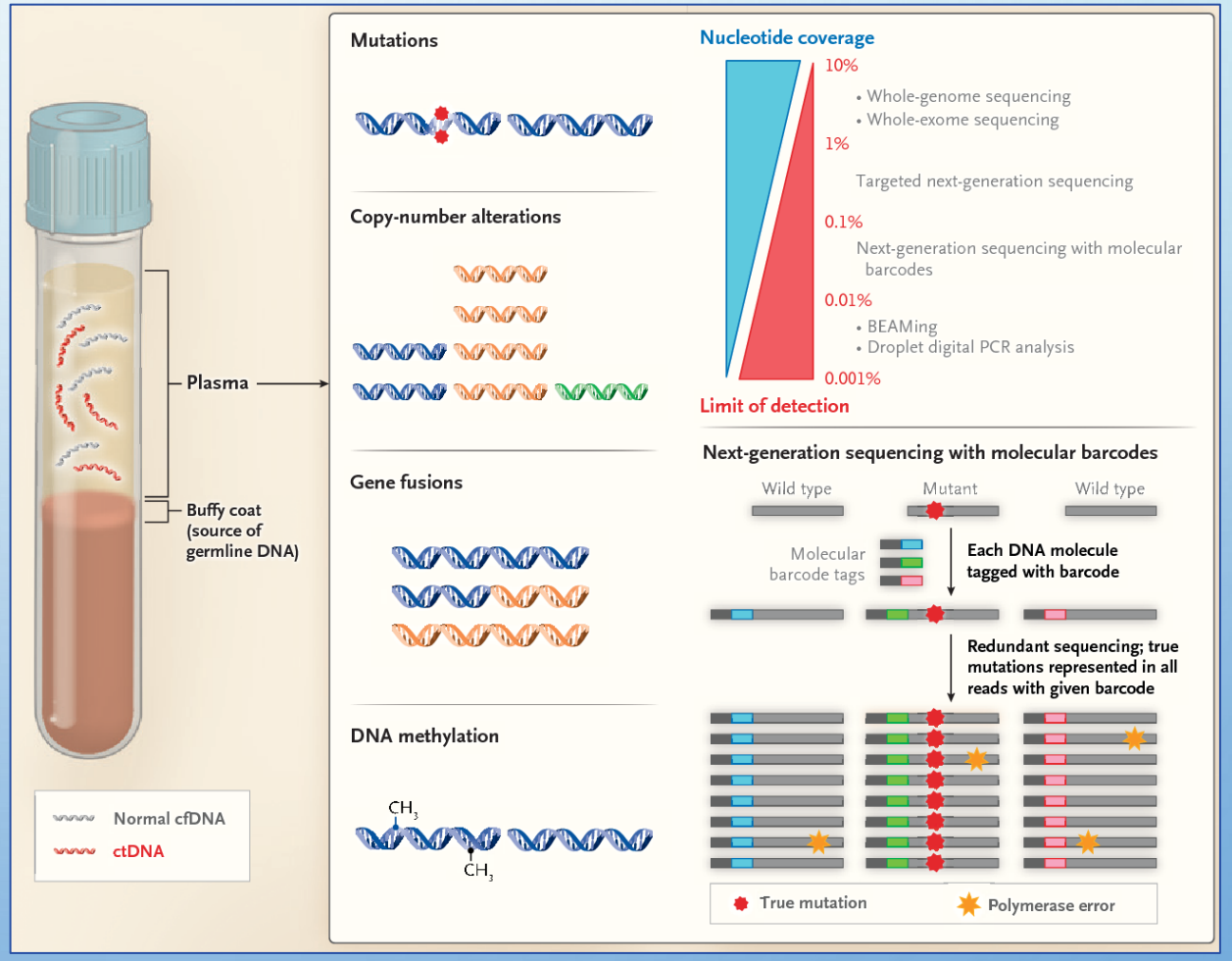
- **Western Blot**: CTC and vesicular biomarkers analysis
- **microBCA:** vesicular proteic biomarkers analysis
- **Mass spectrometry/Raman Technology**: CTC and vesicular proteomic analysis

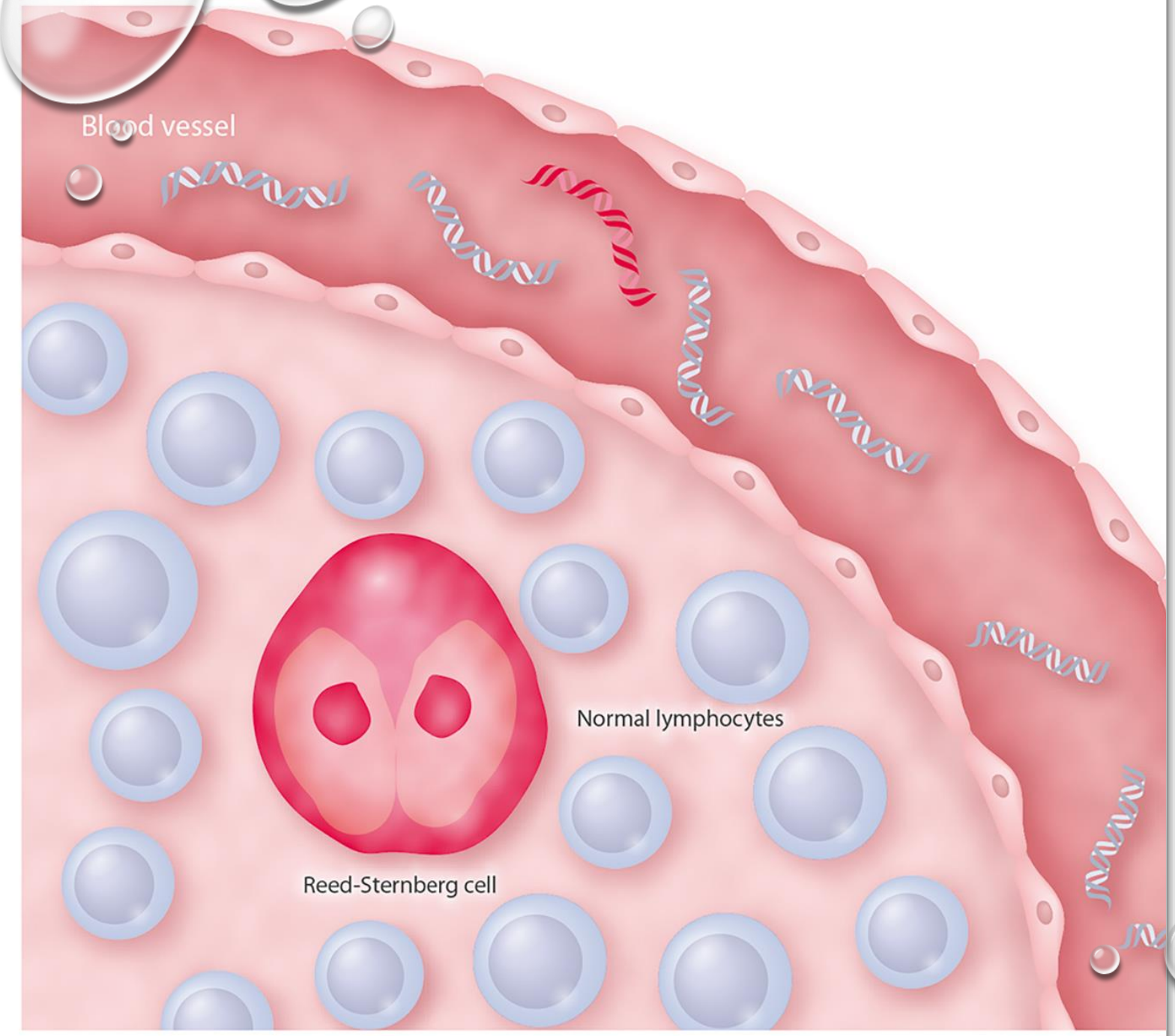


TECHNIQUES FOR LIQUID BIOPSY BIOMARKERS ANALYSIS

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

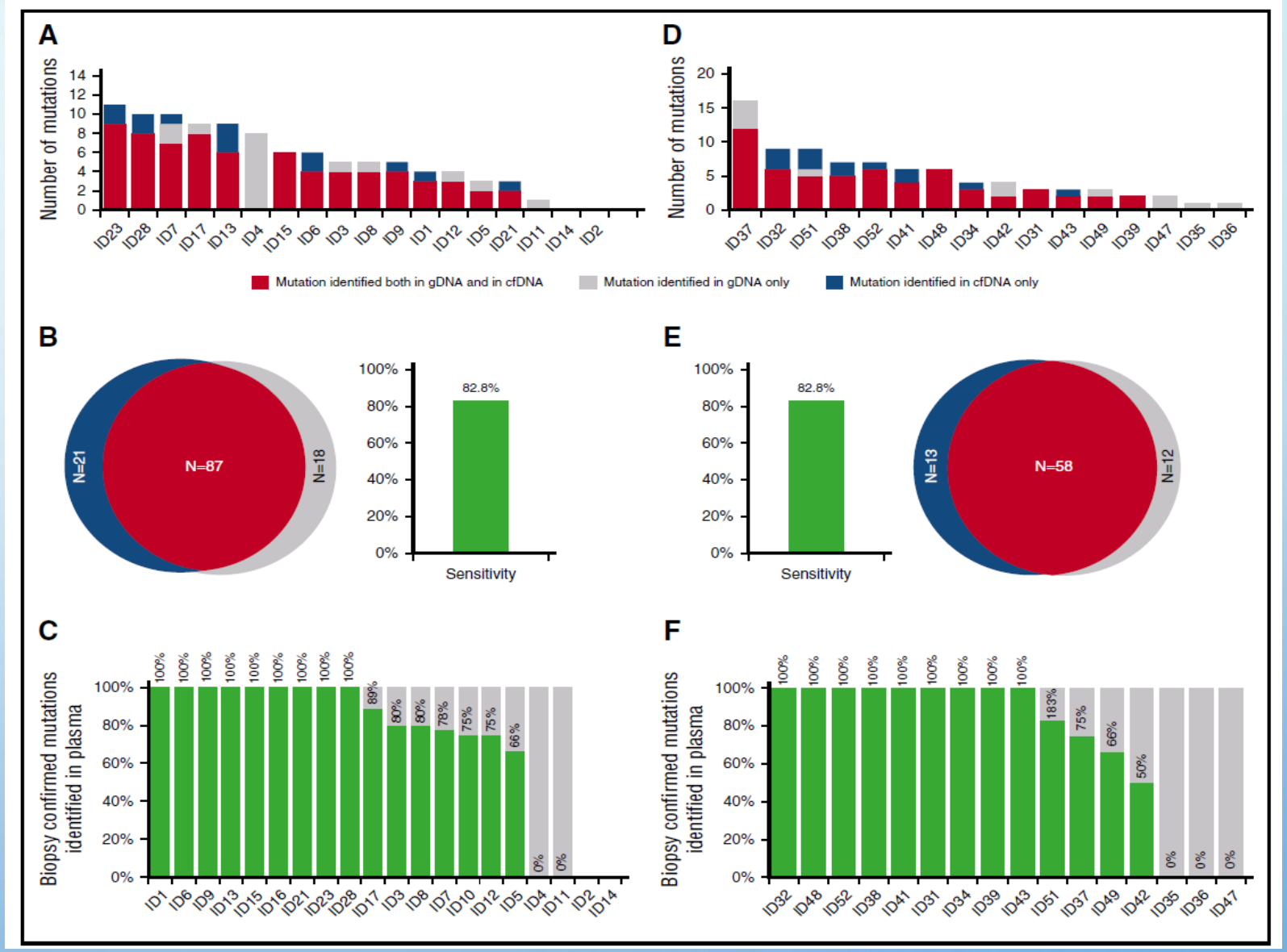




LIQUID BIOPSY IN HEMATOLOGY?

