Exploring the dual analysis of circulating tumour cells and circulating tumour DNA

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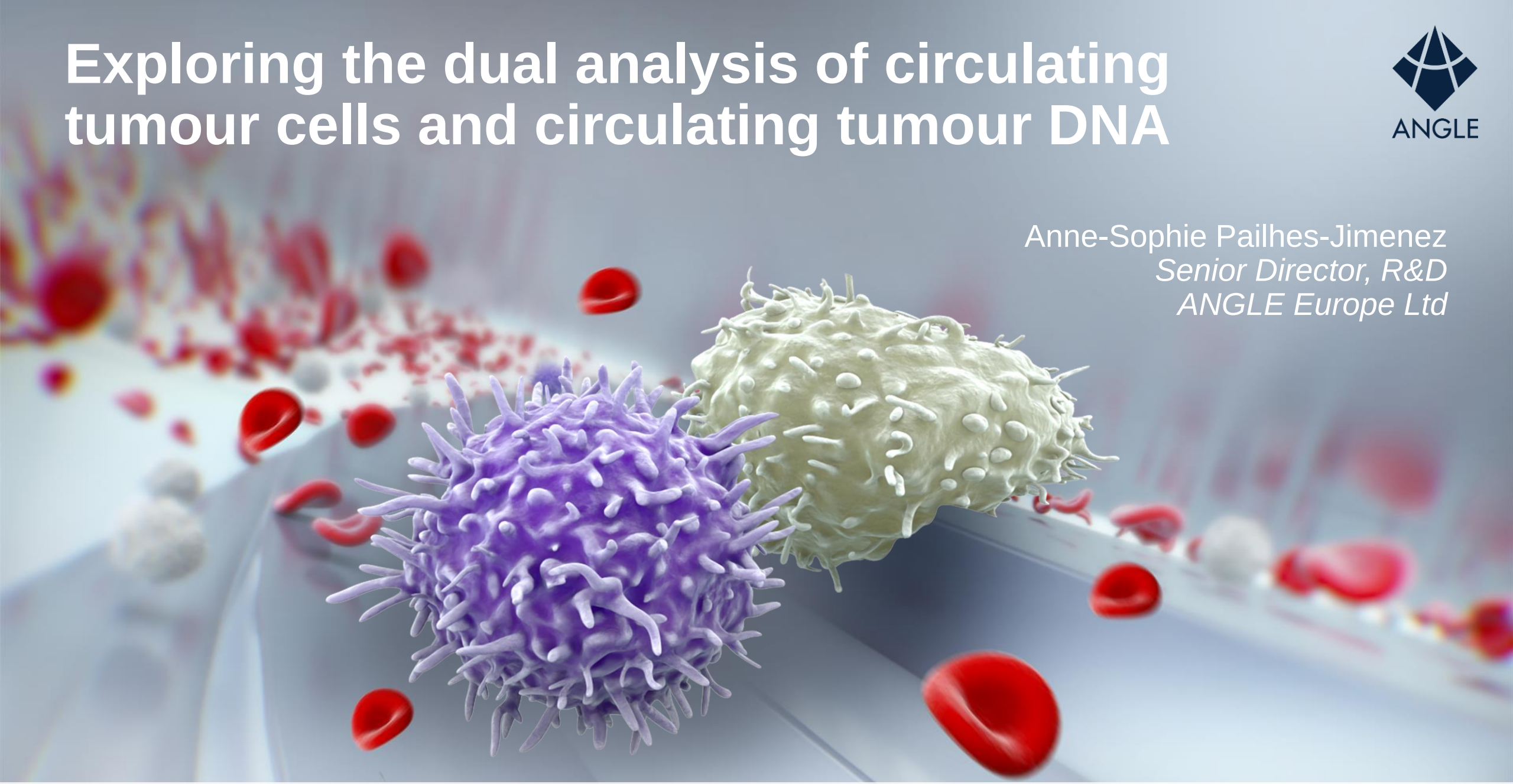

