University of California San Francisco

Masters in Therapeutic Oncology Summit | Breast Edition
March 28, 2025

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A primer on circulating tumor DNA technologies: The message is in the method

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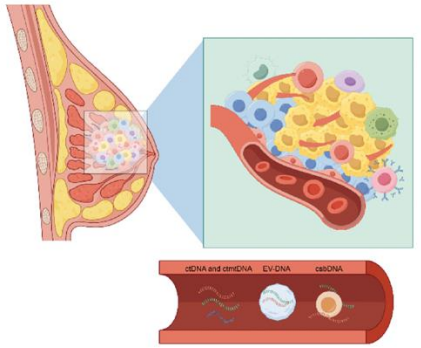
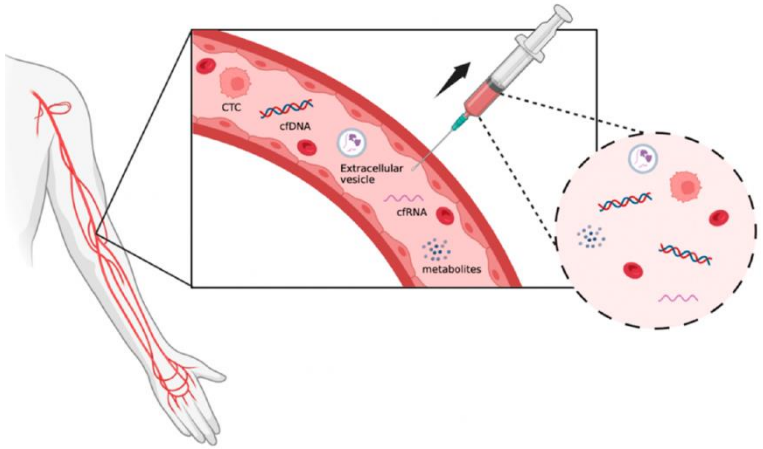
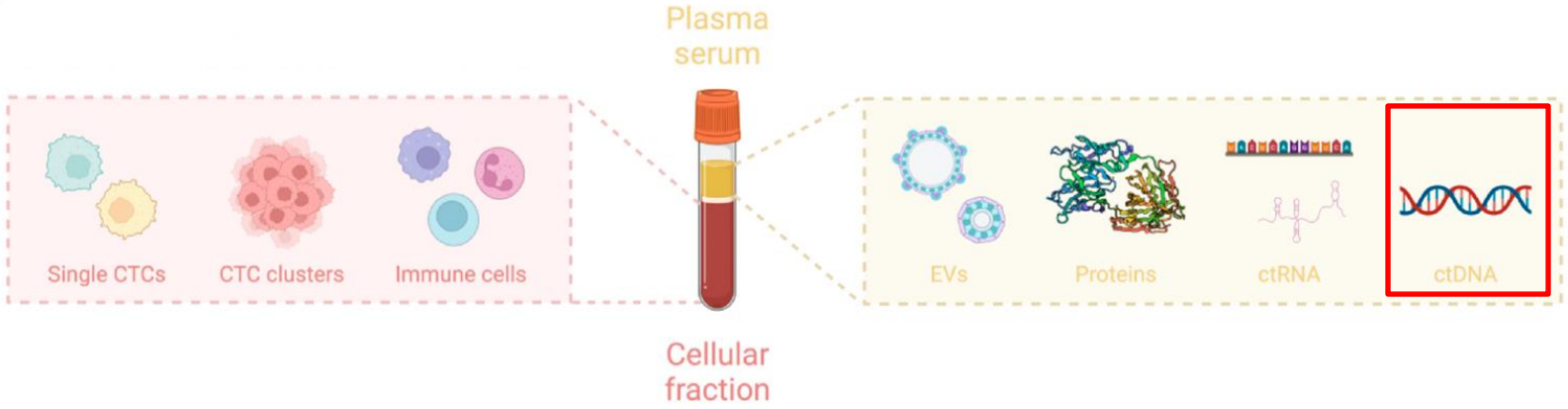
Outline