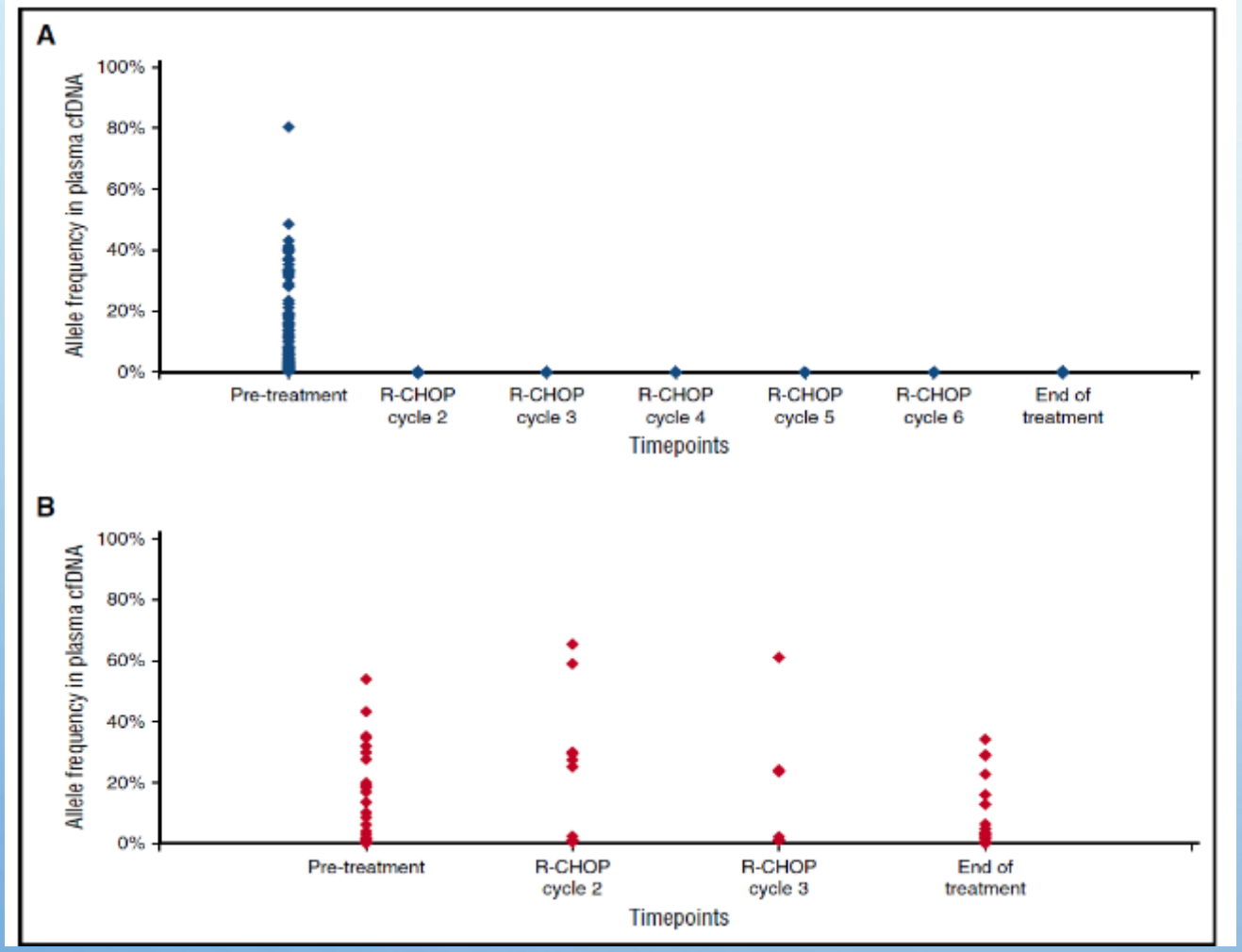
THE IDEAL AND IMMEDIATE APPLICATION IS ON **LYMPHOMAS**, THE SOLID COUNTERPART OF HEMATOLOGIC MALIGNANCIES