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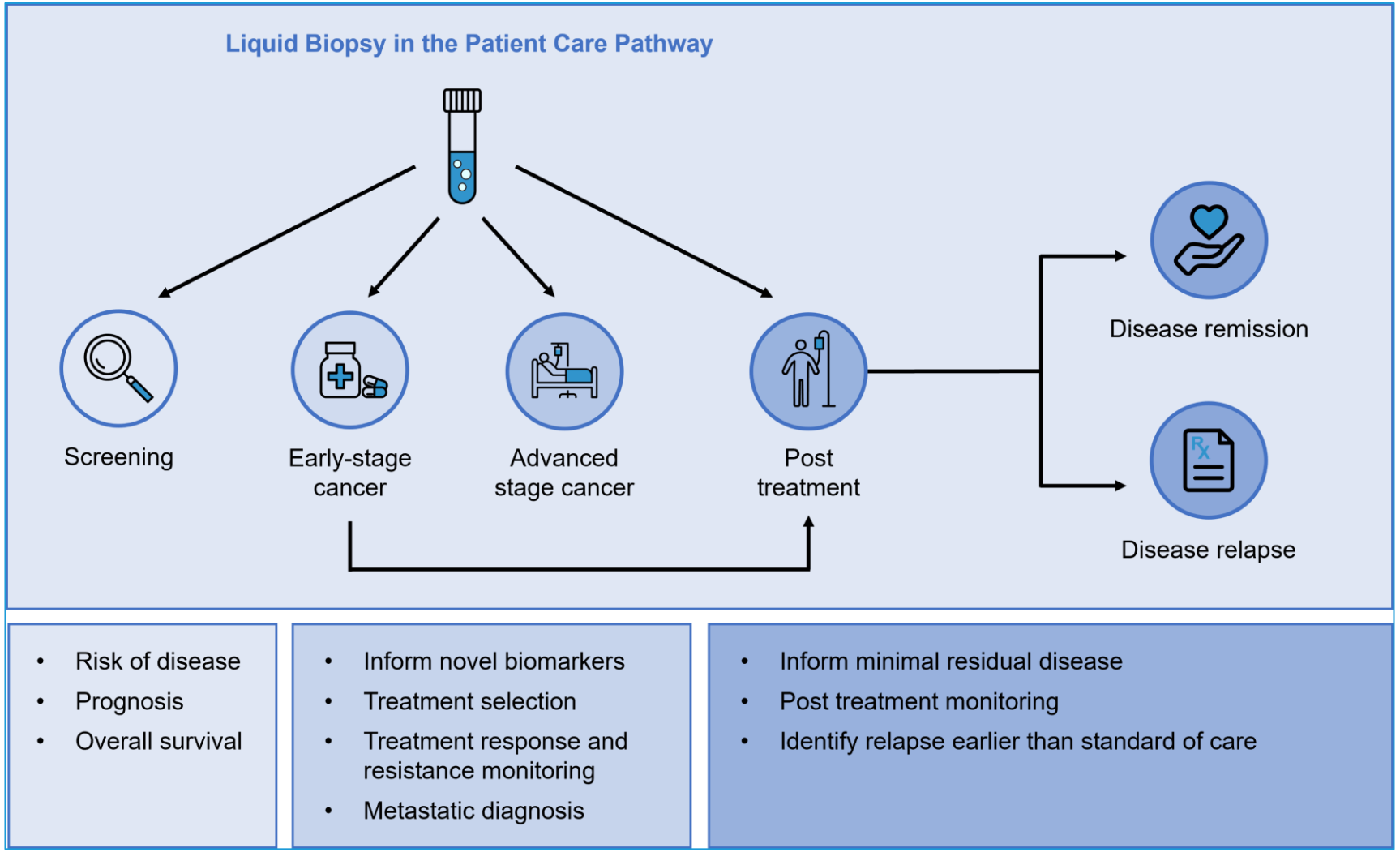
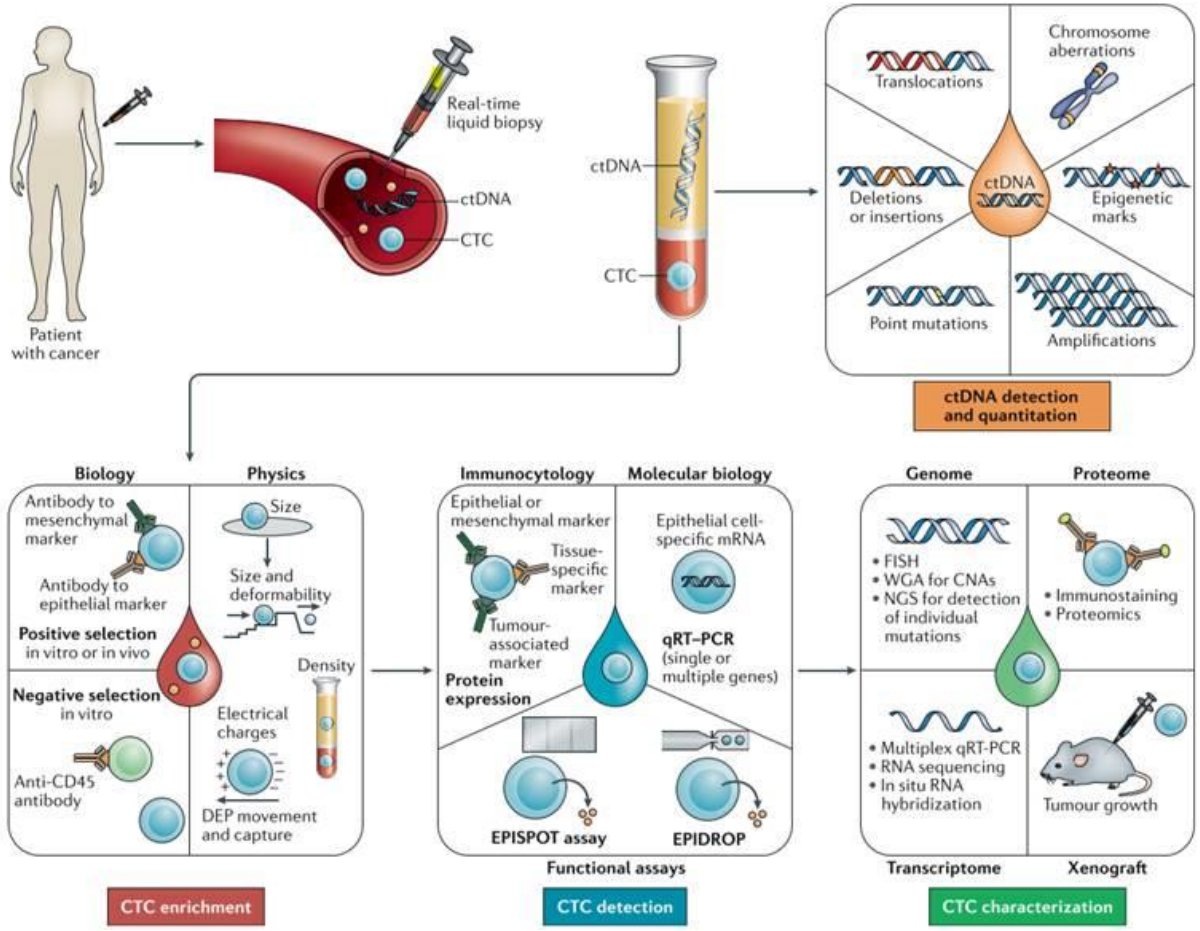
The potential of liquid biopsy throughout the patient care pathway



