XXII ASSEMBLEA MaNGO MILANO

STANDARD TREATMENTS AND NEW DIRECTIONS IN GYNAECOLOGICAL CANCERS

MILANO June 26th-29th, 2025

Responsabili Scientifici:
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MaNGO

Liquid biopsy in gynecological oncology

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Disclosure

I have no actual or potential conflict of interest in relation to this presentation

Liquid biopsy

Liquid biopsy is a biomarker analysis tool that uses the **body fluids** of patients: blood, urine, saliva, cerebrospinal fluid, pleural effusion, etc.

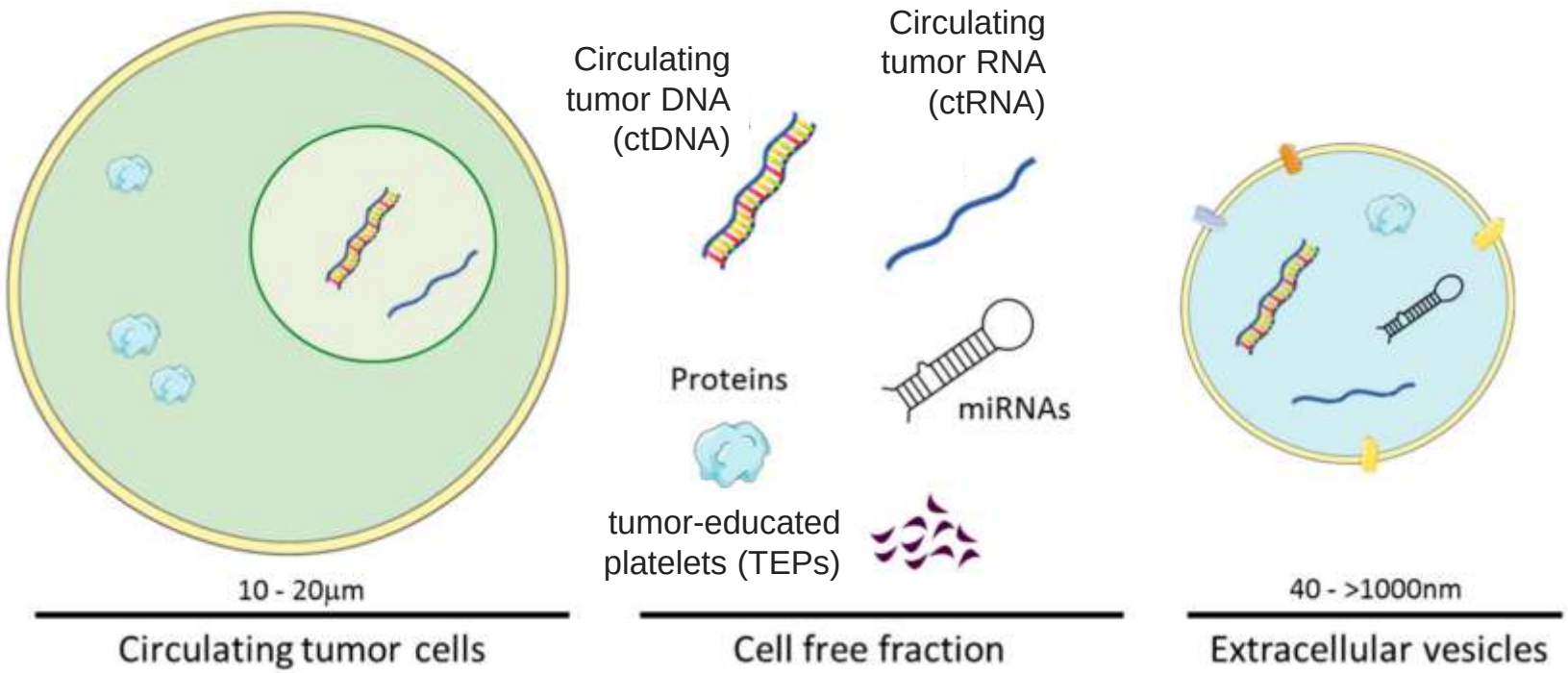
Liquid biopsy may serve as a **surrogate marker** for neoplastic tissue.

Liquid biopsy **can overcome the limitations of traditional tissue biopsy** by:

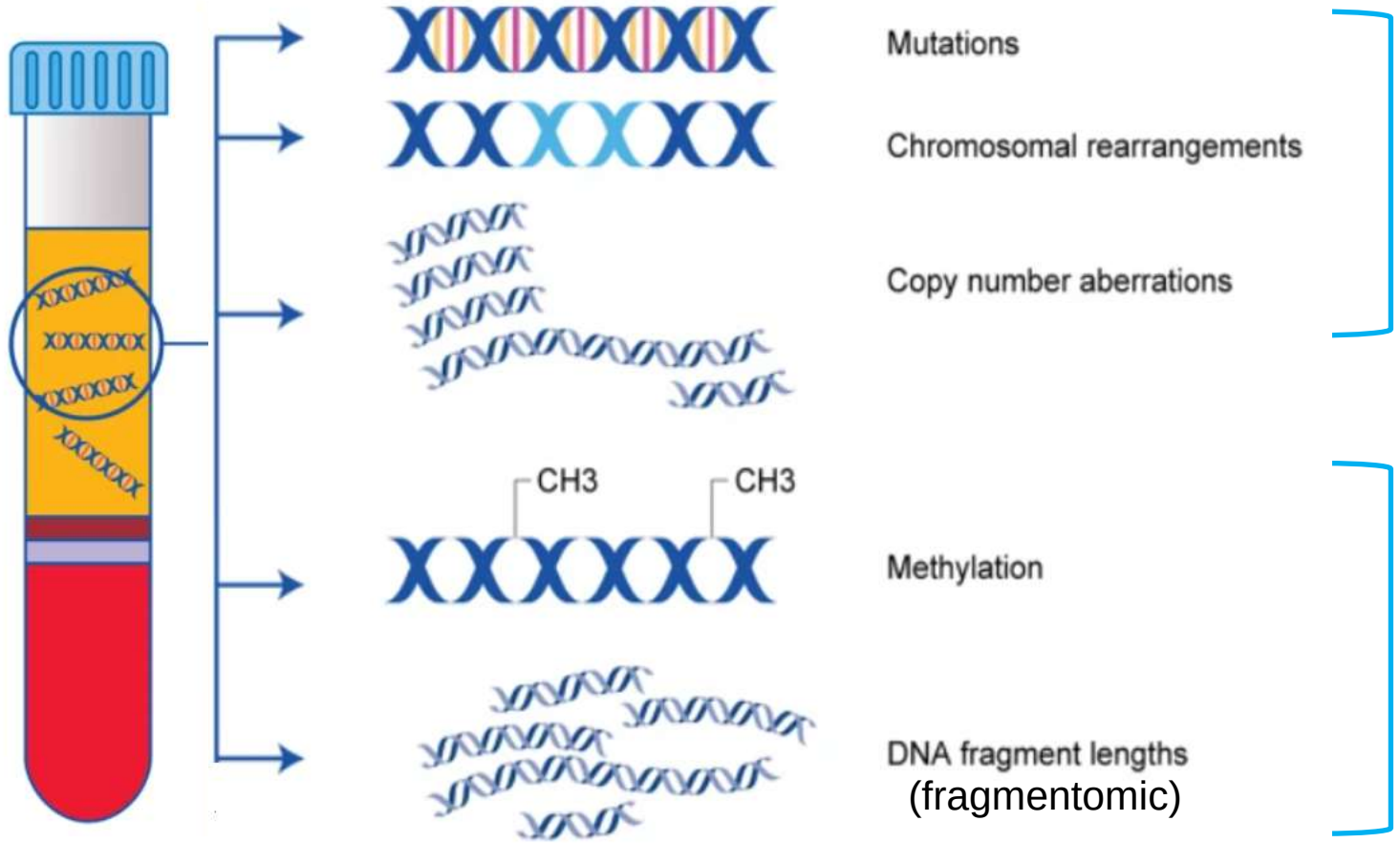
- offering a less invasive and more comfortable procedure;
- reducing risks for the patient;
- circumventing technical challenges related to tumor localization;
- enabling detection of tumor cells that have disseminated to organs and tissues inaccessible to tissue biopsy;
- allowing for serial testing (e.g., monitoring after primary tumor removal);
- lowering overall costs.