THE LIQUID BIOPSY IN LYMPHOMAS



Rossi D., et al, Blood 2017

THE LIQUID BIOPSY IN LYMPHOMAS: THE MONITORING

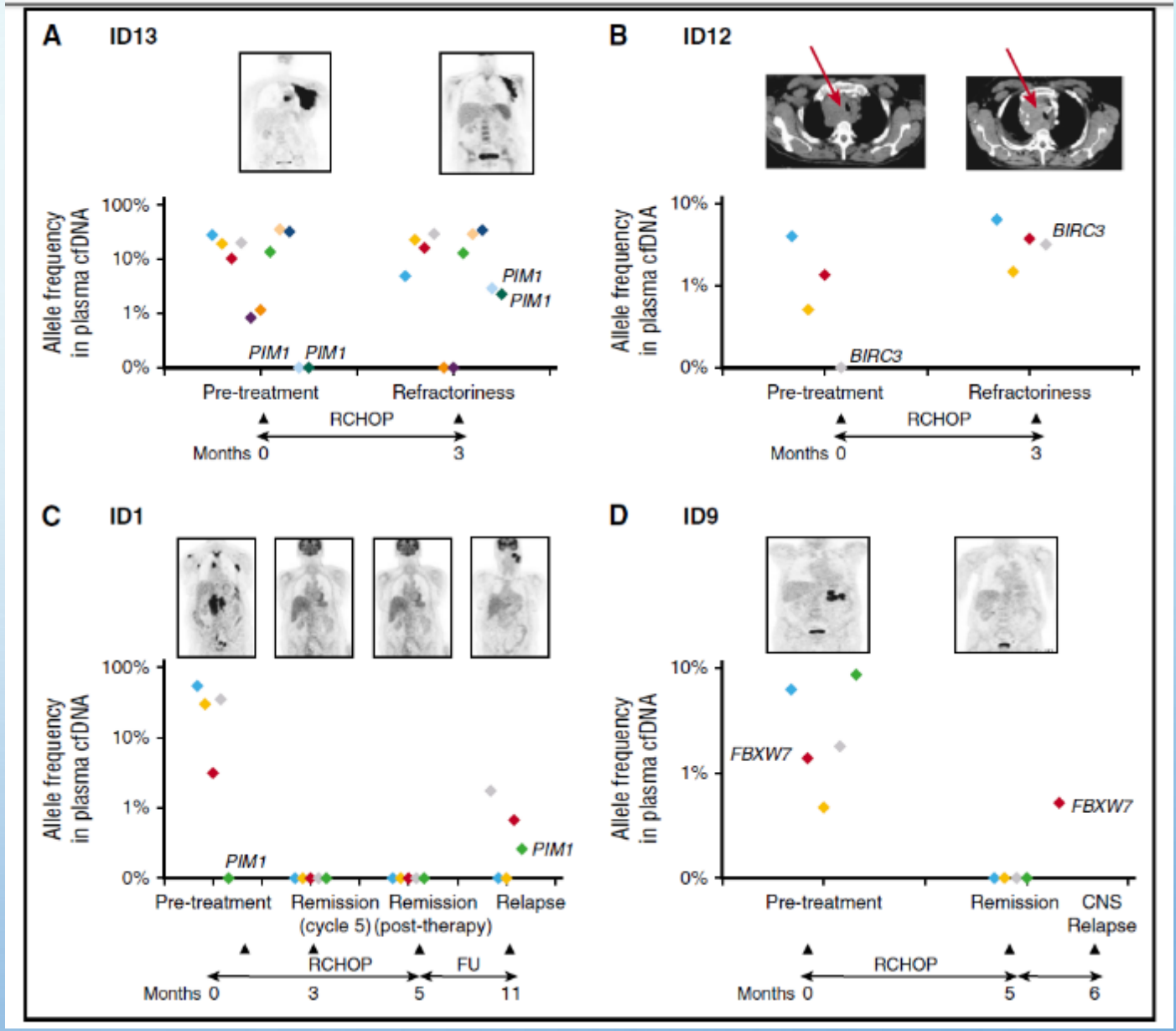


GOOD RESPONDERS

REFRACTORY PATIENTS

Rossi D., et al, Blood 2017

THE LIQUID BIOPSY IN LYMPHOMAS



CLONAL EVOLUTION MONITORING

Rossi D., et al, Blood 2017

LIQUID BIOPSY FOR LYMPHOMAS ANALYSIS IS THE PRESENT

Leukemia www.nature.com/leu

ARTICLE

Check for updates

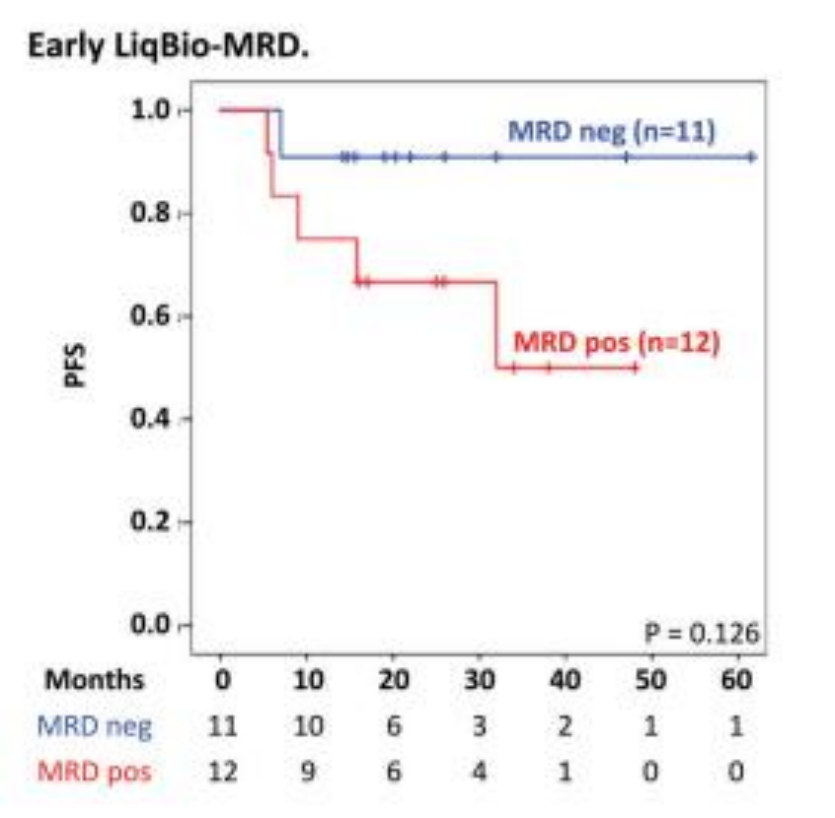
LYMPHOMA

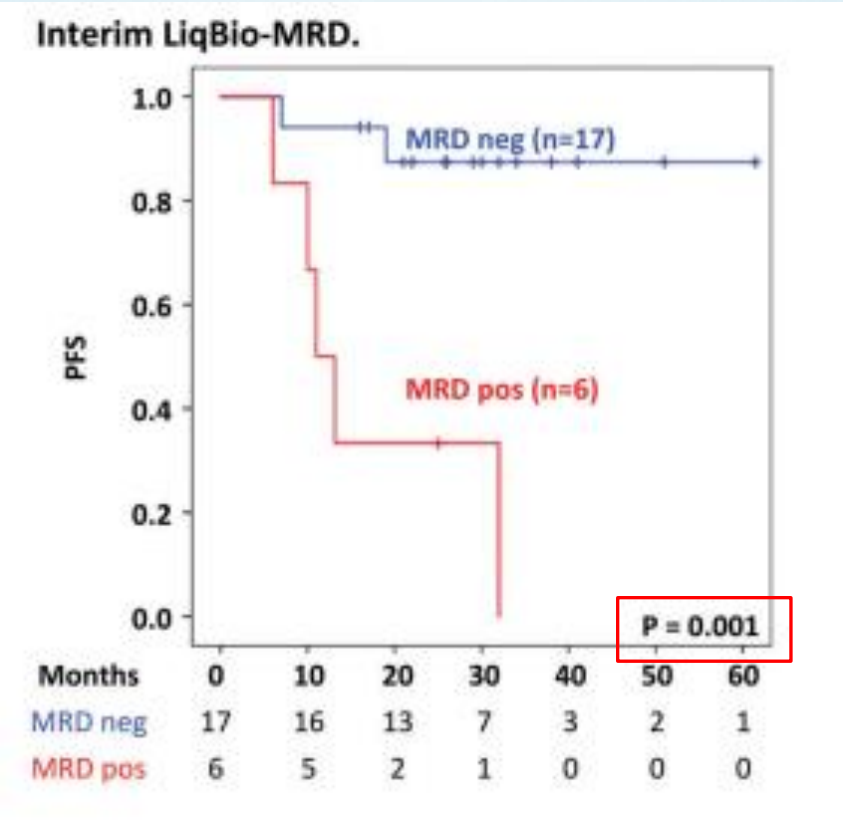
Real-life disease monitoring in follicular lymphoma patients using liquid biopsy ultra-deep sequencing and PET/CT

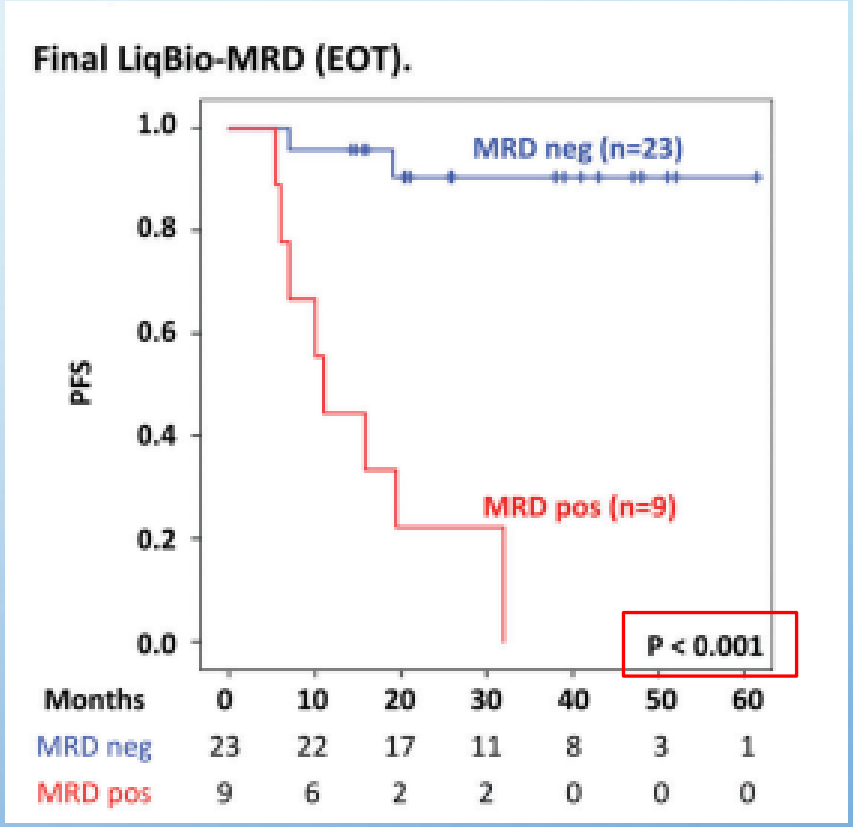
Ana Jiménez-Ubieto[1,10]✉, María Poza[1,10], Alejandro Martin-Muñoz[2], Yanira Ruiz-Heredia[1,2], Sara Dorado[2,3], Gloria Figaredo[4], Juan Manuel Rosa-Rosa[1], Antonia Rodriguez[1], Carmen Barcena[5], Laura Parrilla Navamuel[4], Jaime Carrillo[2], Ricardo Sanchez[1,2], Laura Rufian[1,2], Alexandra Juárez[1,2], Margarita Rodriguez[1,2], Chongwu Wang[6], Paula de Toledo[3], Carlos Grande[7], Manuela Mollejo[4], Luis-Felipe Casado[4], María Calbacho[1], Tycho Baumann[1], Inmaculada Rapado[1], Miguel Gallardo[1,8], Pilar Sarandeses[9], Rosa Ayala[1], Joaquín Martínez-López[1,10] and Santiago Barrio[1,2,10]✉

- 84 FOLLICULAR LYMPHOMA PTS SCREENED BY NGS FOR MRD-SUITABLE MUTATIONS
  - TRACKABLE MUTATIONS IN 95% OF THE LYMPH NODE SAMPLES AND 80% OF THE LIQUID BIOPSY