Liquid Biopsy and CTCs

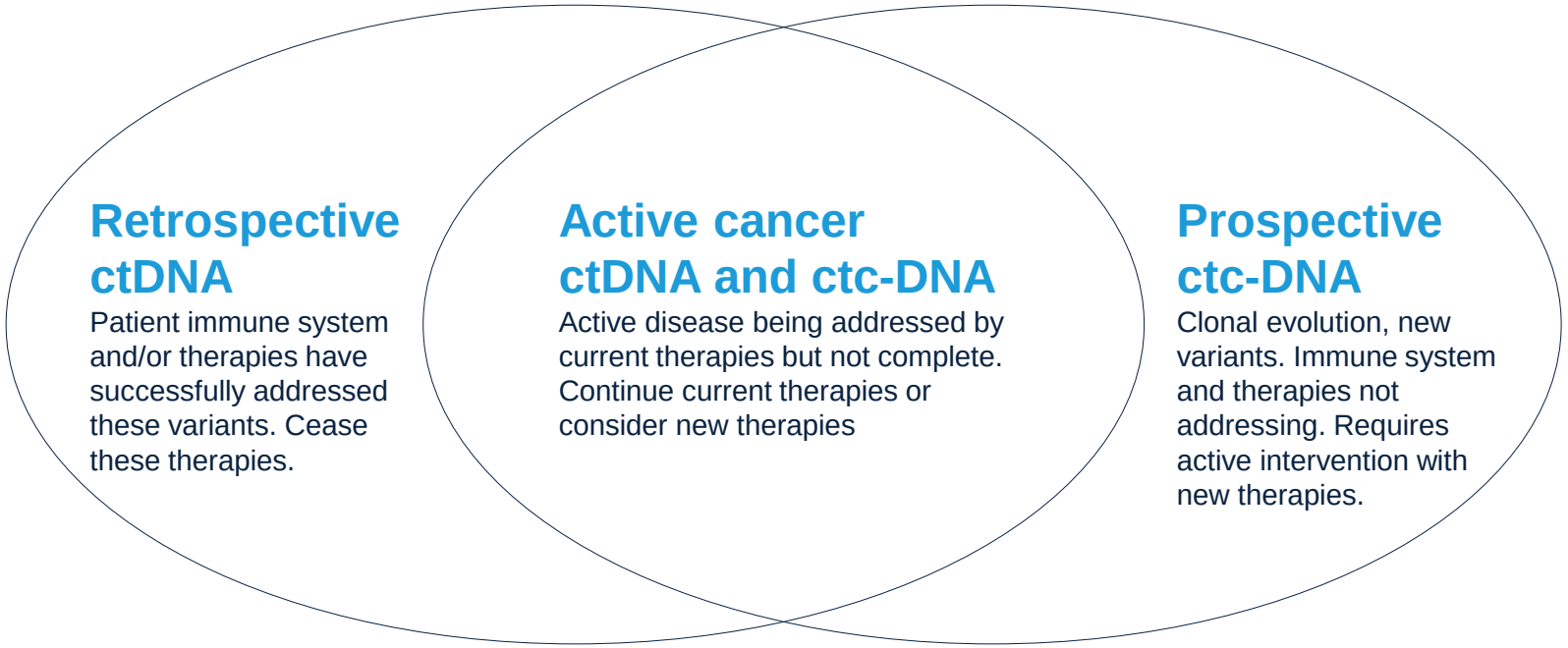
Blood as liquid Biopsy:

- **Minimally-invasive**
- **Repeatable**
- **Representative** of cancer heterogeneity
- **Versatile**
- **Informative**



*Klaus Pantel and Catherine Alix-Panabieres. Liquid biopsy and minimal residual disease — latest advances and implications for cure. Nat Rev Clin Oncol **16,** 409–424 (2019). https://doi.org/10.1038/s41571-019-0187-3*

CTCs and ctDNA provide complementary information



CANCER PROGRESSION OVER TIME

Hypothesis:

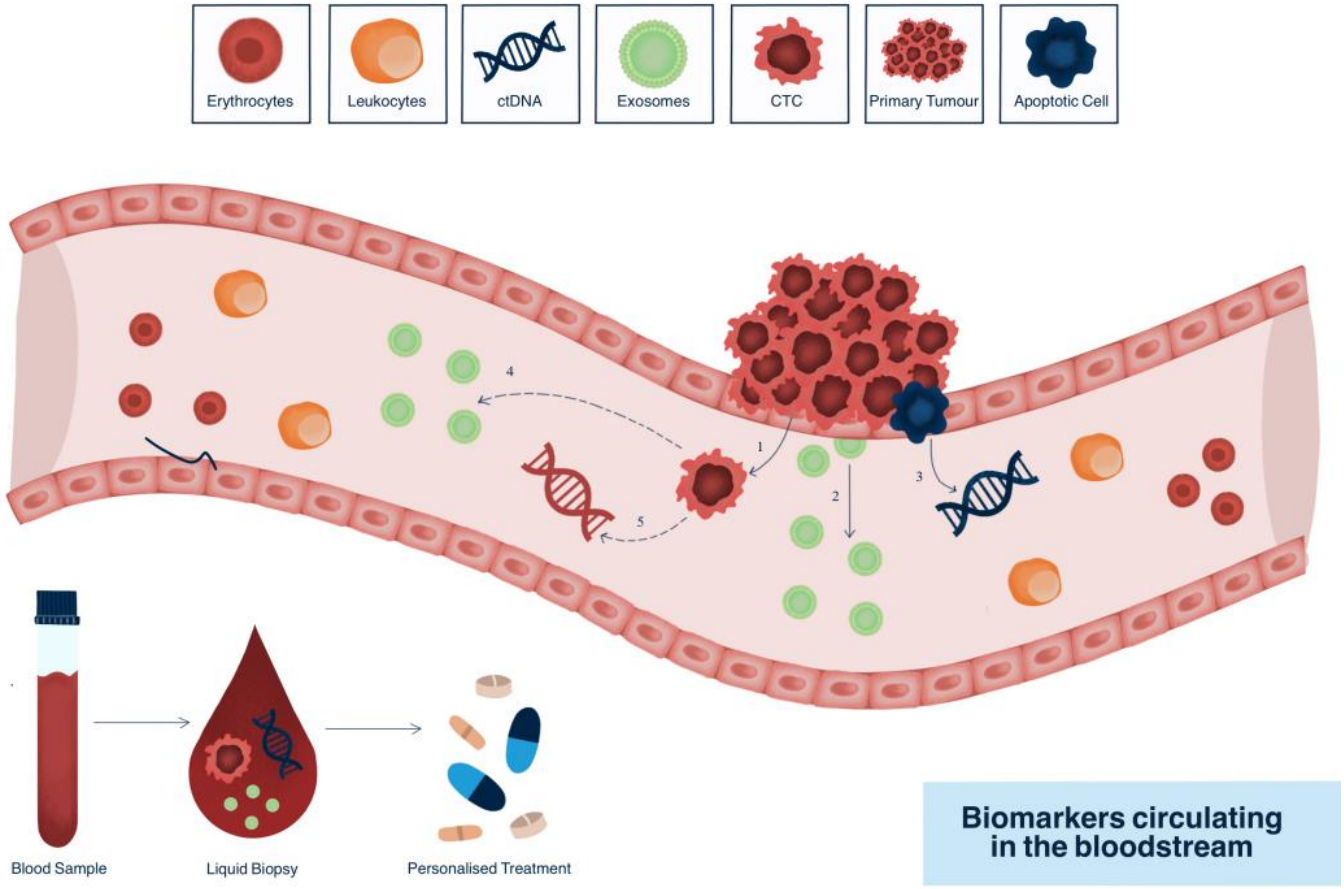
Dual analysis provides critical information not otherwise available on cancer progression and for targeted treatment selection

"**CTCs**, as living cells that are active in the metastatic process, can provide **prospective insight into a patient's cancer.**

In comparison, **ctDNA** derived mainly from dead and dying cells, provides important but **historical** information on patient disease."