Test items in liquid biopsy

Molecules released from **primary tumor** and/or **metastases** into the peripheral blood



Biomarkers in ctDNA

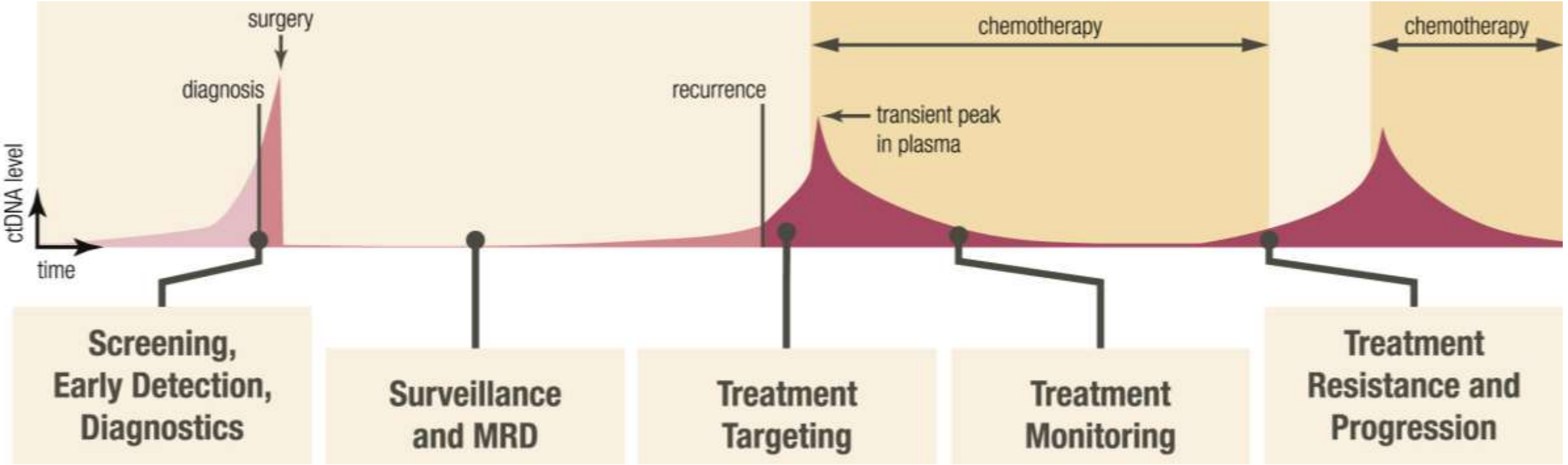


Somatic genomic aberrations

Epigenetic aberrations

Applications of liquid biopsy

Clinical applications in medical oncology of **circulating tumor DNA (ctDNA)**:



The case of alpelisib

The approval of alpelisib by the FDA incorporates a **companion diagnostic** for the selection of patients with breast cancer with ***PIK3CA* mutations:**

- in tumor tissue **AND/OR**
- in ctDNA.

In the SOLAR-1 clinical trial, concordance between PIK3CA mutational status in patient-matched tumor and blood reflected:

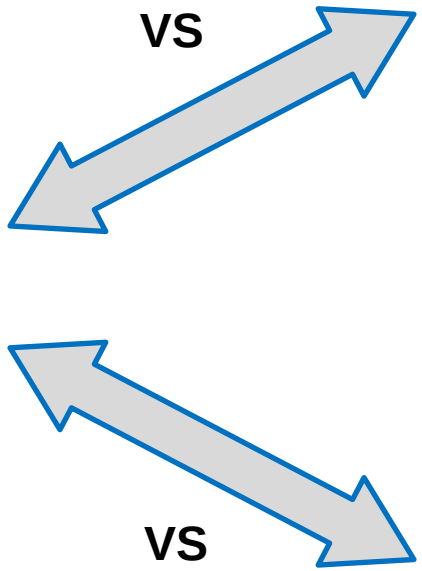
- excellent specificity (negative agreement = 97% (209 of 215),
- less sensitivity (positive agreement = 55% (179 of 328)

for the ctDNA test.

Improvements in PFS were numerically larger when PIK3CA mutations were detected in blood than when they were detected in tissue.

Advantages of liquid biopsy

Potential of **liquid biopsy**



vs **Tissue biopsy**

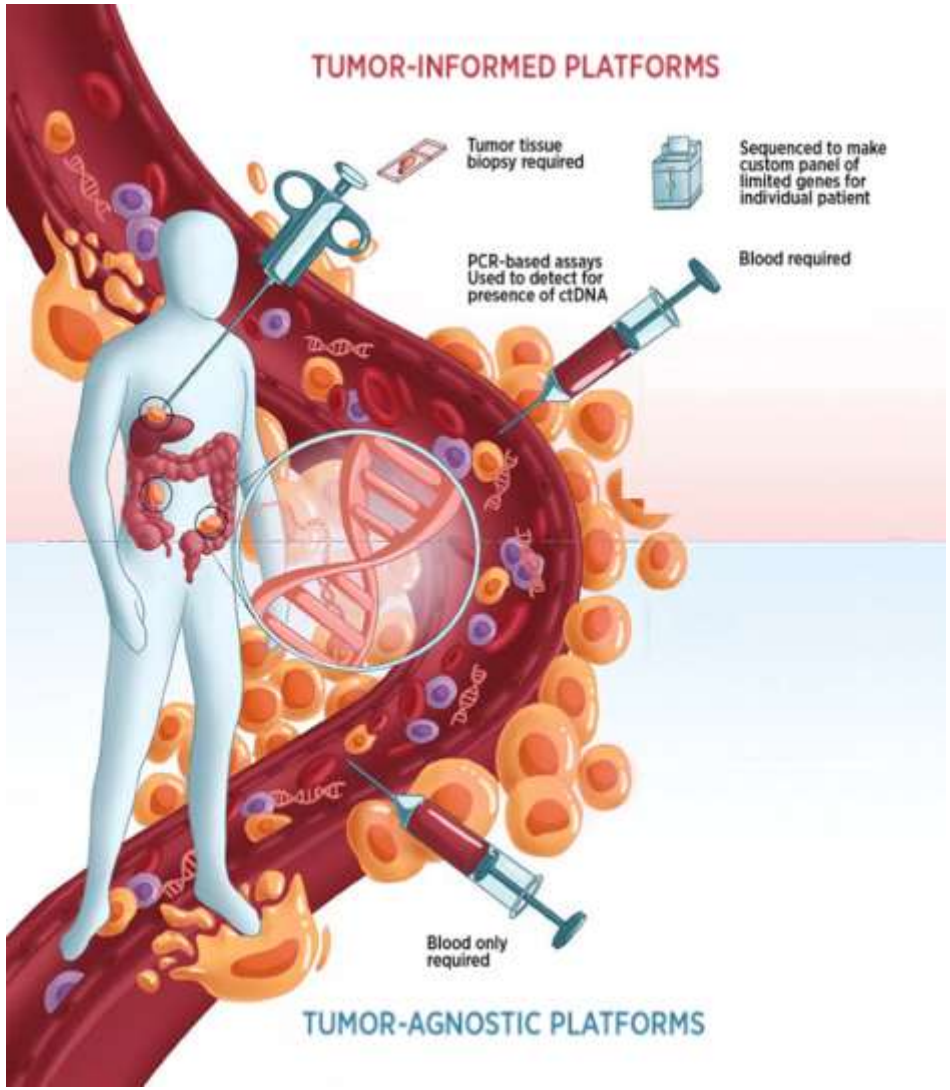
as a **minimally invasive alternative** to

- enable diagnosis
- inform risk stratification
- guide treatment options

vs **Radiographic imaging**

to **facilitate earlier detection** of disease progression.