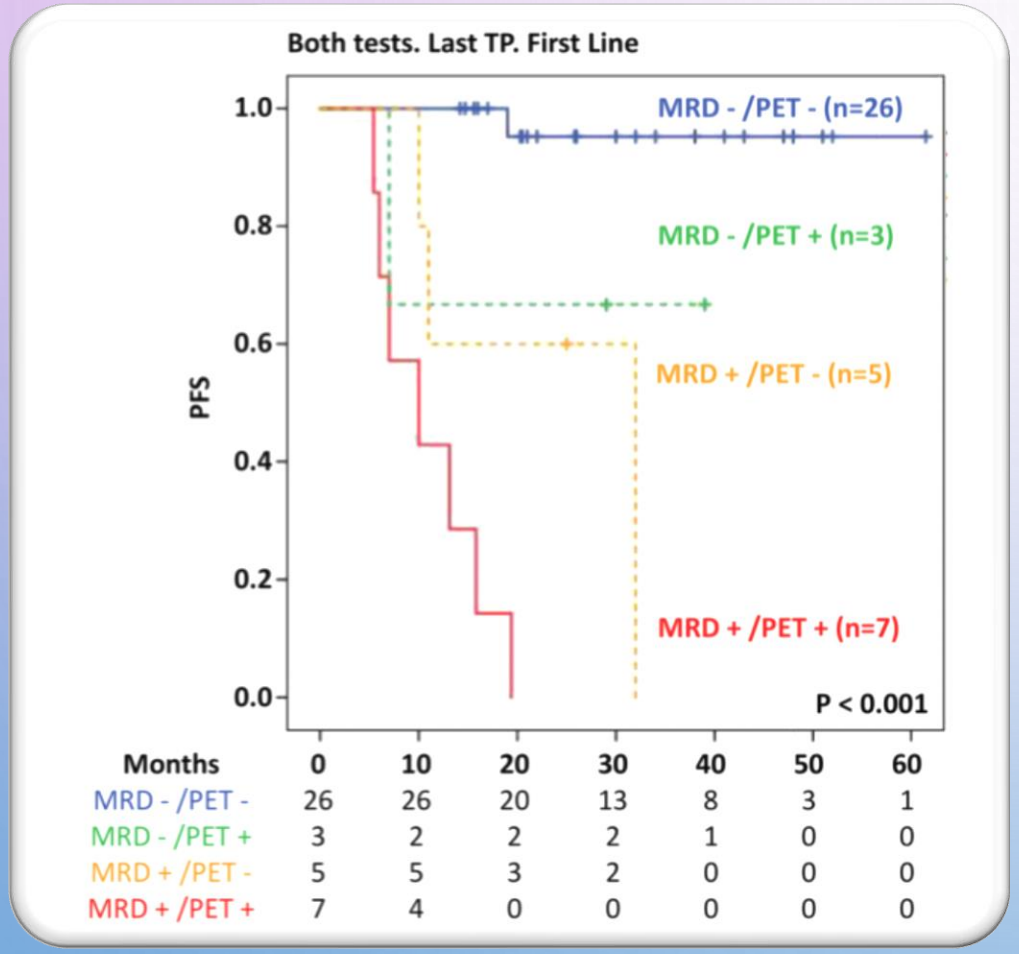
LIQUID BIOPSY by LiqBio-MRD on cfDNA





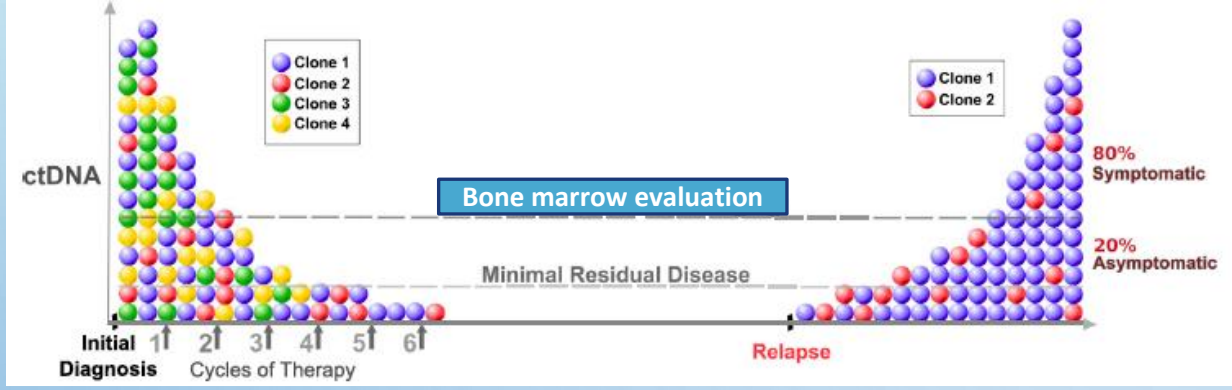


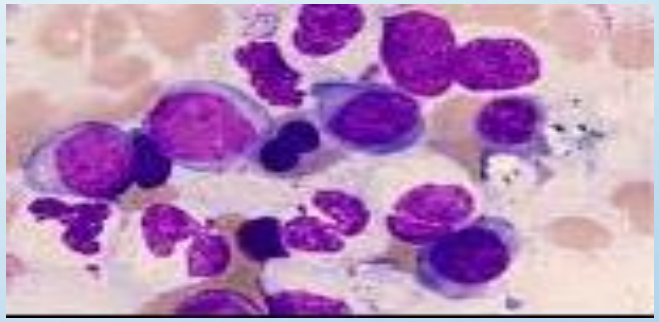
MRD IN FOLLICULAR LYMPHOMA: NEW AND OLD APPROACHES IN SINERGY



- 54 FOLLICULAR LYMPHOMA PTS FOLLOWED-UP BY LIQBIO-MRD: 151 SAMPLES
  - POSITIVE LIQBIO-MRD AT FIRST-LINE THERAPY CORRELATED WITH A HIGHER RISK OF PROGRESSION BOTH AT THE INTERIM EVALUATION ($P = 0.005$) AND AT THE END OF TREATMENT ($P < 0.001$).
  - BY PET/CT DEAUVILLE SCORE, WITH A MEDIAN PFS OF 19 MONTHS VS. NR ($P < 0.001$) AT THE INTERIM AND 13 MONTHS VS. NR ($P < 0.001$) AT EOT.
  - LIQBIO-MRD AND PET/CT COMBINED IDENTIFIED THE PATIENTS THAT PROGRESSED IN LESS THAN TWO YEARS WITH 88% SENSITIVITY AND 100% SPECIFICITY.

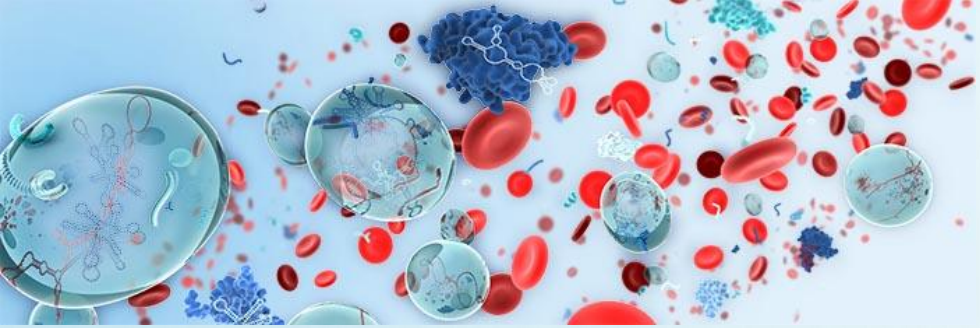
LIQUID APPROACH FOR LIQUID DISEASES???



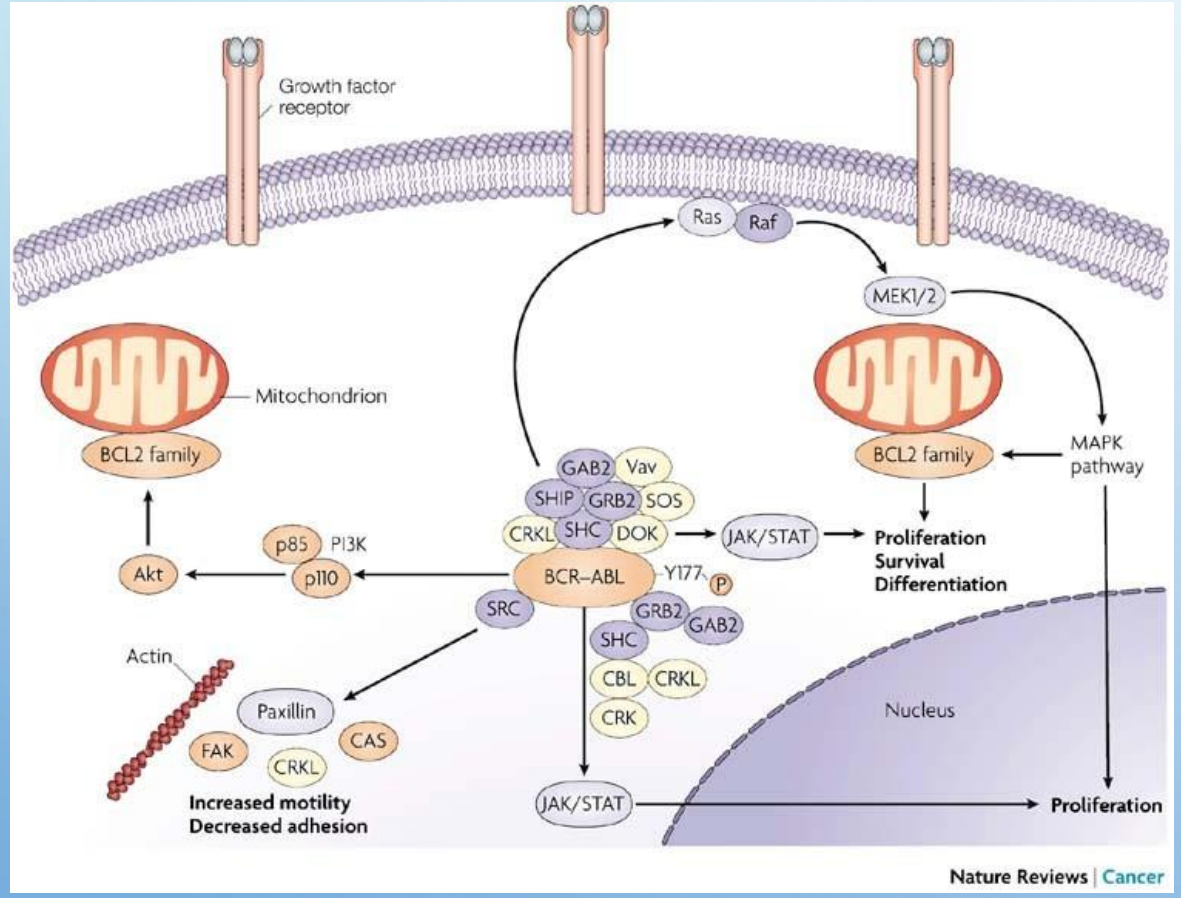


Myelodysplastic syndromes

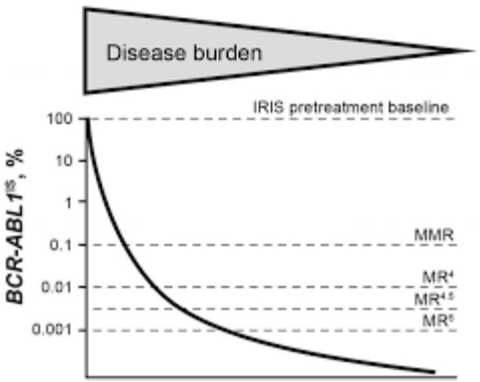
Punctio sicca



EXOSOMES: the experience in Chronic Myeloid Leukemia



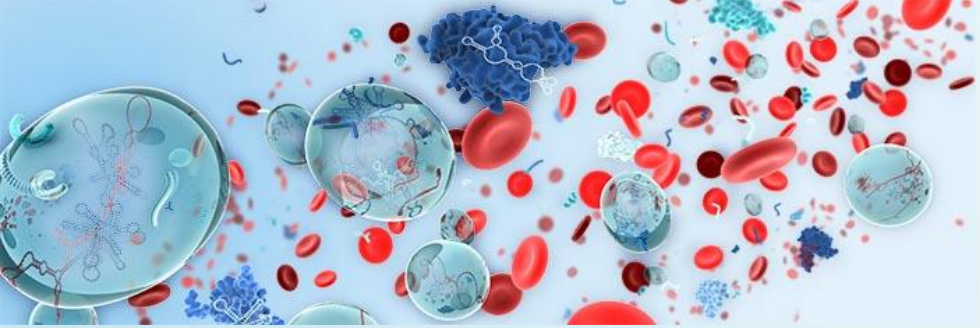
Minimal Residual Disease monitored by RT-qPCR quantifing BCR::ABL1 transcript.