Prof. Evi Lianidou
Head of the Molecular Diagnostics Laboratory
National and Kapodistrian University of Athens

Circulating tumour cells (CTCs)



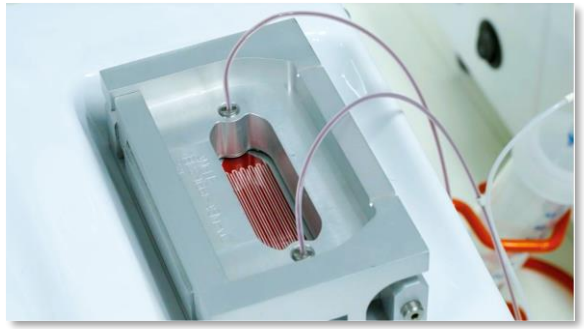
CTC and CTC clusters:

- Reflective of **metastatic** tumor
- Source of **biomarkers** (suitable for multiomics analysis)
- **Rare** event: 1 CTC / 10^9 blood cells

→ Importance of upstream workflow to generate quality sample.

Parsortix® PC1 system

The first FDA cleared / CE-IVD medical device for the capture and harvest of circulating tumor cells from metastatic breast cancer patient blood for user-validated subsequent analysis.



Intended use

"The Parsortix® PC1 system is an in vitro diagnostic device intended to enrich circulating tumor cells (CTCs) from peripheral blood collected in K_2EDTA tubes from patients diagnosed with metastatic breast cancer. The system employs a microfluidic chamber (a Parsortix cell separation cassette) to capture cells of a certain size and deformability from the population of cells present in blood. The cells retained in the cassette are harvested by the Parsortix PC1 system for use in subsequent downstream assays.

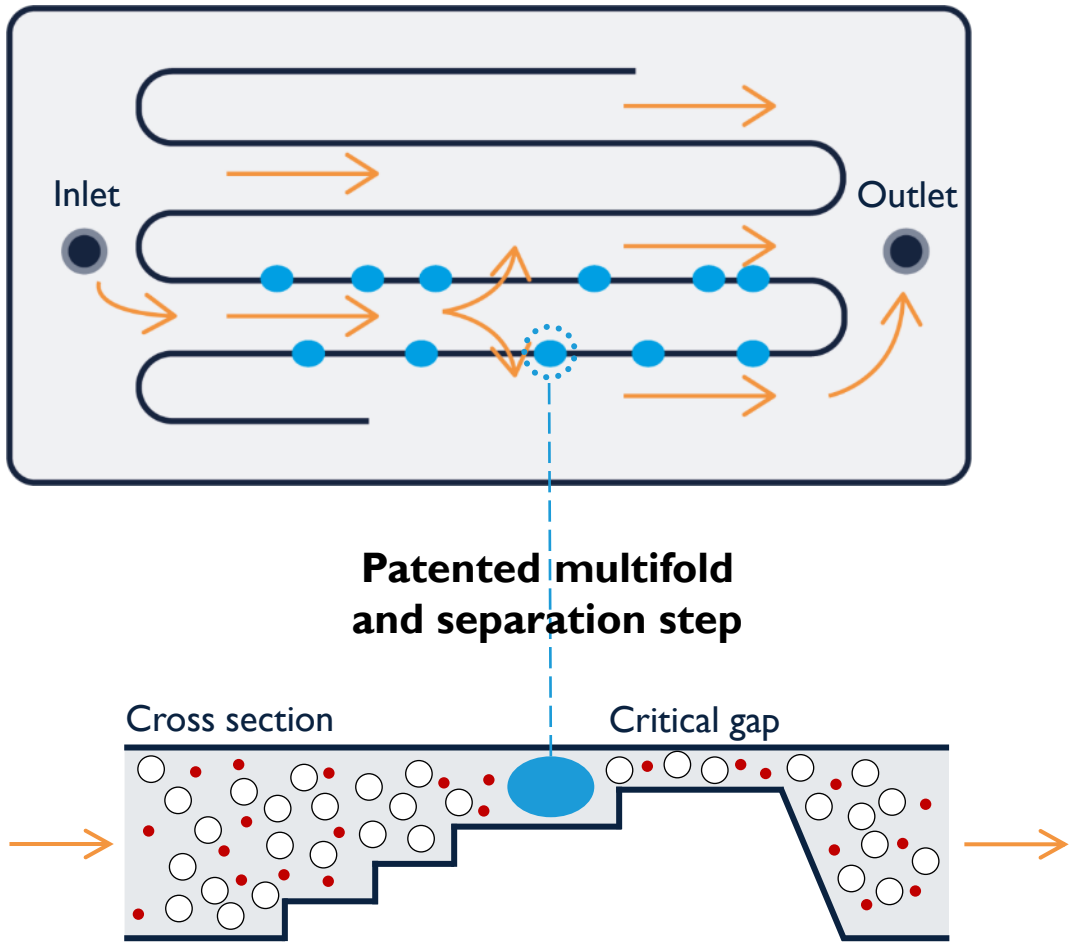
The end user is responsible for the validation of any downstream assay. The standalone device, as indicated, does not identify, enumerate or characterize CTCs and cannot be used to make any diagnostic/prognostic claims for CTCs, including monitoring indications or as an aid in any disease management and/or treatment decisions.

*FDA Clearance for use with metastatic breast cancer patients only

Parsortix systems: capturing and harvesting living cancer cells

ANGLE

Plan view

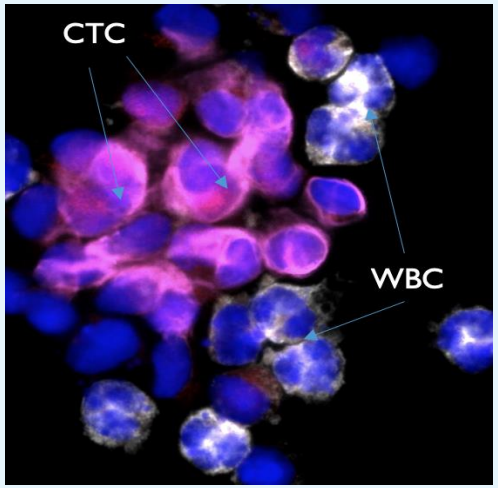


Platform technology

The Parsortix system harvests **cancer cells from blood** based on their larger size and lack of deformability