Type of assay - 1



Tumor-informed assays

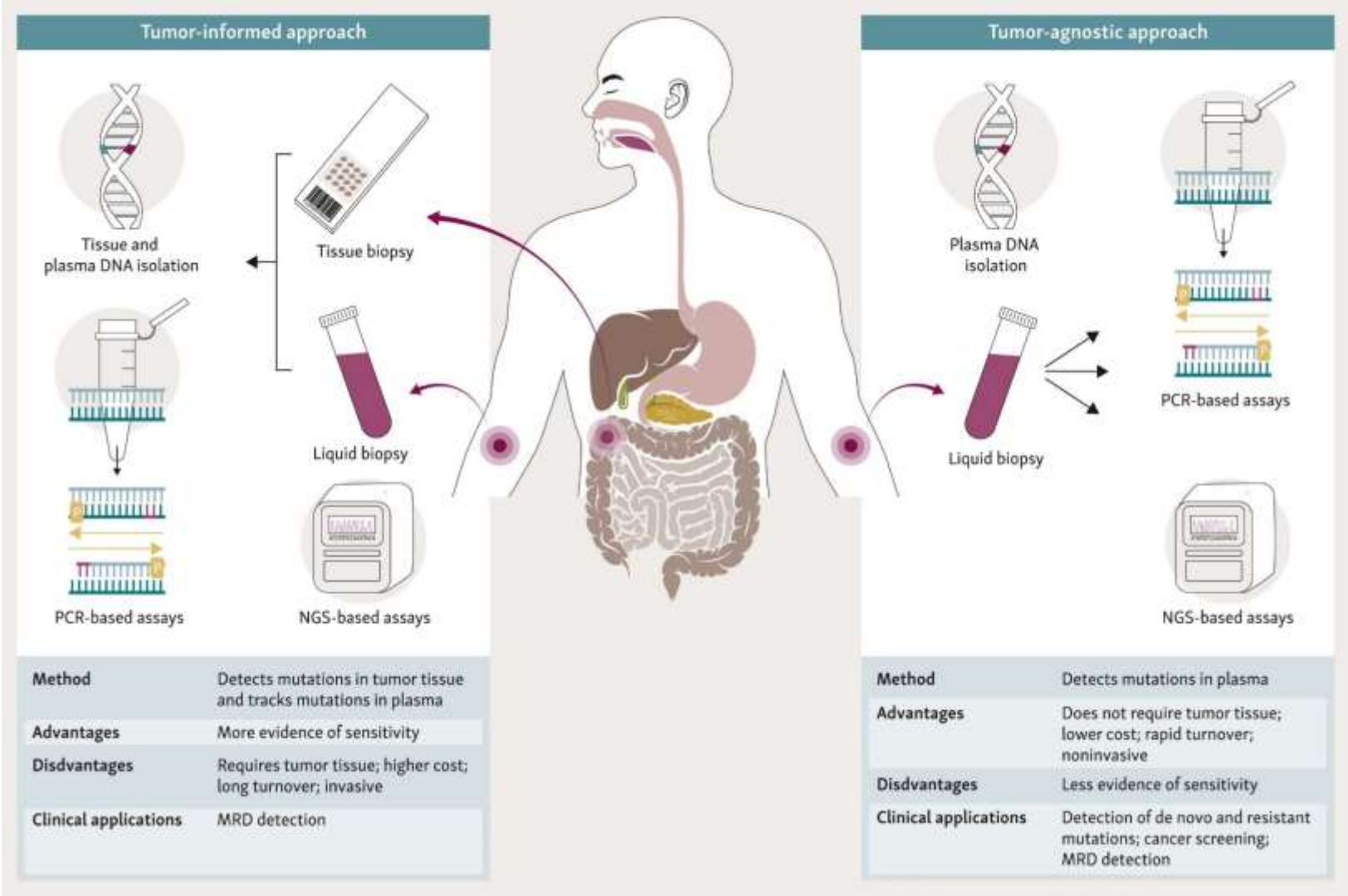
based on detecting **tumor-specific molecular alterations.**

- High **specificity**.
- Lower analytic sensitivity is needed when tumor load is high and DNA is released into circulation at an early stage.
- **needs to be tailored** for each patient according to their specific tumor mutation profile.

Tumor-agnostic assays

- allow for the detection of variants in multiple genes, regardless of the primary tumor pattern.
- can be applied to cancer patients across all stages and histotypes.

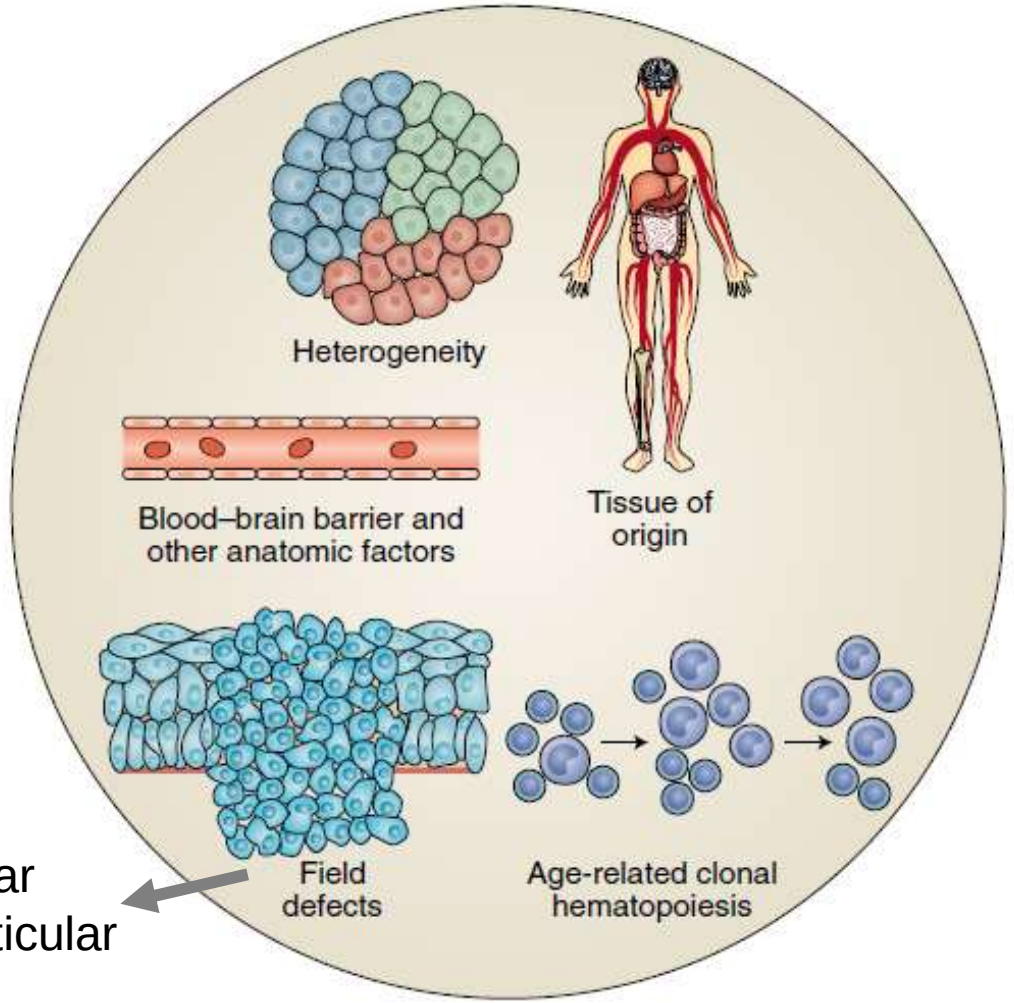
Type of assay - 2



Tumor-informed approach	
Method	Detects mutations in tumor tissue and tracks mutations in plasma
Advantages	More evidence of sensitivity
Disdvantages	Requires tumor tissue; higher cost; long turnover; invasive
Clinical applications	MRD detection