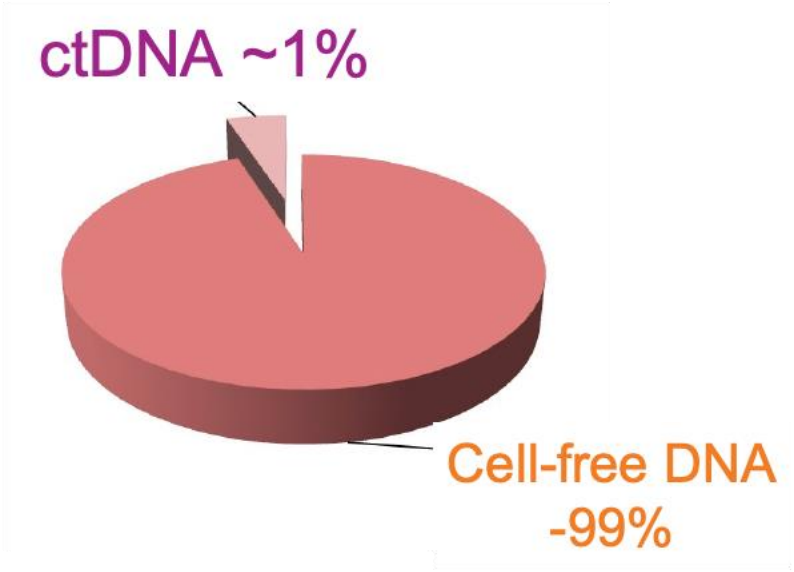
I. ctDNA 101: A historical perspective
II. Technologies to optimize preanalytical conditions
III. Technologies for ctDNA detection
IV. Challenges in ctDNA detection
V. Future directions
VI. Summary