Other cells can be captured:

- **white blood cells** associated with the tumor microenvironment
- **megakaryocytes** (frequency may relate to cancer)
- **fetal cells** from maternal blood



CTC and WBC cluster

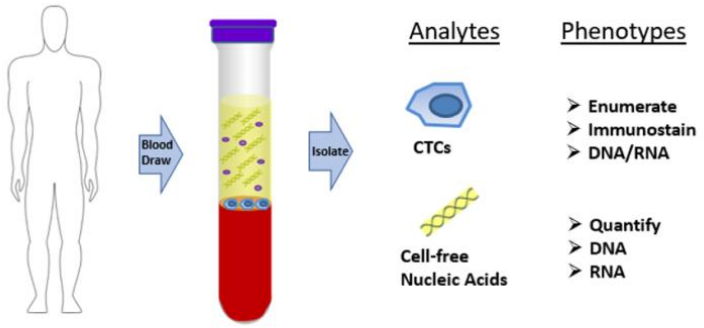
Key

- Captured CTCs
- White blood cells
- Red blood cells
- Blood flow

Workflow

Workflow allowing for CTCs and ctDNA analysis

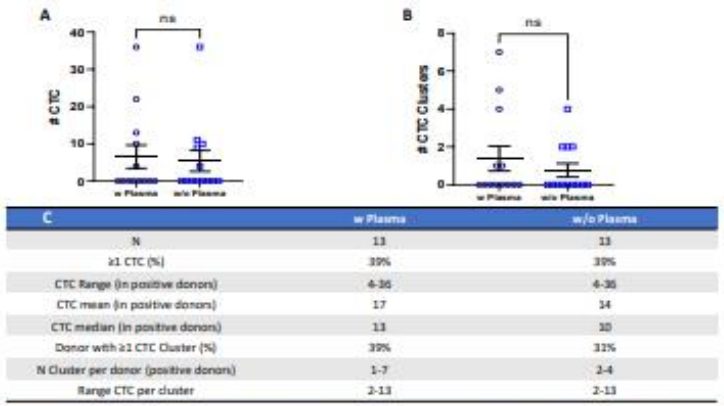
Dual analysis of biomarkers present in plasma and cellular component



https://uscnorriscancer.usc.edu/liquid-biopsy-research-core/

Proof Of Concept work:

No CTC loss following plasma removal, prior to Parsortix enrichment.



C	w Plasma	w/o Plasma
N	13	13
≥1 CTC (%)	39%	39%
CTC Range (in positive donors)	4-36	4-36
CTC mean (in positive donors)	17	14
CTC median (in positive donors)	13	10
Donor with ≥1 CTC Cluster (%)	39%	31%
N Cluster per donor (positive donors)	1-7	2-4
Range CTC per cluster	2-13	2-13

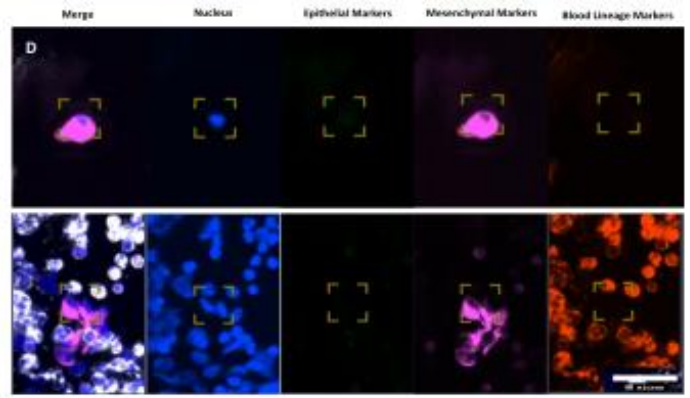


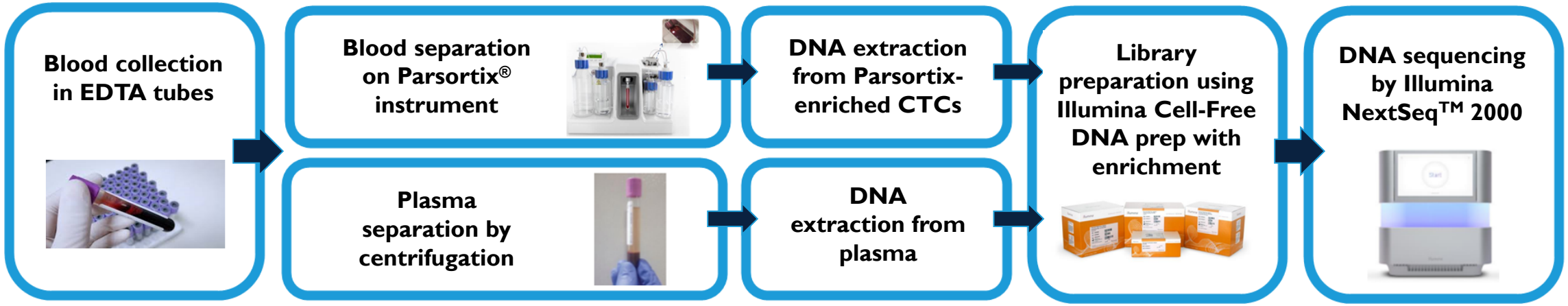
Figure 5. CTC detection in MBC patients blood samples processed with or without plasma. Dot plot shows mean ± SEM of the number of **A)** CTCs and **B)** CTC clusters identified in each donor per condition. No statistically significant difference was observed, *ns= p≥0.05, Paired T-test.* **C)** Table showing number of donors included in each cohort (N), percentage (%) of donors with ≥1 CTC, range, mean and median of CTCs captured within the positive group, percentage of donors with ≥1 CTC cluster, range of CTC clusters per donor, and range of number of CTCs per cluster; **D)** Representative images of a single mesenchymal CTC (top) and a cluster of mesenchymal CTCs (bottom). Epithelial markers (FITC) in green, Mesenchymal markers (Cy7) in magenta, Blood lineage markers (Cy5) in red or white in the merge, Nucleus (DAPI) in blue.

https://angleplc.com/wp-content/uploads/2023/05/PTX-Poster-A-ISLB-2022-Bidetect-002.pdf

Can we allow for dual detection of targets/variants which may have an impact on patient care?