Treatment time with selective TKI

Needs:

- **More sensitivity**
- **Detection of active leukemic cells resident in BM**



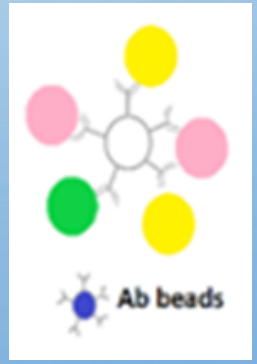
EXOSOMES: the experience in Chronic Myeloid Leukemia

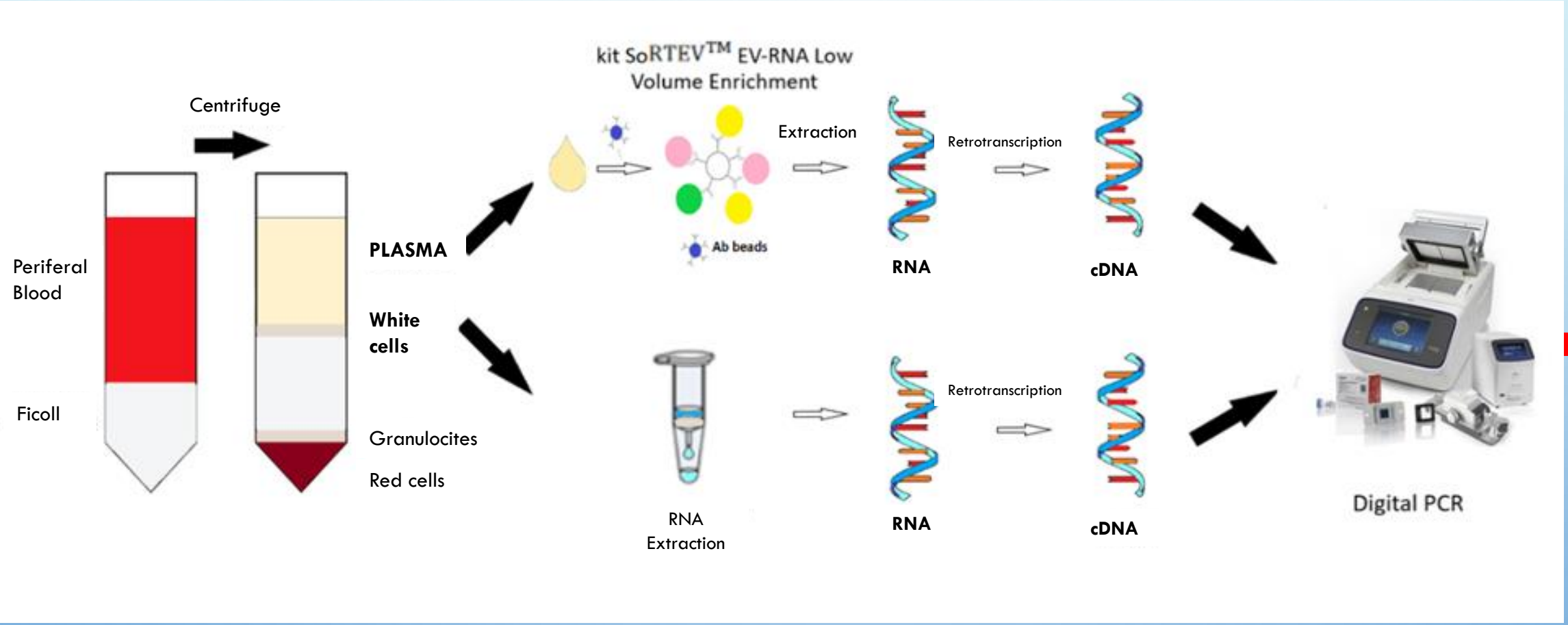
Taking advantage from two innovative approaches

Digital PCR

Are we able to detect «something» more and not circulating cells?

Tumor-exosomes enrichment




kit SoRTEV™ EV-RNA Low Volume Enrichment
Centrifuge
Periferal Blood
Ficoll
PLASMA
White cells
Granulocites
Red cells
Ab beads
Extraction
RNA
Retrotranscription
cDNA
RNA Extraction
RNA
Retrotranscription
cDNA
Digital PCR

BCR::ABL1

FEASABILITY: PATIENTS AND CONTROLS

- 10 CHRONIC MYELOID LEUKEMIA PATIENTS
- 1 CHRONIC MYELOID LEUKEMIA PATIENT MONITORED FROM DIAGNOSIS
- 10 HEALTHY CONTROLS
- 4 PATHOLOGIC CONTROLS

	Ph⁻ 1	Ph⁻ 2	Ph⁻ 3	Ph⁻ 4
Sex	M	F	F	M
Diagnosis	Acute lymphoblastic leukemia B	Acute myeloid leukemia	Myelofibrosis	Multiple myeloma
Age (years)	38	67	64	49
Disease phase	Diagnosis	CR after consolidation therapy	CR at 3 months post allo-HSCT	Relapse

Ph⁻, Philadelphia-negative; CR, complete remission; HSCT, hematopoietic stem cell transplantation.

Results on tumor derived exosomes

	RT-qPCR *BCR-ABL1/ ABL1*		dPCR PB cells *BCR-ABL1*		dPCR EXO *BCR-ABL1*			dPCR EXO *Y4*		
Case no.	MR	IS	DOTS	Copies/μl of reaction	DOTS	Copies/μl of reaction	Copies/ml plasma	DOTS	Copies/μl of reaction	Copies/ml plasma
1	4.5	0.0013	2	0.161	3	0.256	6.67	294	28.239	1,058.9
2	4.5	0	1	0.0857	1	0.091	4.74	100	10.579	661.18
3	4.5	0.0031	3	0.226	2	0.158	8.23	251	22.711	1,419.44
4	5.0	0	5	0.439	4	0.324	20.25	2	0.299	37.37
5	5.0	0	0	0	2	0.226	14.12	11	0.848	35.3
6	5.0	0	2	0.188	3	0.219	13.69	6	0.432	54.0
7	5.0	0	1	0.0773	1	0.072	4.5	30	2.39	298.75
8	5.0	0	1	0.0781	5	0.407	12.72	11	0.915	57.19
9	5.0	0.0008	1	0.0853	4	0.329	10.28	8	0.613	38.31
10	4.0	0.0053	5	0.441	5	0.378	14.76	76	5.945	928.9

Improvement of sensitivity and detection

Bernardi S et al, IntJMolMed 2019

Results on tumor derived exosomes

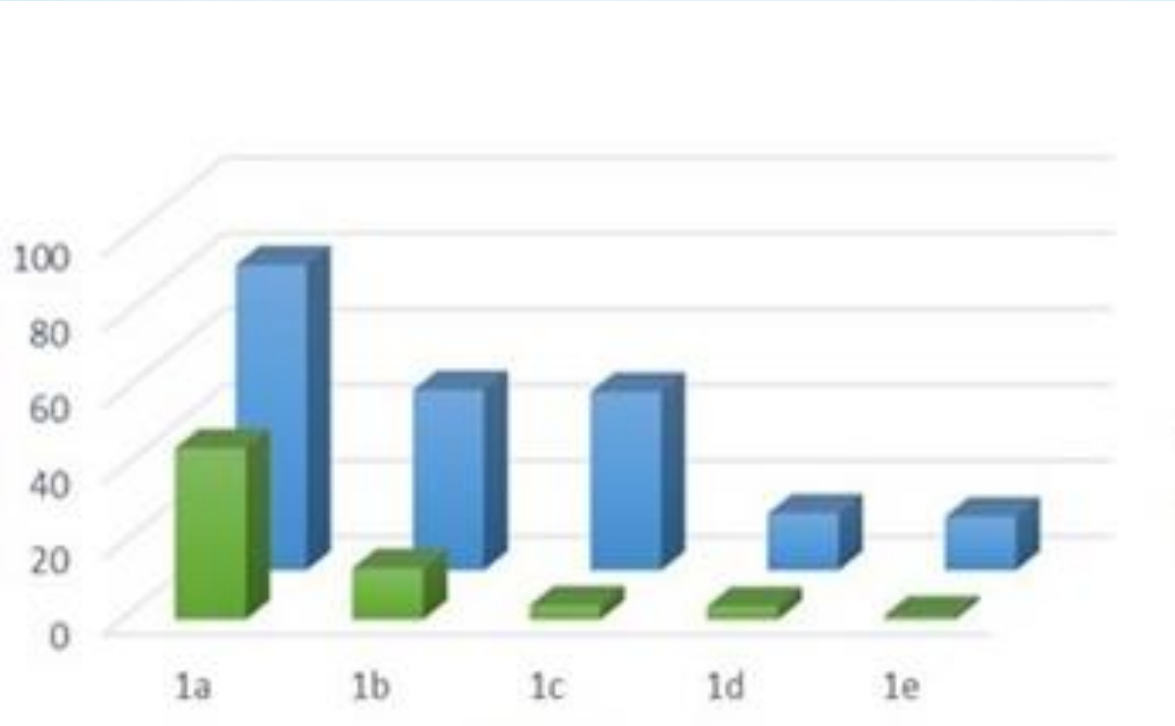
	RT-qPCR *BCR-ABL1/ ABL1*		dPCR PB cells *BCR-ABL1*		dPCR EXO *BCR-ABL1*			dPCR EXO *Y4*		
Case no.	MR	IS	DOTS	Copies/μl of reaction	DOTS	Copies/μl of reaction	Copies/ml plasma	DOTS	Copies/μl of reaction	Copies/ml plasma
1a	0.0	19.528	49	3.014	8	0.645	80.625	97	9.146	1,143.25
1b	2.0	0.1021	11	0.905	5	0.381	47.625	58	4.973	621.625
1c	3.0	0.0112	3	0.250	5	0.377	47.125	18	1.288	161
1d	3.0	0.0707	3	0.234	3	0.241	15.0625	24	1.734	108.375
1e	4.0	0.0054	1	0.0725	3	0.228	14.25	11	0.909	56.813

- **Decrease of BCR::ABL1 transcript in every evaluated matrix**
- **Linear correlation in case of high leukemic burden**