Circulating tumor DNA (ctDNA) is a subset of cell-free DNA shed from tumors





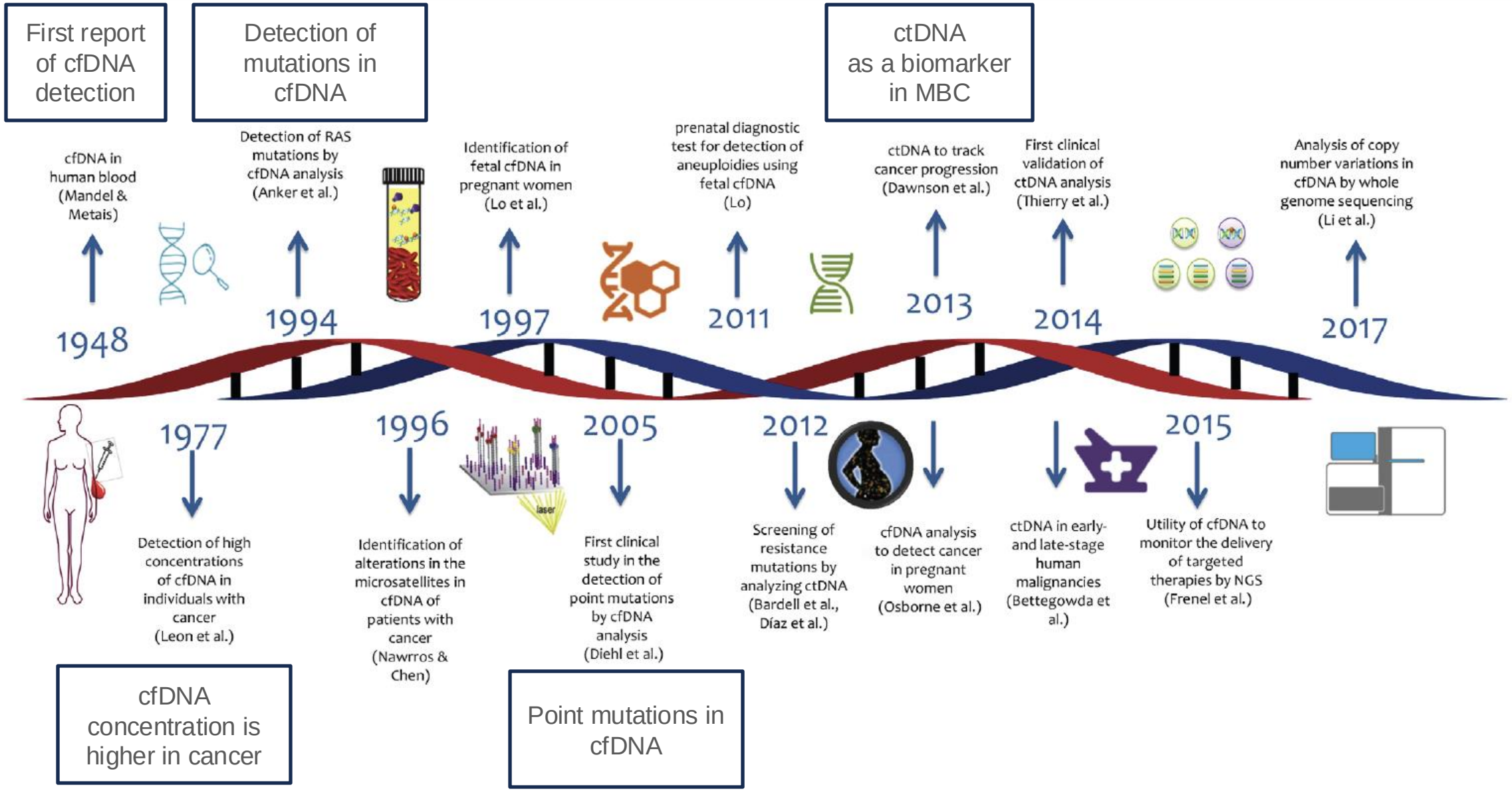




- ctDNA is a subset of cell-free DNA shed exclusively by tumor cells.
- The total amount of DNA floating in the blood is called cell-free DNA.
- Most DNA (70%) in the blood comes from dying hematopoietic cells.
- The fraction of ctDNA depends on many factors, including tumor characteristics (e.g., subtype and size).

Ho et al. *Int. J. Mol. Sci. 2024,* 25, 8594; Zavarykina et al. *Int. J. Mol. Sci. 2024,* 25, 17073; Zeng et al. *Cancer Letters* 2025; 616: 217574;

The story of cell-free DNA and circulating tumor DNA (ctDNA)



Dominguez-Vigil et al. *Oncotarget*. 2018;9 (2):2912-2933.

Circulating tumor DNA analysis is a fast-growing area of research

Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer

Sarah-Jane Dawson, F.R.A.C.P., Ph.D., Dana W.Y. Tsui, Ph.D., Muhammed Murtaza, M.B., B.S., Heather Biggs, M.A., Oscar M. Rueda, Ph.D., Suet-Feung Chin, Ph.D., Mark J. Dunning, Ph.D., Davina Gale, B.Sc., Tim Forshew, Ph.D., Betania Mahler-Araujo, M.D., Sabrina Rajan, M.D., Sean Humphray, B.Sc., Jennifer Becq, Ph.D., David Halsall, M.R.C.Path., Ph.D., Matthew Wallis, M.B., Ch.B., David Bentley, D.Phil., Carlos Caldas, M.D., F.Med.Sci., and Nitzan Rosenfeld, Ph.D.