Molecular analysis of CTCs and ctDNA

ctDNA and Parsortix-derived CTC-DNA can be analysed from single blood sample across multiple cancer types to detect mutations using Illumina Cell-Free DNA prep with enrichment combined with custom panel targeting Lung Cancer.



Illumina Cell-Free DNA prep with Enrichment and custom 79 gene panel enables a flexibility in the targeted genes.

DNA dual analysis
Assay performance (1/2)

Study design for evaluation of performance of DNA dual analysis

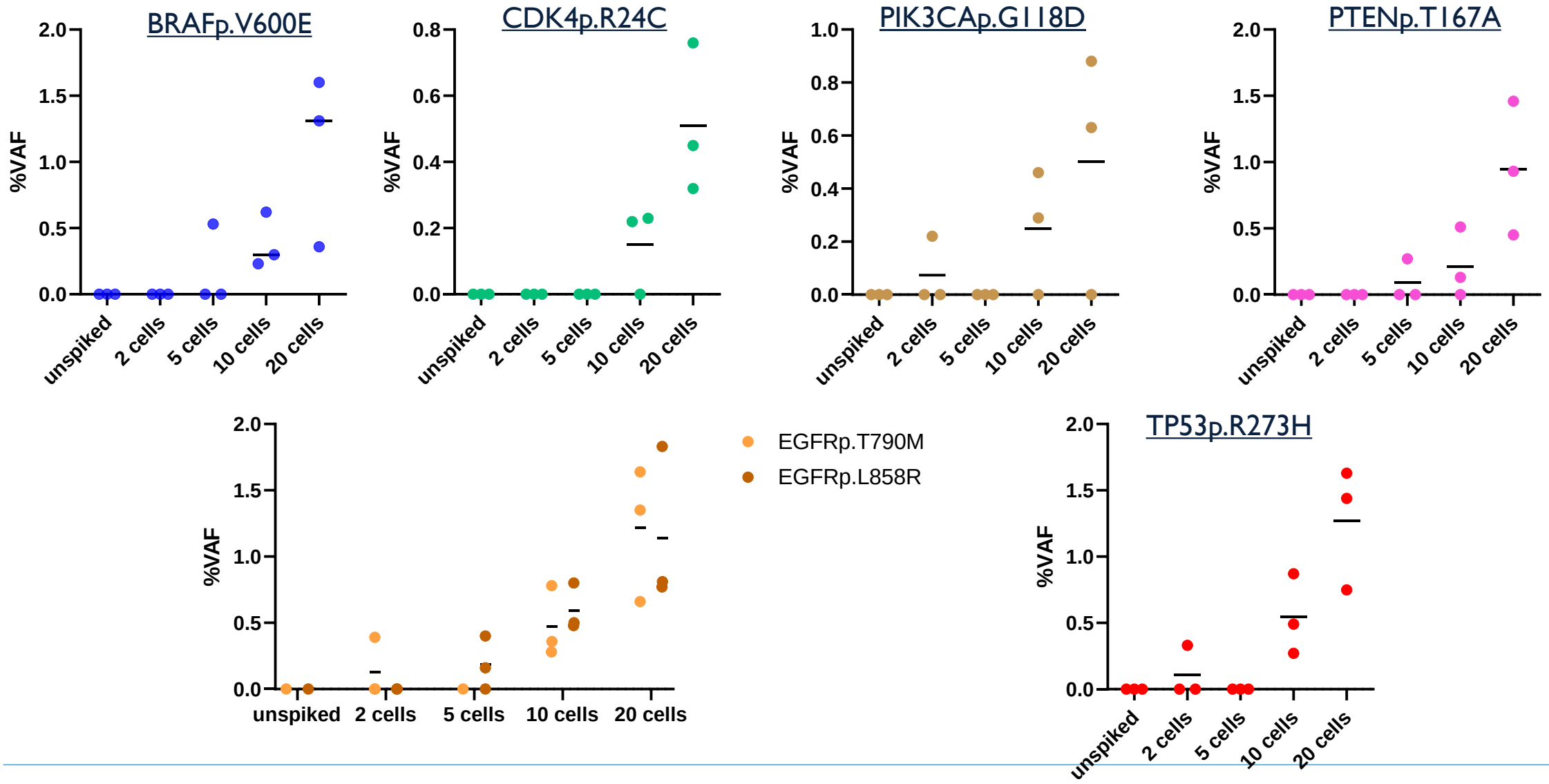
Contrived samples were used to assess the analytical performance of the assay:

- Two cell lines with known mutations, were spiked into Parsortix-enriched samples
- DNA samples from CTC were extracted and processed following Illumina Cell-Free DNA prep with Enrichment procedure.

Table: expected mutations to be detected in spiked samples

Cell line 1	Cell line 2
pTEN p.T167A	EGFR p.T790M
BRAF p.V600E	EGFR p.L858R
CDK4 p.R24C	PIK3CA p.G118D
	TP53 p.R273H

DNA dual analysis
Assay performance (1/2)



DNA dual analysis of Patients with Lung cancer

Patient data 1/4

2 cohorts of Lung Cancer patient

- **1st cohort: 8 Lung Cancer patient samples (no treatment)**
- **2nd cohort: 19 Lung Cancer patient samples (may be under treatment)**

✓ **Good quality** samples

✓ Overall, **93% of samples with at least one oncogenic gene detected.**

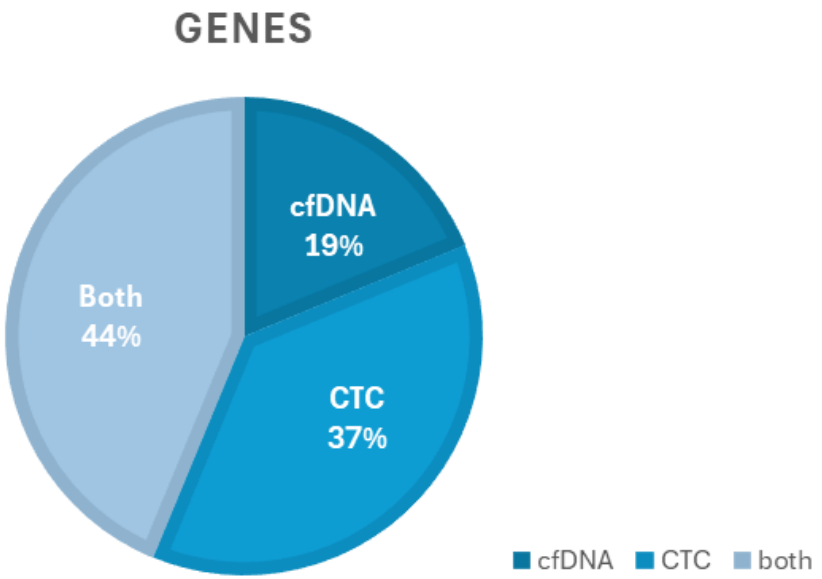
DNA dual analysis of Patients with Lung cancer
Patient data 2/4

1st cohort - Lung cancer panel (n=8):

CTC(average)= 49 and CTC(median)= 20

100% patients showed oncogenic mutations signal for one or more targets.