Bernardi S et al, IntJMolMed 2019

Results on tumor derived exosomes monitoring

Exosomal BCR::ABL1 transcript

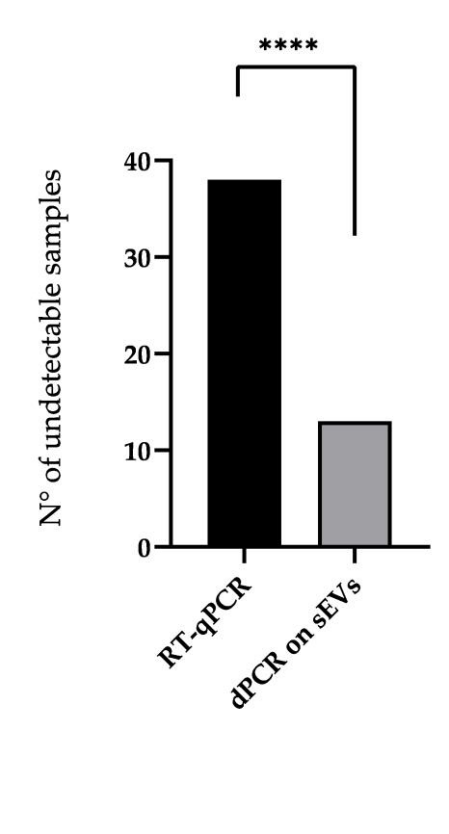


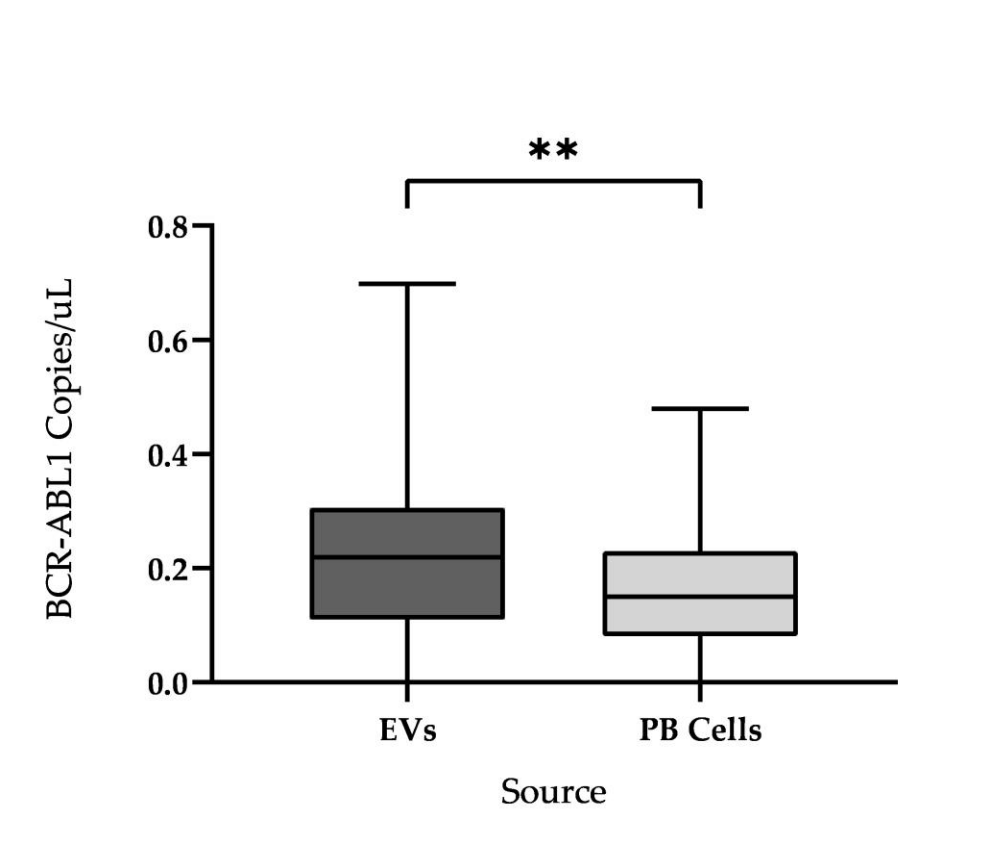
Absolute number of exosomal BCR-ABL1 transcript

Cellular BCR-ABL1 transcript normalized by ABL

VALIDATION: PATIENTS AND CONTROLS

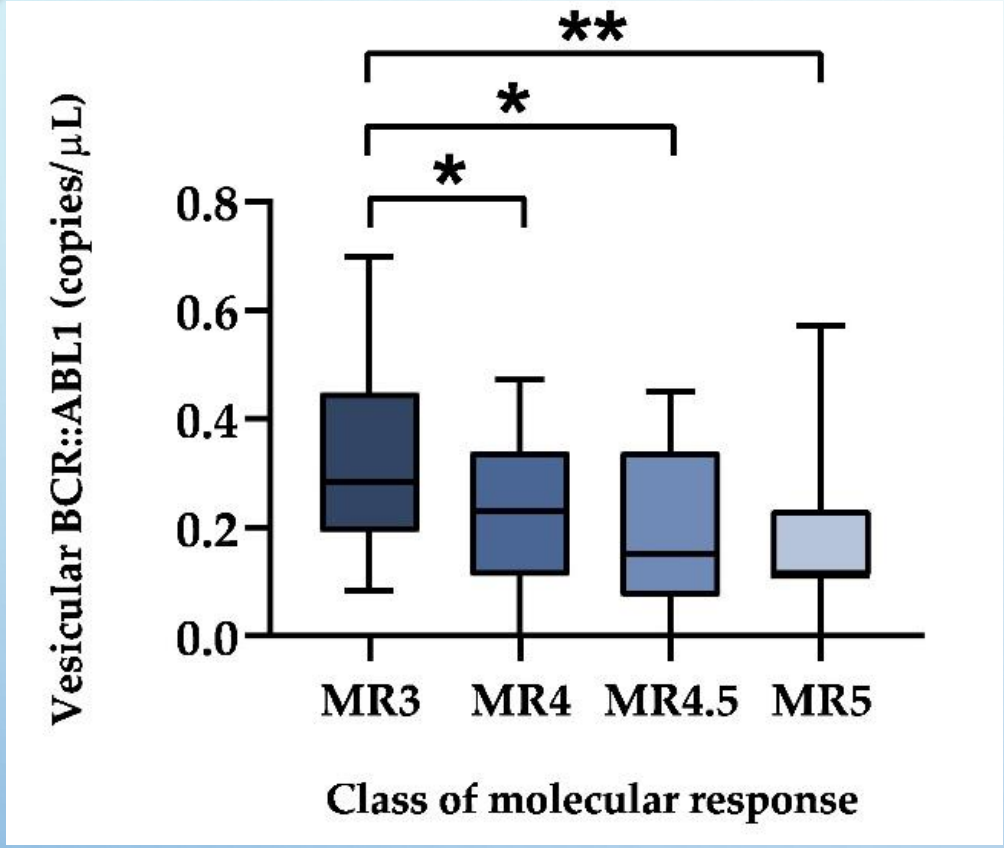
Variable	Total number of samples= 104
Sex (F-M)	42-62 (40%-60%)
Age	64.5 (34 – 90)
Ongoing Therapy	
Imatinib	21 (20%)
Nilotinib	14 (13.5%)
Dasatinib	6 (12%)
Bosutinib	1 (1%)
Intermittent	27 (26%)
TFR	24 (23%)
Class of MR	
MR^{3}	18 (17%)
MR^{4}	27 (26%)
MR^{4} undetectable	0 (0%)
$MR^{4.5}$	23 (22%)
$MR^{4.5}$ undetectable	6 (26% of total $MR^{4.5}$)
$MR^{5.0}$	36 (35%)
$MR^{5.0}$ undetectable	32 (89%of total $MR^{5.0}$)