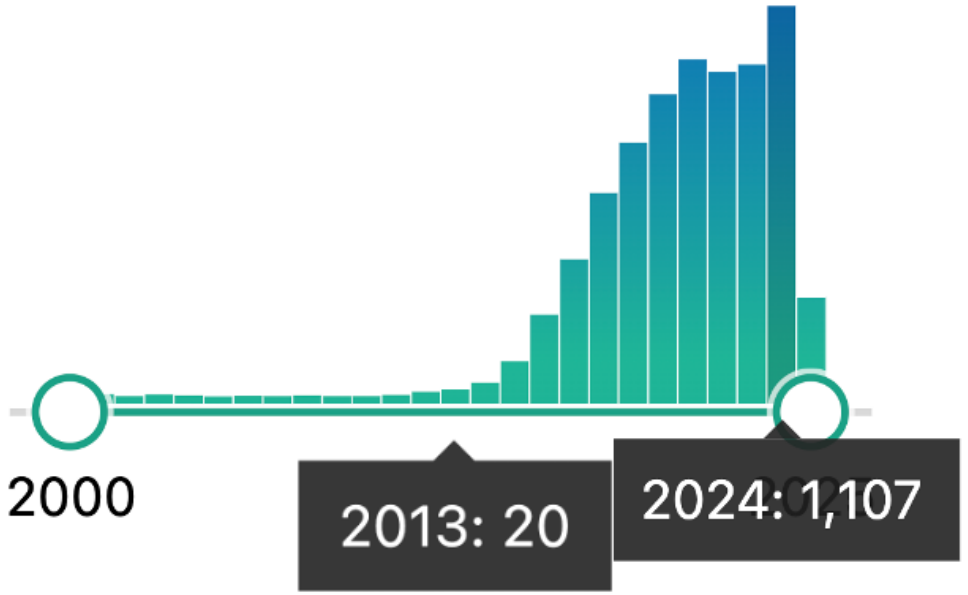
aliquots (2 ml) of plasma with the use of the [redacted] To measure the DNA carrying specific somatic genomic alterations in plasma, we carried out a microfluidic digital polymerase-chain-reaction (PCR) assay[17,23-25] ([redacted]) or direct plasma sequencing by means of tagged-amplicon deep sequencing[22] [redacted] (see the Supplementary Appendix).