32 genes (74 variants) seem to be specifically detected in this cohort:



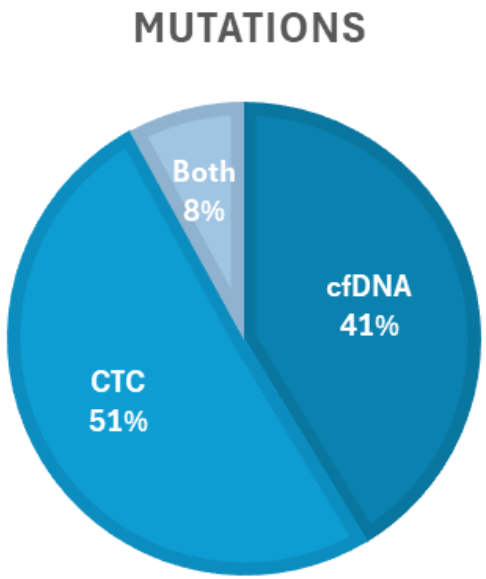


Table: example of detected mutations in specific analyte

CTC	cfDNA
CHEK1*	MSH6
FBXW7*	POLE
MLH1	BRAF
ESR1	APC
ROS1	ATM

** predominant*

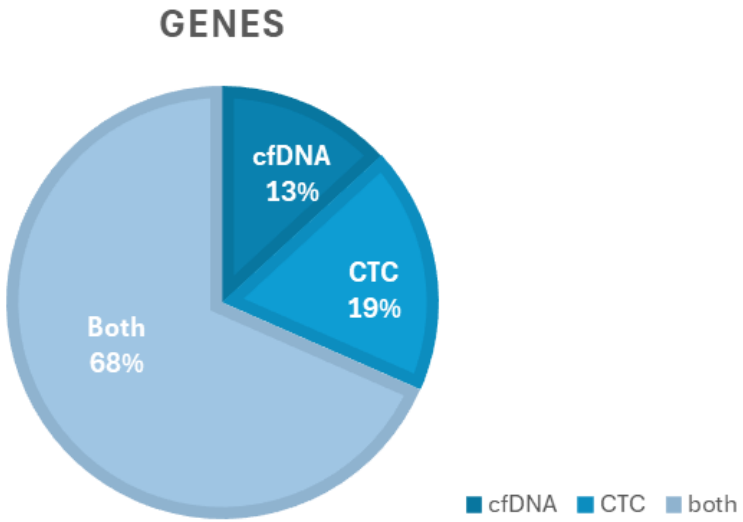
Dual analysis of Patients with Lung cancer
Patient data 3/4

2nd cohort - Lung cancer panel (n=19):

CTC(average)= 4 and CTC(median)= 3

90% of patients with at least one oncogenic mutations in CTC for one or more targets.

38 genes (214 variants) have been specifically detected in this cohort:



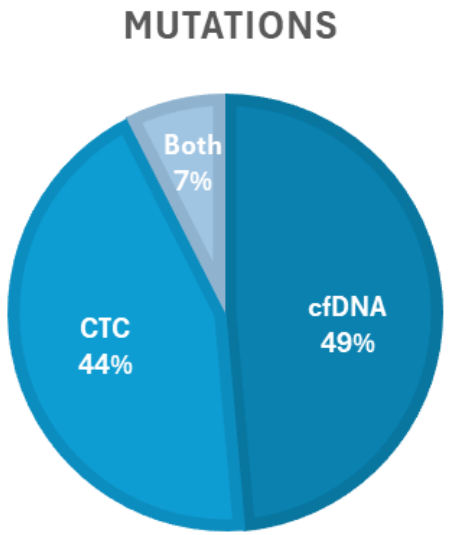


Table: example of detected mutations in specific analyte

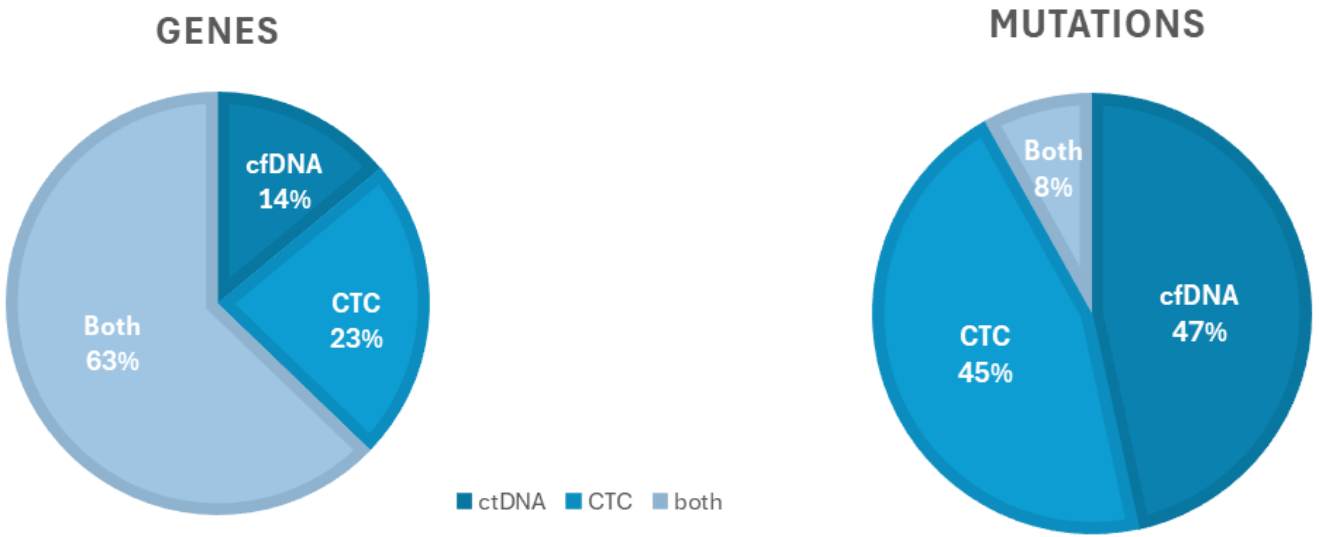
CTC	cfDNA
EpCAM	CDKN2A
MYCN	NTRK
FOXL2	PIK3CA
HRAS	APC
CHEK1*	RB1*

** predominant*

DNA dual analysis of Patients with Lung cancer
Patient data 4/4

Full cohort - Lung cancer panel (n=27):

43 genes (247 variants) have been specifically detected in this cohort:



Analysing both analytes give access to **twice** as many **mutations**, which could be investigated and/or use as "potential" biomarkers.