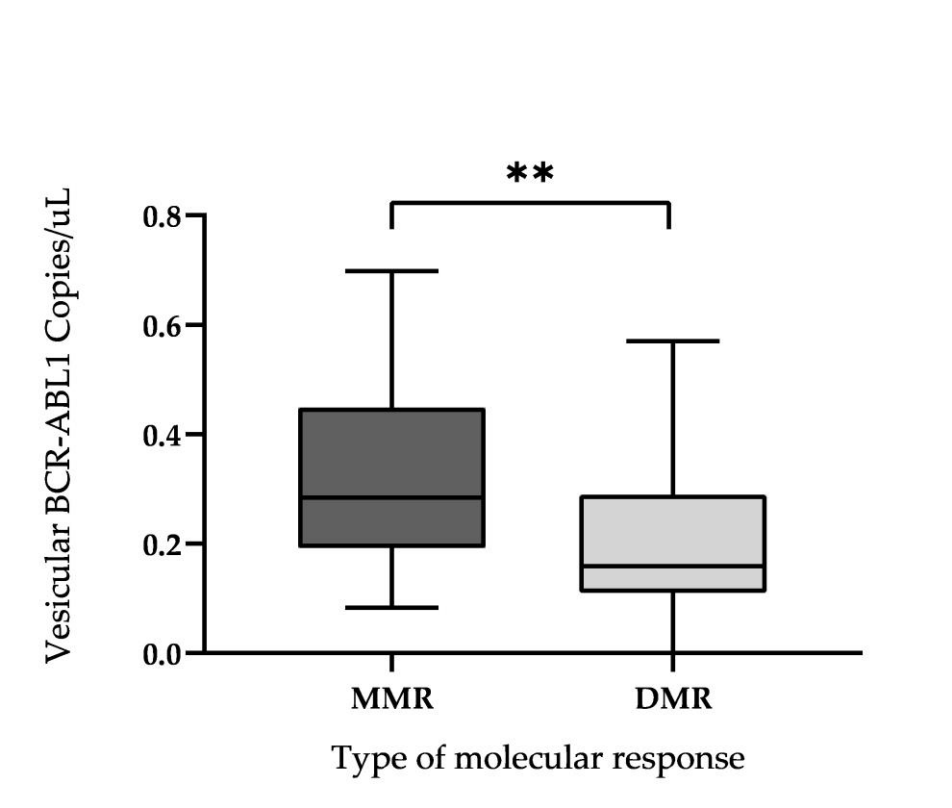




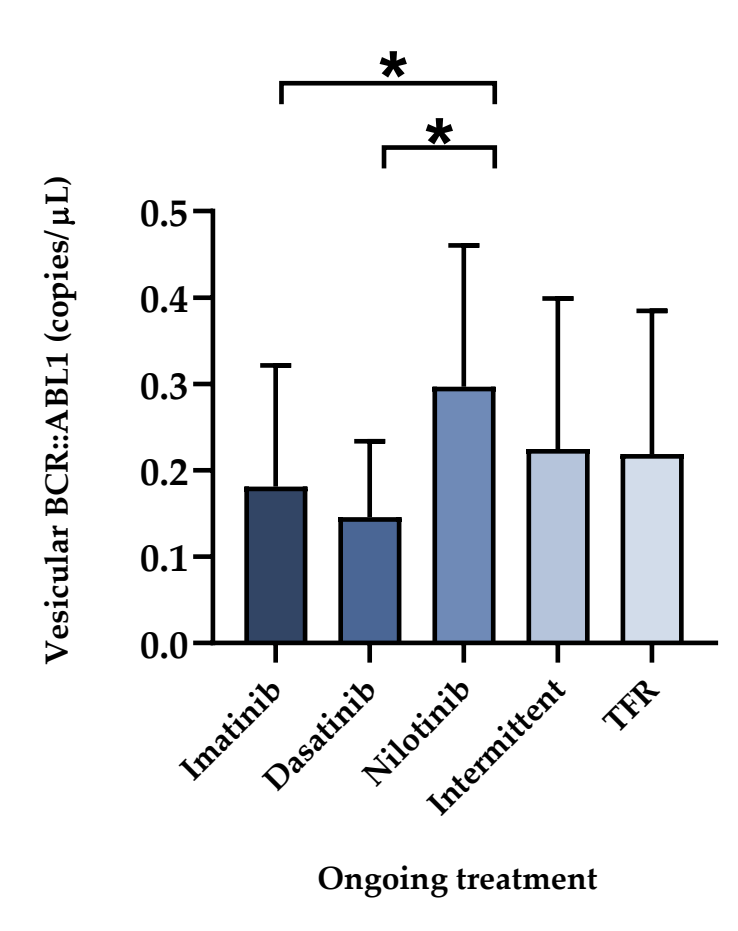
dPCR IS MORE SENSITIVE THAN RT-qPCR

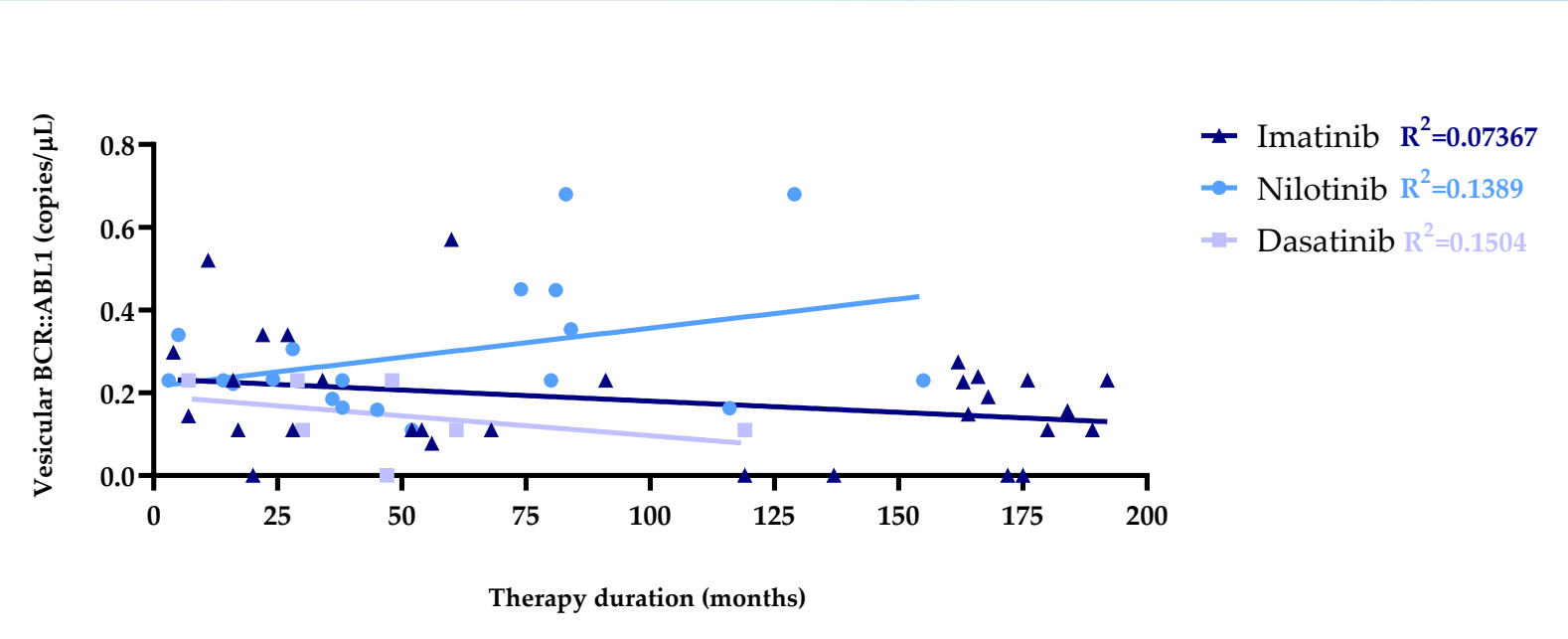
VESICULAR BCR::ABL1 QUANTIFICATION IN DIFFERENT MR CLASSES

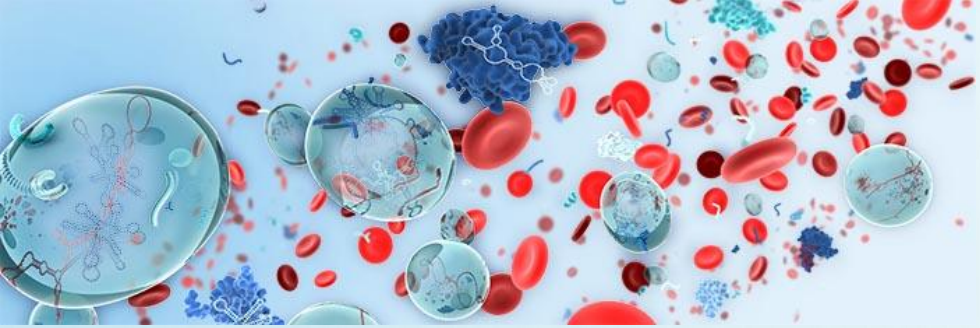




VESICULAR BCR::ABL1 QUANTIFICATION IS INFLUENCED BY the ONGOING THERAPY





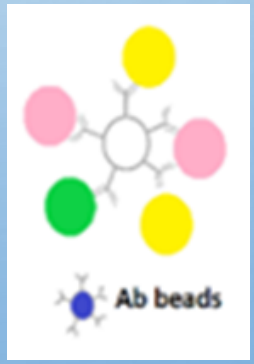


EXOSOMES: the experience in Acute Myeloid Leukemia

Taking advantage from two innovative approaches

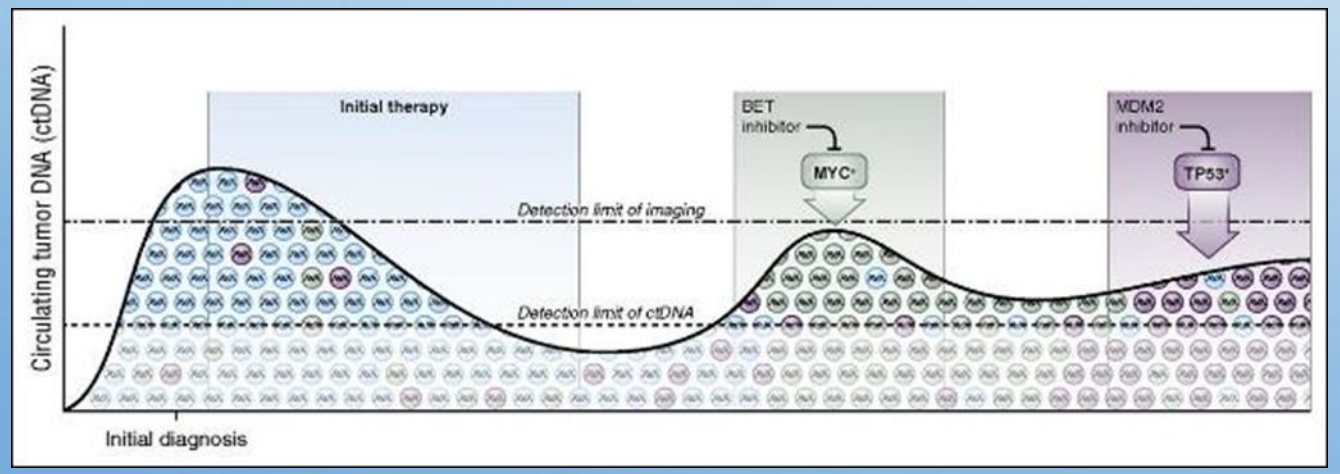
NGS

Tumor-exosomes enrichment



FEASABILITY: PATIENTS AND CONTROLS

- 14 ACUTE MYELOID LEUKEMIA PATIENTS
- 8 ACUTE MYELOID LEUKEMIA PATIENTS MONITORED AT DIFFERENT DISEASE PHASES
- 3 HEALTHY CONTROLS



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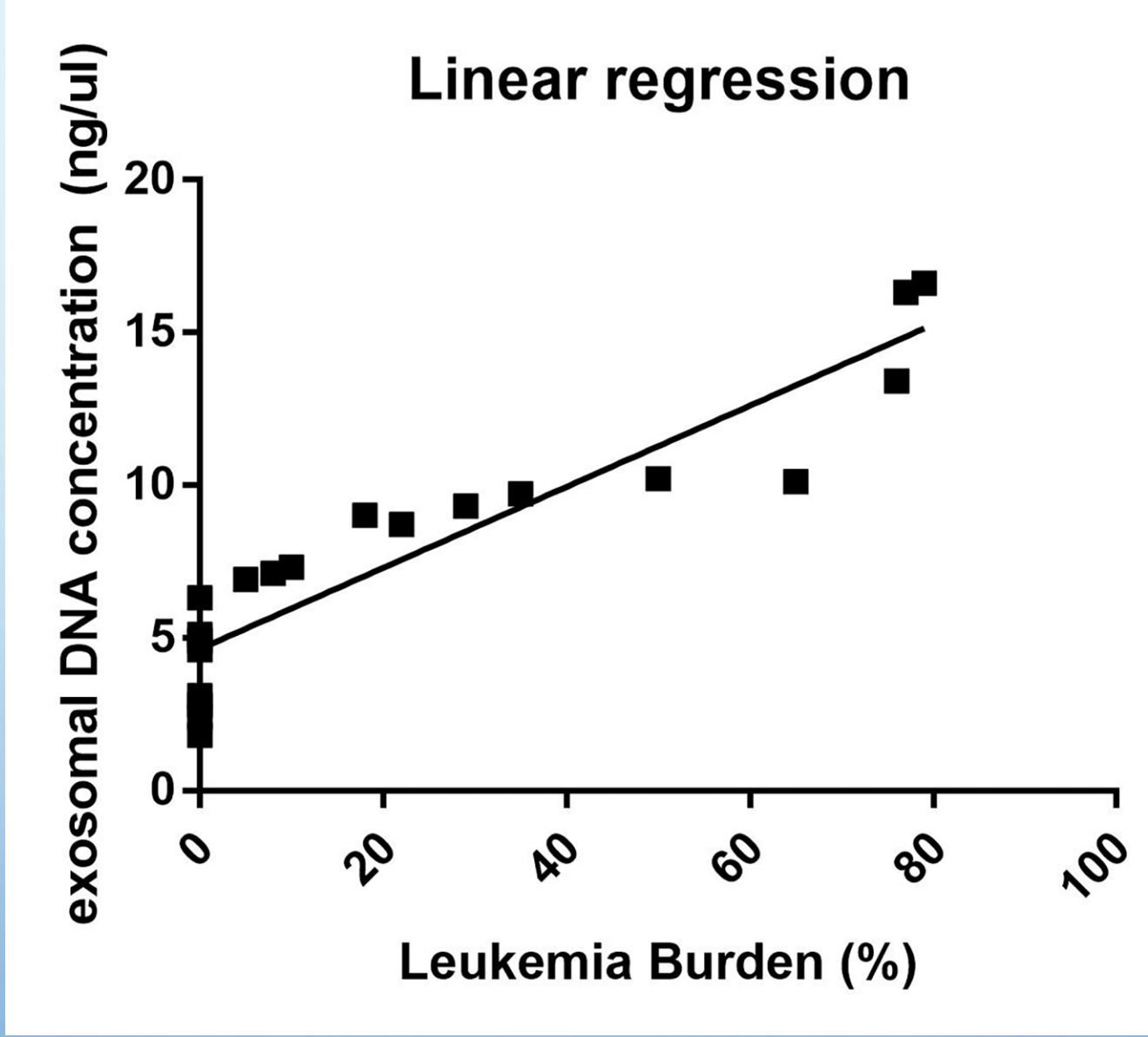