Tumor-agnostic approach	
Method	Detects mutations in plasma
Advantages	Does not require tumor tissue; lower cost; rapid turnover; noninvasive
Disdvantages	Less evidence of sensitivity
Clinical applications	Detection of de novo and resistant mutations; cancer screening; MRD detection

Limitations of liquid biopsy



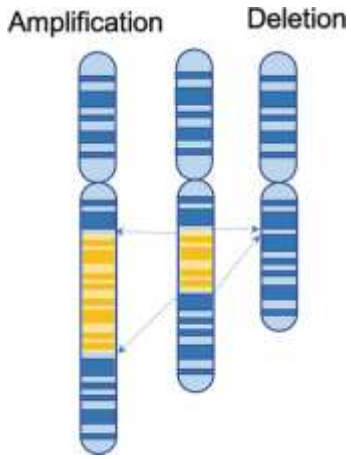
(i.e., pre-malignant molecular aberrations related to a particular etiological factor)

Our experience

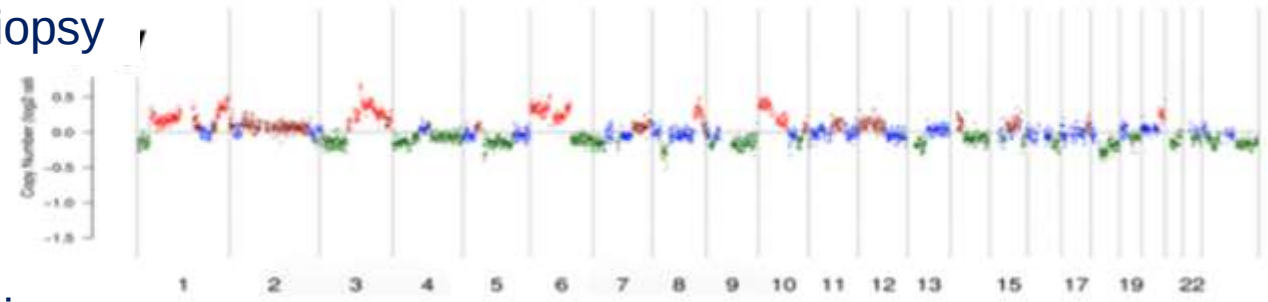
Title	**Genome-wide CNAs in ctDNA as a novel biomarker for patients with HGSOC**
Type of study	Retrospective
Aim	To identify biomarker suitable monitor response to therapy
Population	46 pts with HGSOC
Sampling	Plasma samples taken • before any treatment • at different time points during follow-up

CNAs in ctDNA as a biomarker to monitor disease - 1

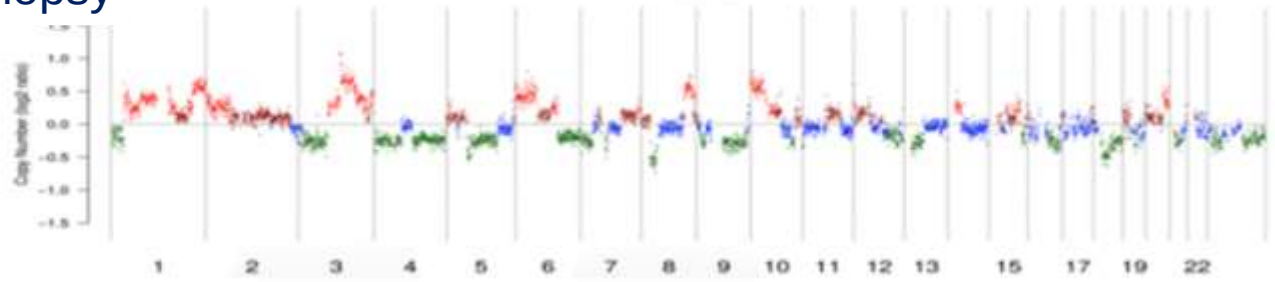
Copy Number Alterations (CNAs) is a **promising standalone biomarker** for liquid biopsy.