DNA dual analysis of Patients with Lung cancer
Validation of data using another technology

<u>Validation</u> of patient data using another cohort of patient samples (under treatment – n=10)

Use of a pan-CANCER NGS panel, with blocker displacement amplification

20 genes (27 variants) have been specifically detected in this cohort:

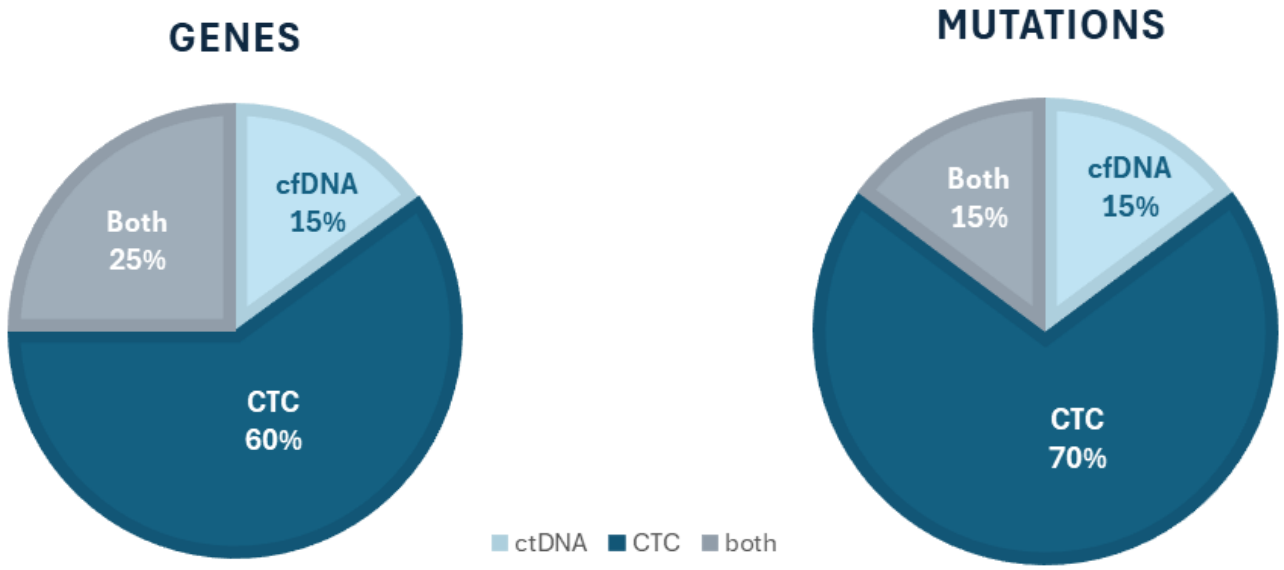


Table: example of detected mutations in CTC fractions

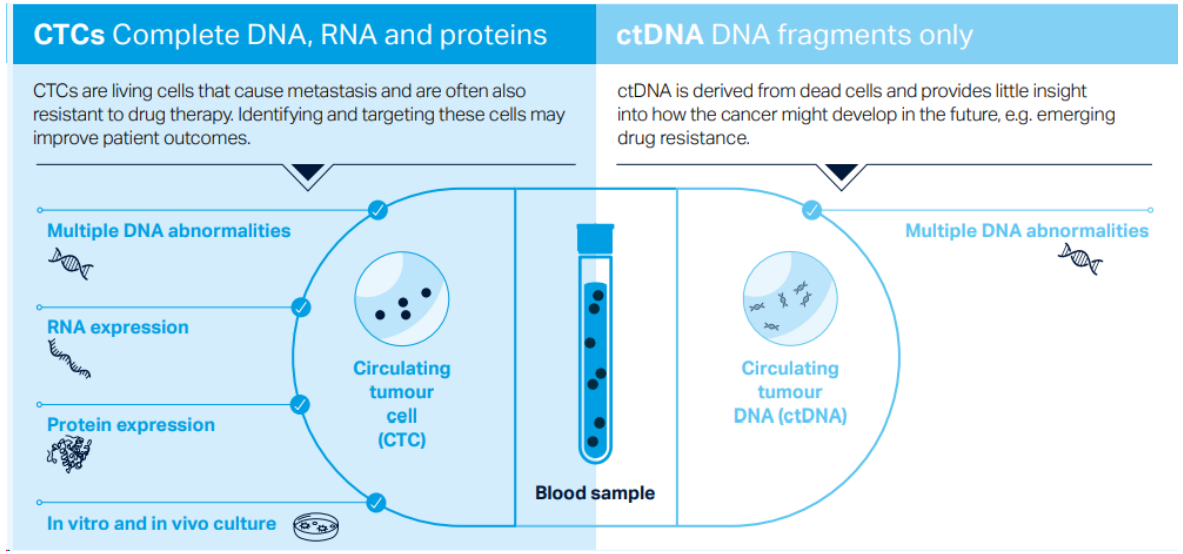
CTC	cfDNA
PiK3CA	MYC
MTOR	MET
ESR1	EGFR*

* *predominant*

Confirmation of the utility of using CTC as well as cfDNA to have access to a larger number of potential biomarkers

Conclusions

Parsortix DNA dual analysis strategy
CONCLUSION



- ✓ Successful detection of potential actionable targets in cfDNA and CTC, from the same sample.
- ✓ CTC samples provide **a unique detection** of actionable targets in most of the patient samples.
- ✓ This study highlights the potential for multiomic analysis.
- ✓ More data to come…

Parsortix system is allowing for CTC enrichment and **DNA analysis from plasma and CTC, from the same blood sample, with high analytical sensitivity and specificity.**
Dual analysis provides the **possibility to access (new) biomarkers**, which may help guide patient care.

Thank you for your attention!

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