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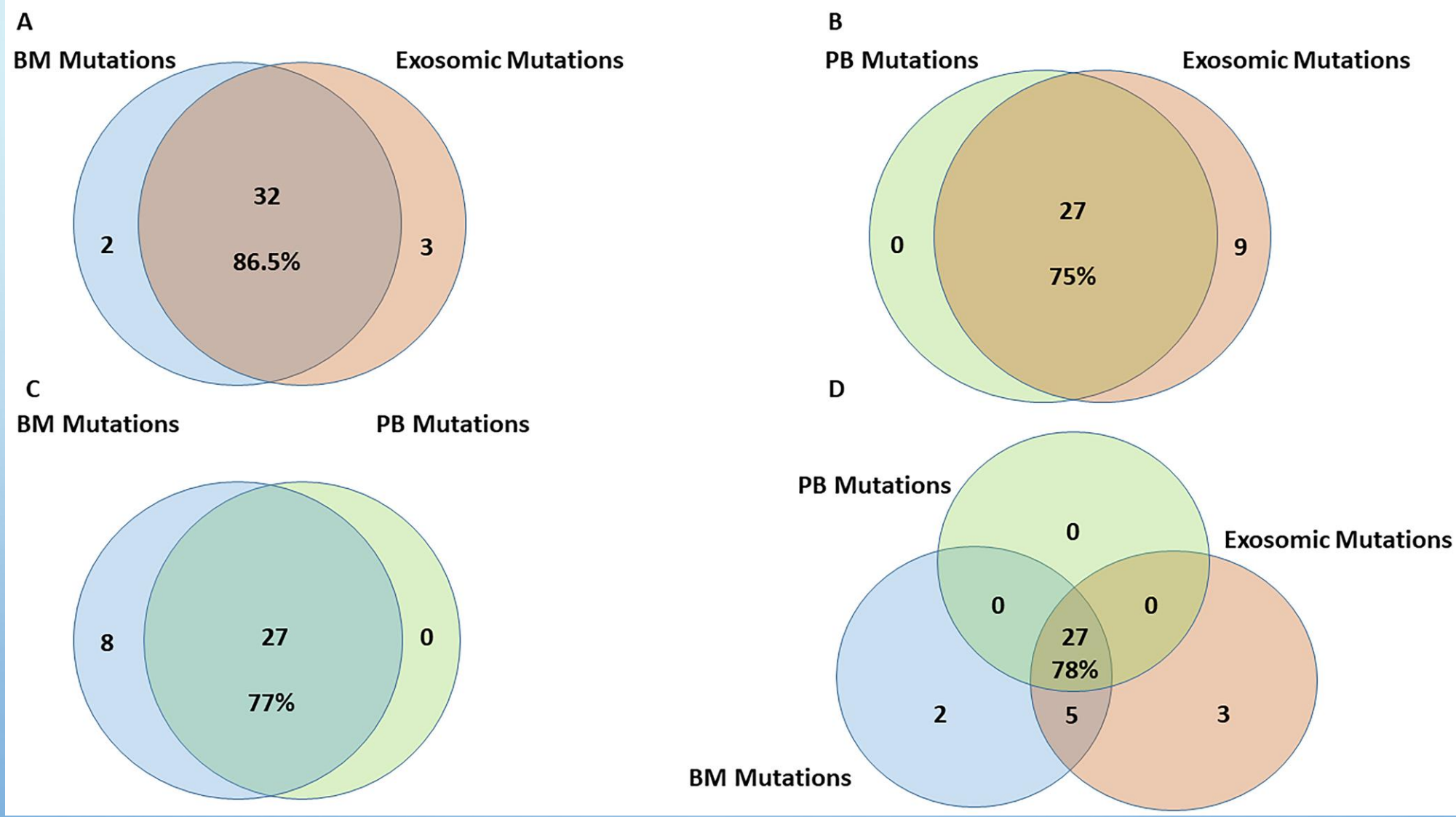

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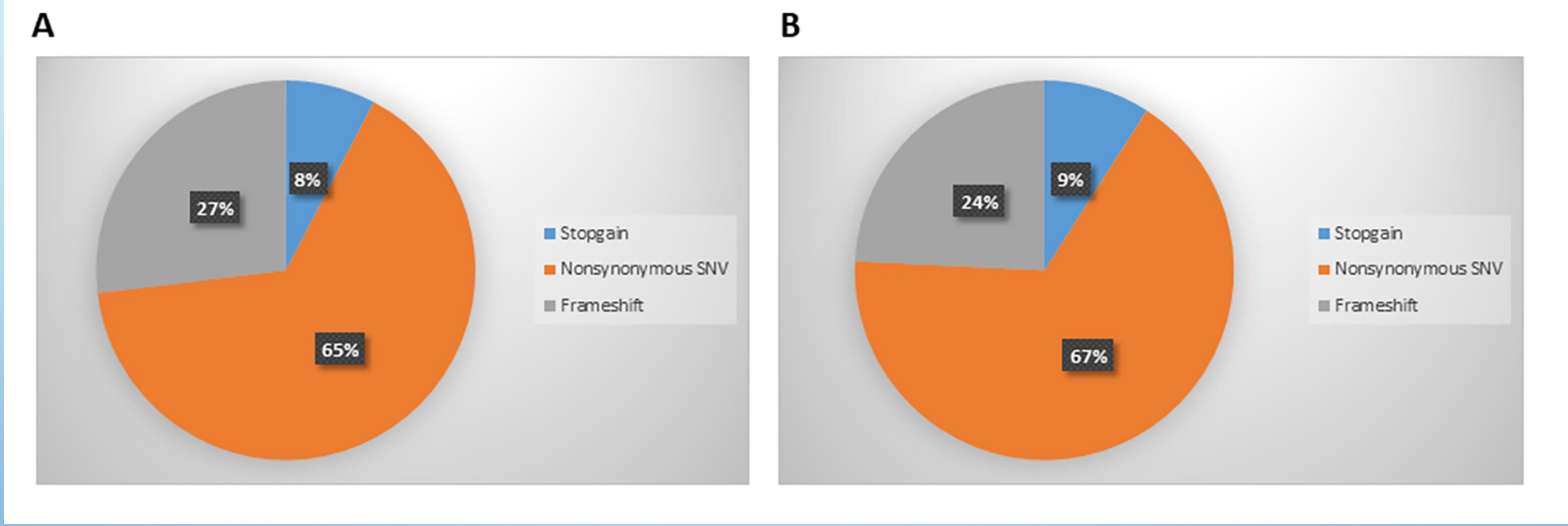
EVERY TYPES OF MUTATION ARE SHUTTLED



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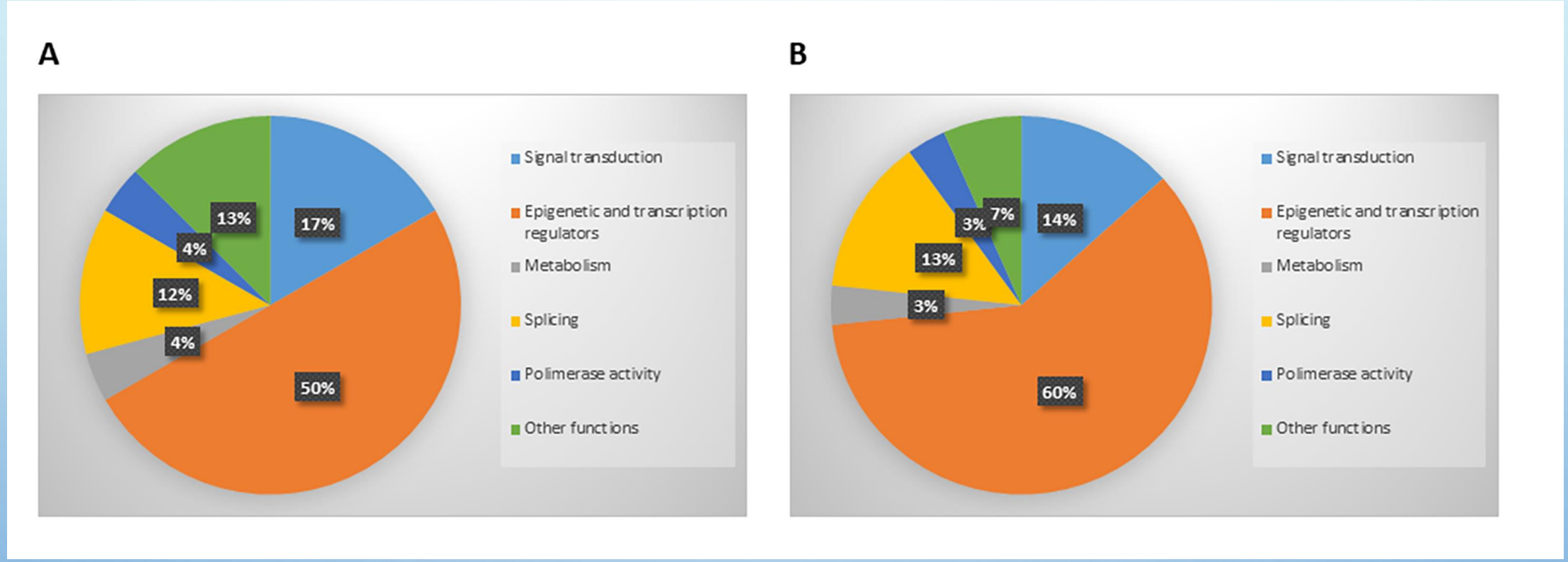
EVERY TYPES OF GENE ARE SHUTTLED



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LIQUID BIOPSY IN HEMATOLOGY:

- IT IS FEASIBLE
- IT IS USEFUL
- IT IS DIFFERENT
- IT IS INNOVATIVE
- IT IS RELIABLE