Liquid biopsy

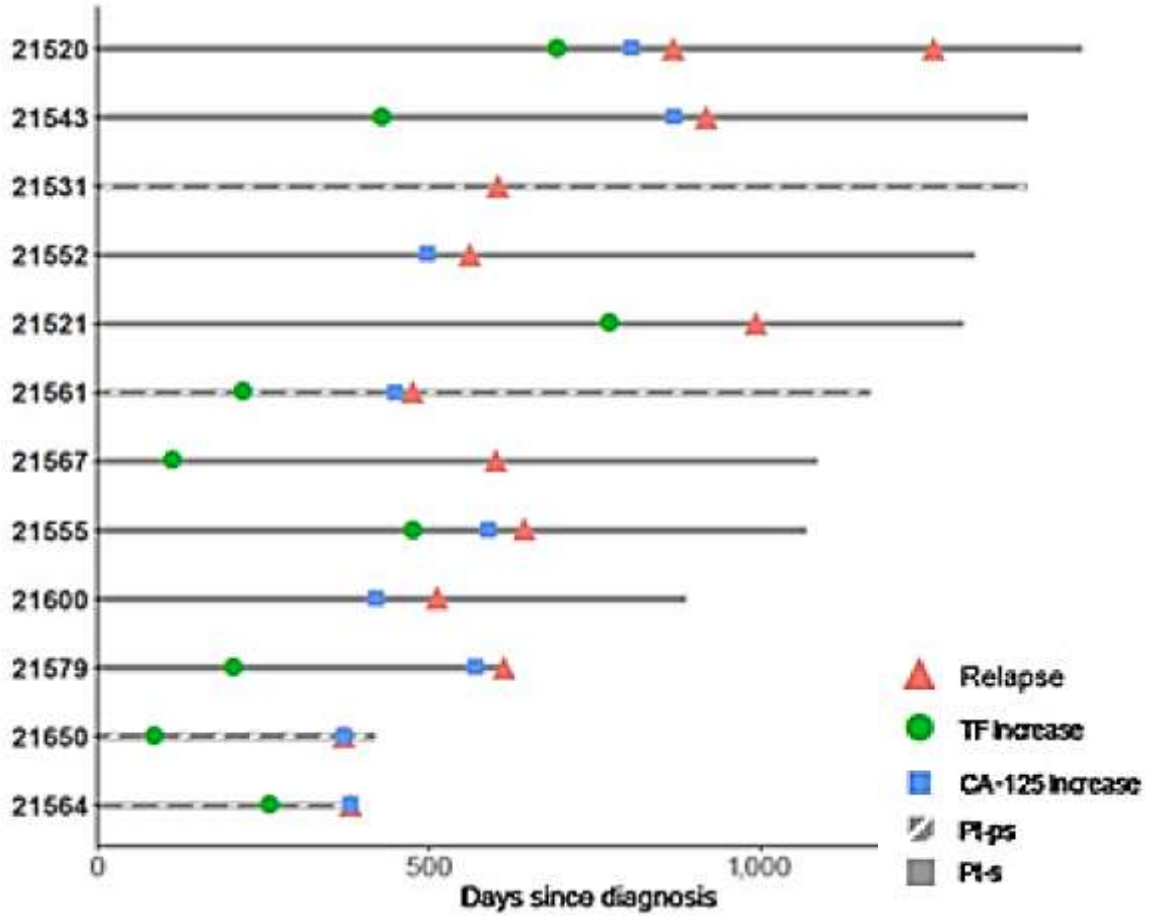


Tumor biopsy

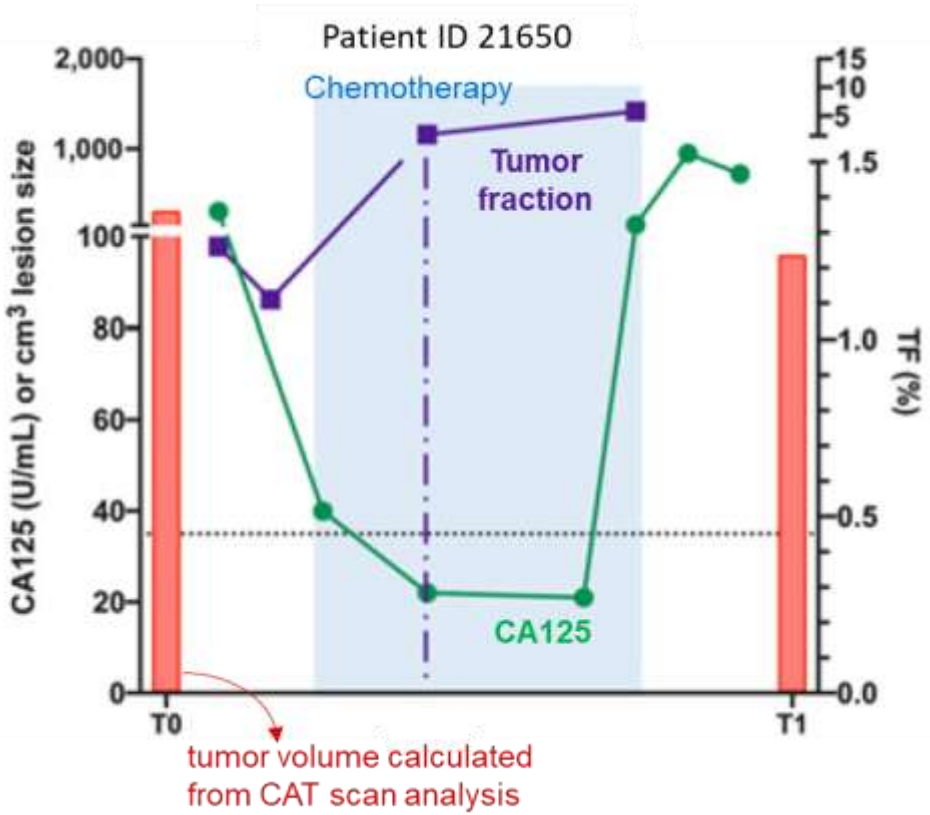


CNA measurement in ctDNA can be used to determine the amount of tumor DNA in plasma = **tumor fraction**

CNAs in ctDNA as a biomarker to monitor disease - 2



Tumor fraction can anticipate time of relapse better than CA-125 and CT scan.



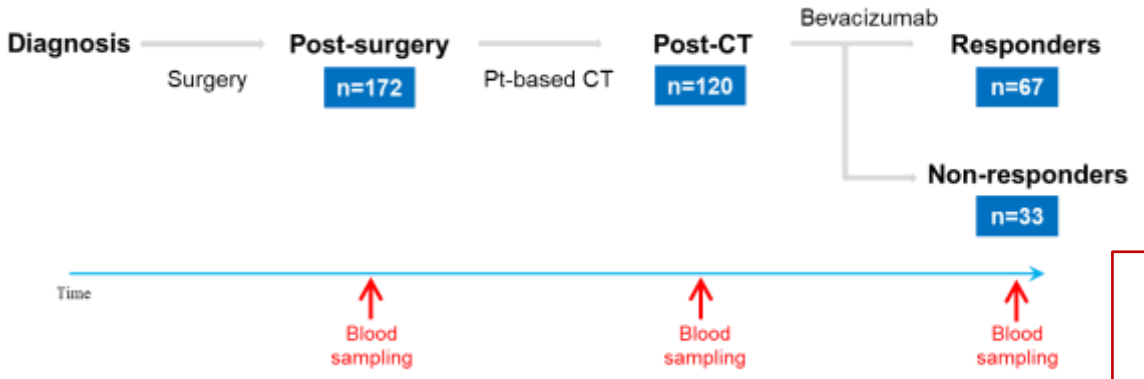
Paracchini, Beltrame, Grassi et al. Clin Cancer Res 2021

Our experience

Title	**Multimodal analysis of ctDNA for the detection of prognostic markers in ovarian cancer**
Type of study	Translational project of the **MITO16a/ MaNGO-OV2 study**
Aim	To identify prognostic biomarkers
Population	172 pts with OC receiving carboplatin +paclitaxel with bevacizumab
Sampling	Plasma samples taken • before any treatment • at different time points during follow-up

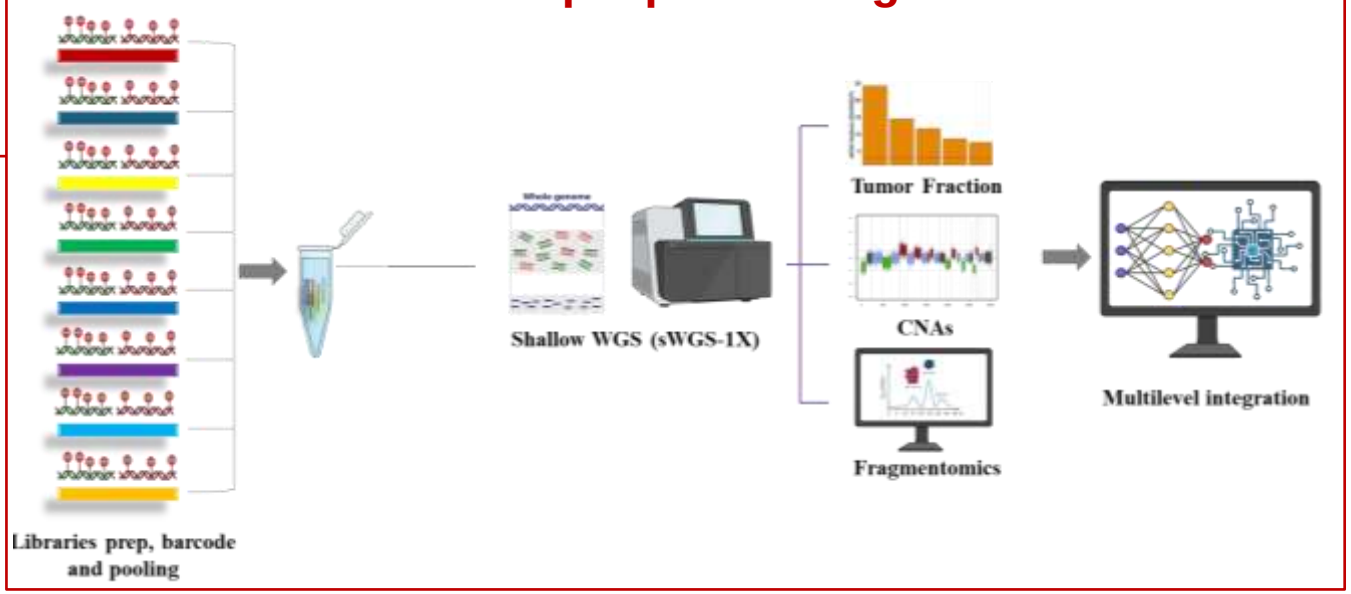
Multiparametric analysis of ctDNA to identify prognostic biomarkers - 1

Sampling

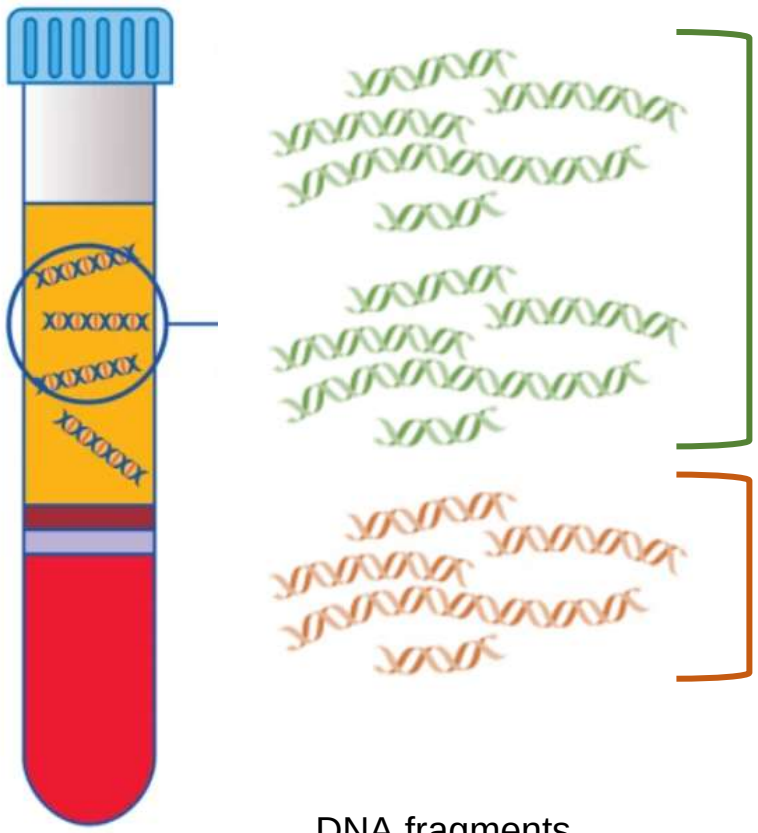


MITO16a/MaNGO-OV2 study

Sample processing



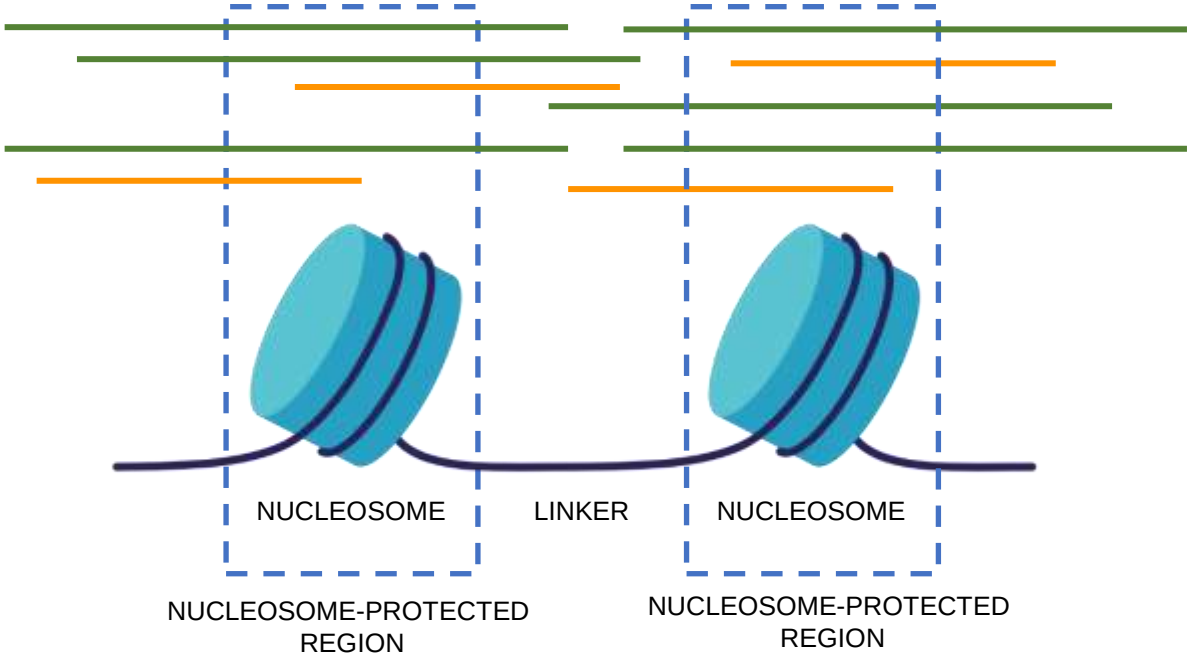
Fragmentomics



Hematopoietic system origin

Cancer tissue origin

DNA fragments



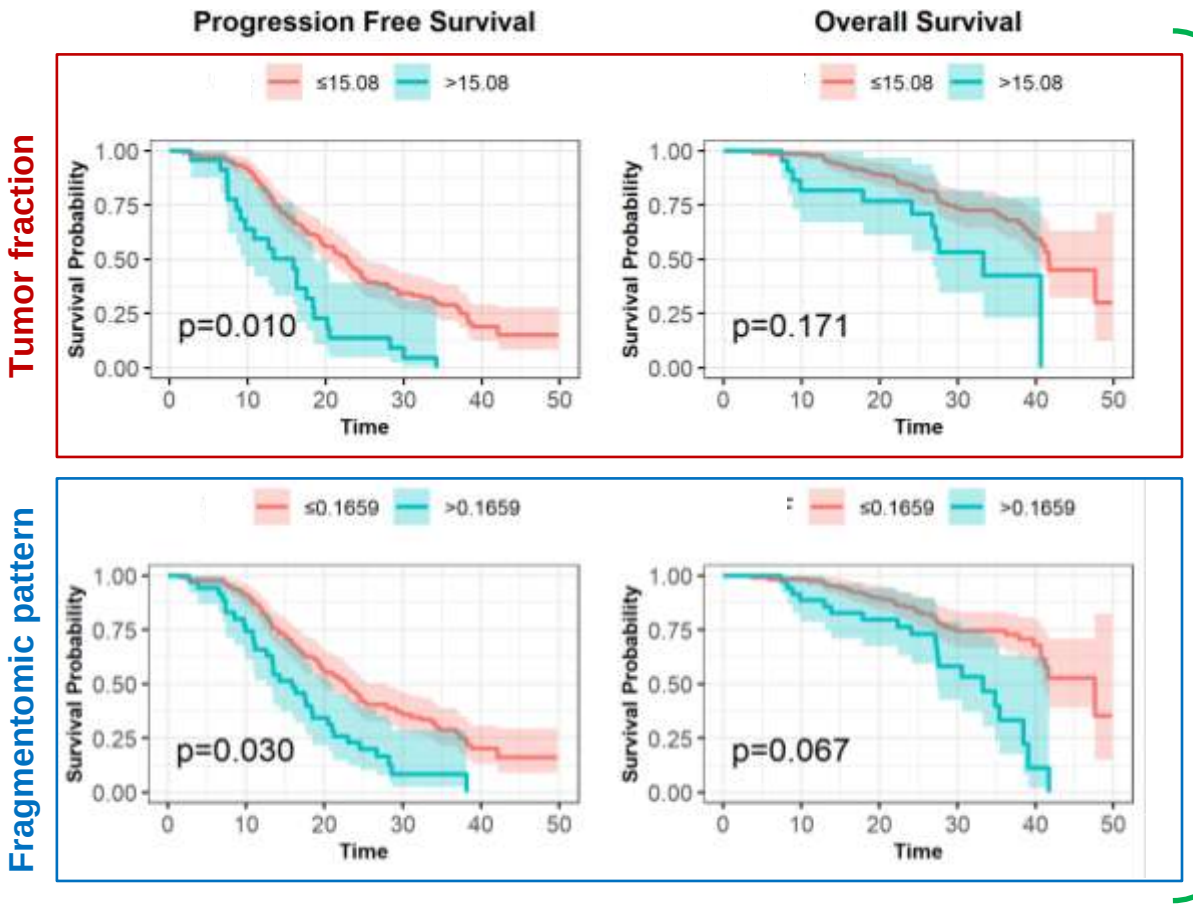
Cancer DNA fragments are:

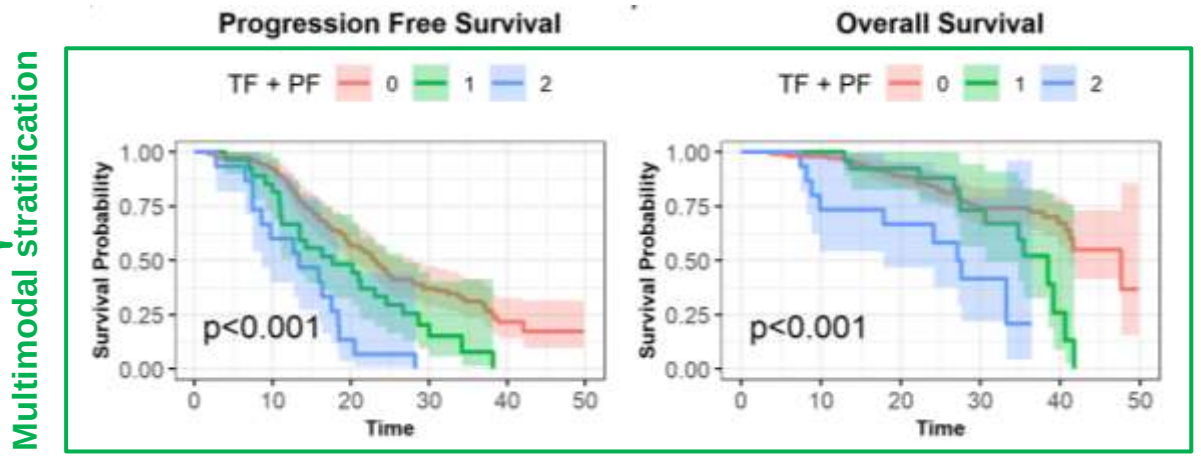
- Shorter (<150 bp)
- Different coverage patterns in regulatory regions
- Enriched in specific sequence at the ends of DNA fragments (end motif)

Applications:

- To develop sensitive cancer detection models.
- To aid in cancer diagnosis
- Cancer monitoring

Multiparametric analysis of ctDNA to identify prognostic biomarkers - 2



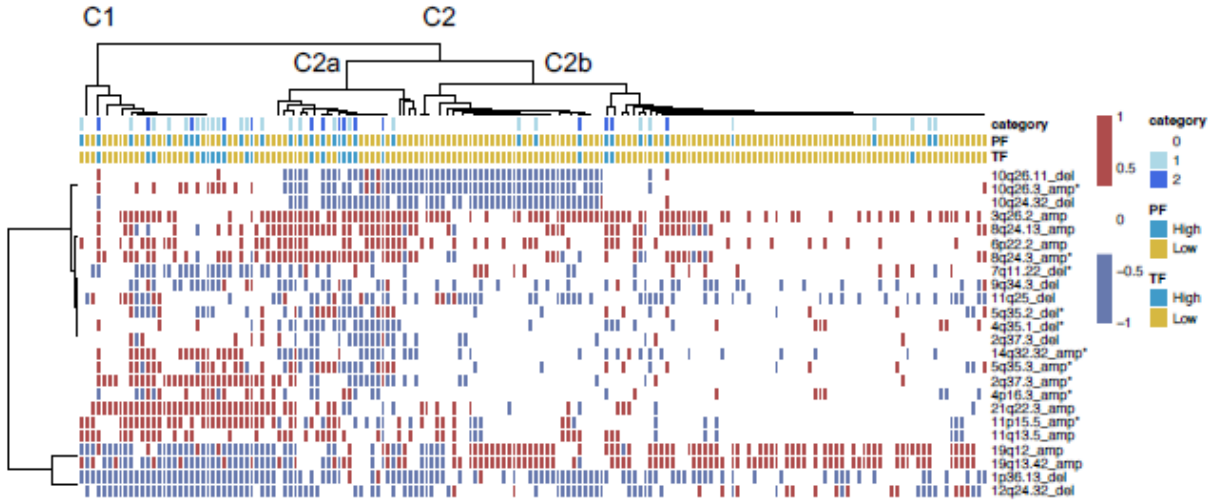


Incorporating tumor fraction and fragmentomic profiling of plasma ctDNA collected post-surgery can effectively stratify HGSOC patients by prognosis

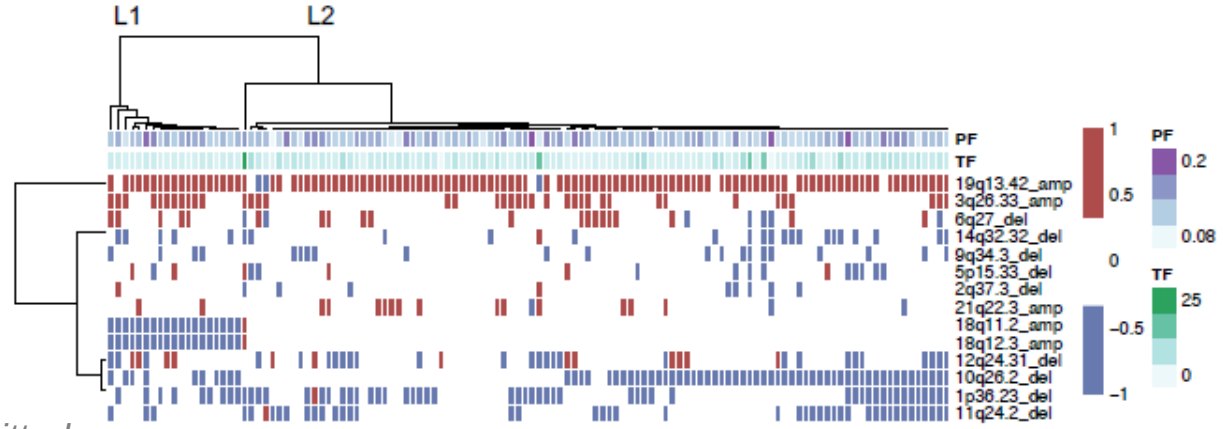
Paracchini, et al. submitted

Multiparametric analysis of ctDNA to identify prognostic biomarkers - 3

Post-surgery



At the end of first-line therapy



Paracchini, et al. submitted

Unsupervised cluster analysis of genomic alterations

- amplifications
- deletions

Amplifications at 19q13.42 were observed following chemotherapy, suggesting a connection between this locus and chemoresistance/poor prognosis

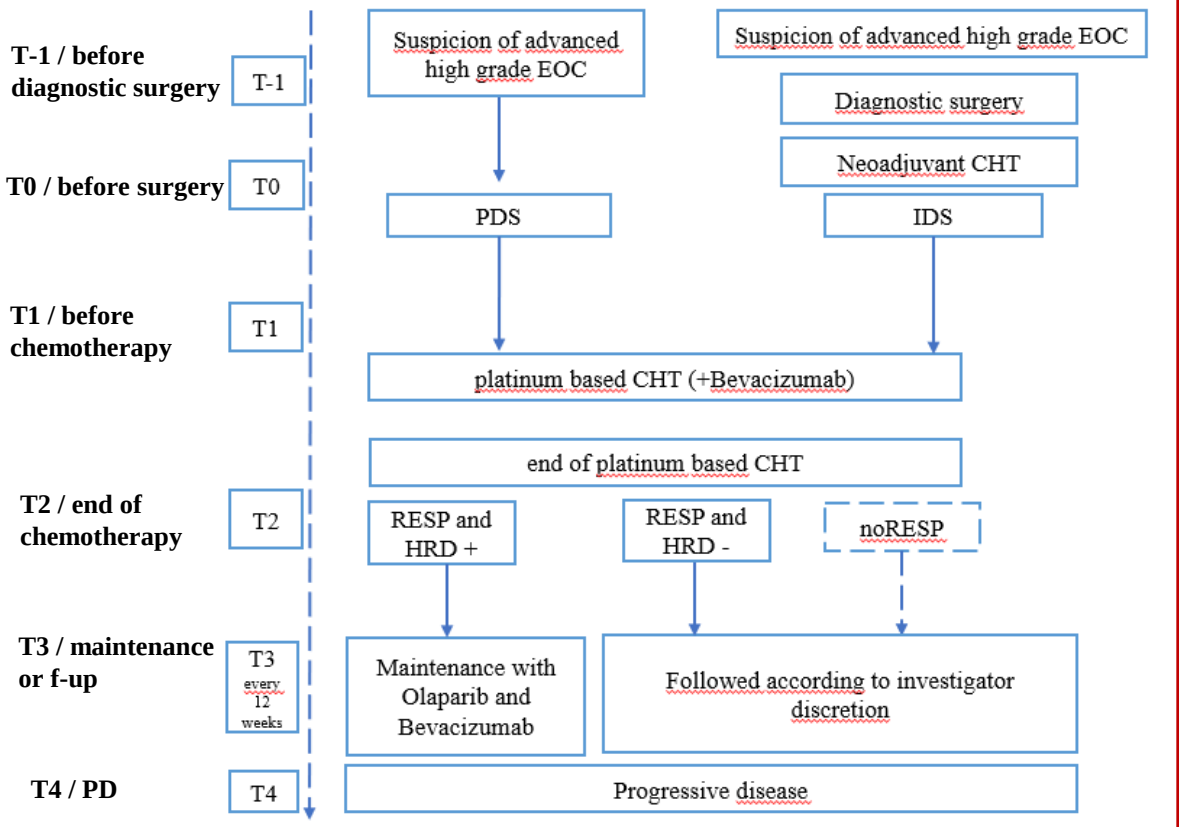
Our experience

Title	**Multimodal analysis of ctDNA from patients with HGSOC to understand disease progression**
Type of study	Translational project of the **IOLANTHE study (ongoing)**
Aim	To assess and track disease progression and the alterations in the mutational status of HR-related genes that may influence sensitivity to PARP inhibitors
Population	Expected 190 pts with HGSOC
Sampling	Plasma samples taken • before any treatment • at different time points during follow-up

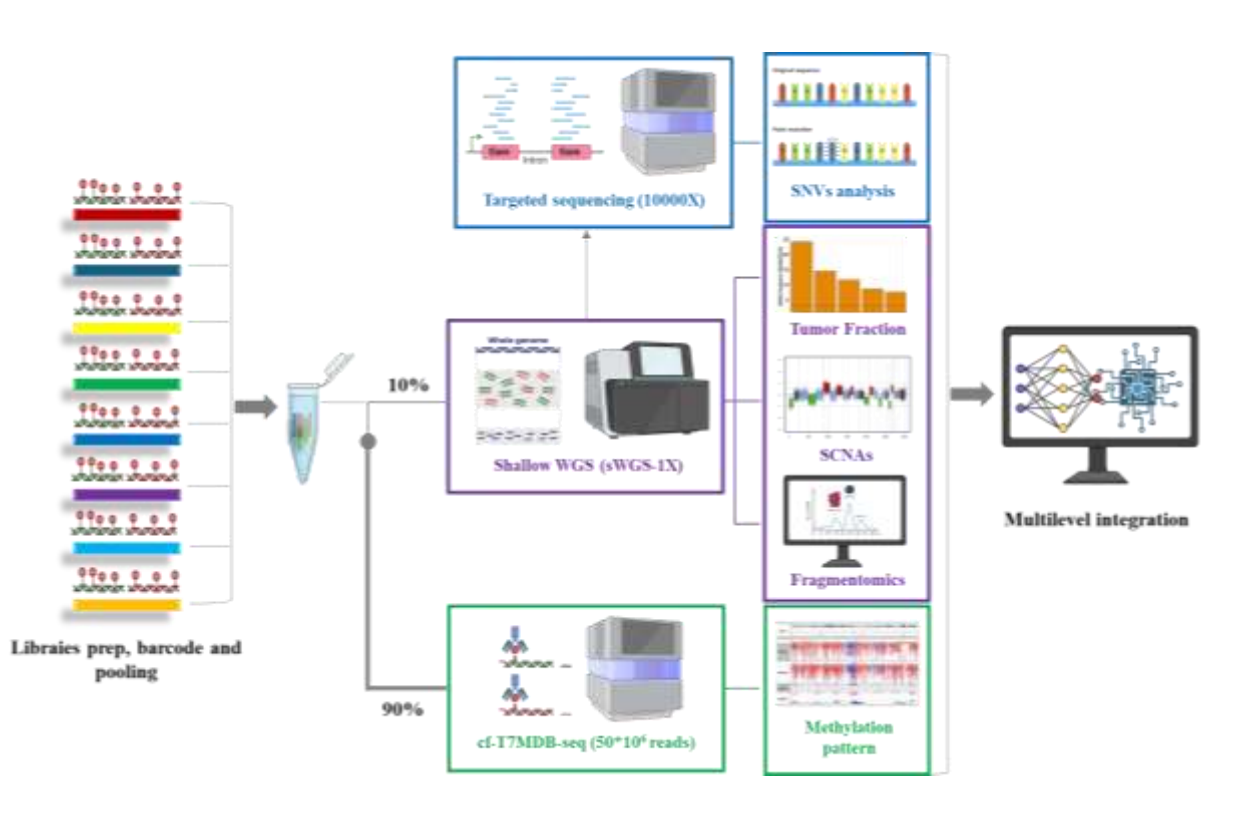
Multiparametric analysis of ctDNA to understand disease evolution - 1

IOLANTHE study

Sampling



Sample processing



Multiparametric analysis of ctDNA to understand disease evolution - 2

No. of sites involved: 13

First patient-in: 15-Sep-2023

No. of pts registered: 198

No. of screening failures: 25

No. of pts eligible for step 1: 134

Closure of enrolment: end of Sep. 2025

Samples collected by the Sponsor – June 2025

Timepoint	Tissue	Blood		Plasma	
	No. of patients/samples	No. of patients	No. of samples	No. of patients	No. of samples
T-1	14	33	101	34	157
T0	36	11	32	11	51
T1	-	17	51	36	179
T2	-	-	-	23	113
T3	-	-	-	4	23
T3 (1)	-	-	-	11	53
T3 (2)	-	-	-	4	18
T3 (3)	-	-	-	1	6
T4	-	-	-	4	20

Concluding remarks - 1

- **Liquid biopsy** is a powerful **minimally invasive** approach that holds the potential to address several clinical challenges associated with **gynecological tumors**, including:
  - **monitoring therapeutic responses** in metastatic settings,
  - **minimal residual disease (MRD) tracking**,
  - refining our understanding of the **heterogeneity of clinical responses**.

- Despite the promise of liquid biopsies, there are existing challenges related to **technical limitations** and the **need to establish their clinical value**. These challenges represent active areas of research that require interdisciplinary approaches to effectively connect scientific discoveries with clinical applications.

Concluding remarks - 2

- Current research focuses on **improving molecular assays** to detect tumor components in liquid biopsy with greater sensitivity and exploring **additional biofluids** like ascites to broaden diagnostic options.

- **Multiparametric analysis** of data from liquid biopsy in a tumor-agnostic platform could help to detect molecular relapse and to analyze the clonal architecture of relapsed disease.

- **Integrating liquid biopsy with traditional tissue analysis** may allow clinicians to leverage real-time genetic information alongside the detailed insights from tissue analysis, enhancing diagnosis, staging, and targeted therapy strategies.