N ENGL J MED 368;13 NEJM.ORG MARCH 28, 2013

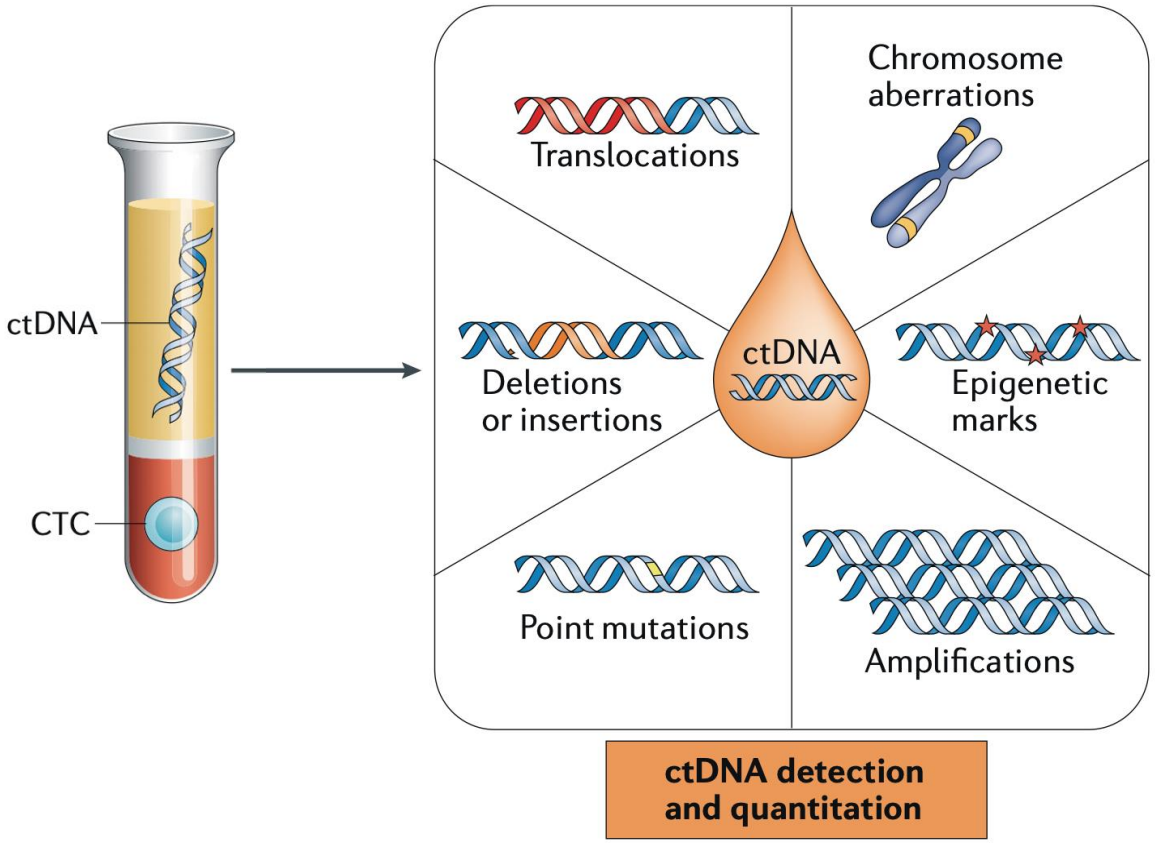
digital PCR

Next generation sequencing

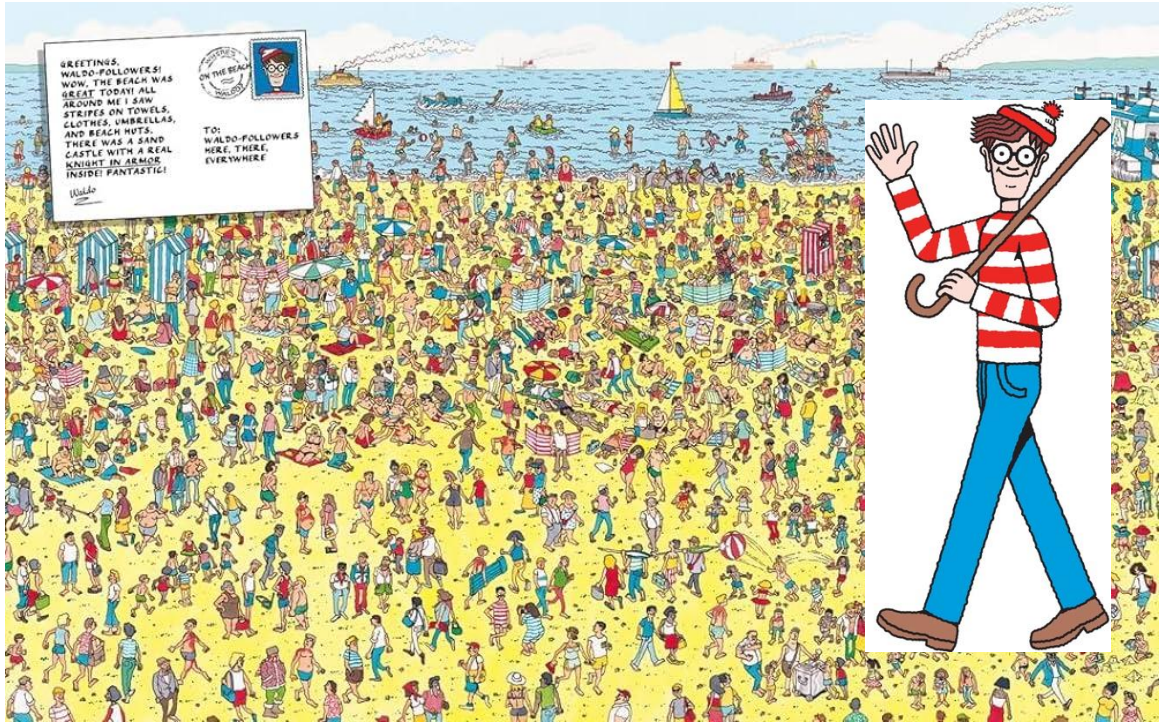
>50-fold increase in publications in a decade



ctDNA carries genetic information (e.g., mutations) found in the tumor of origin



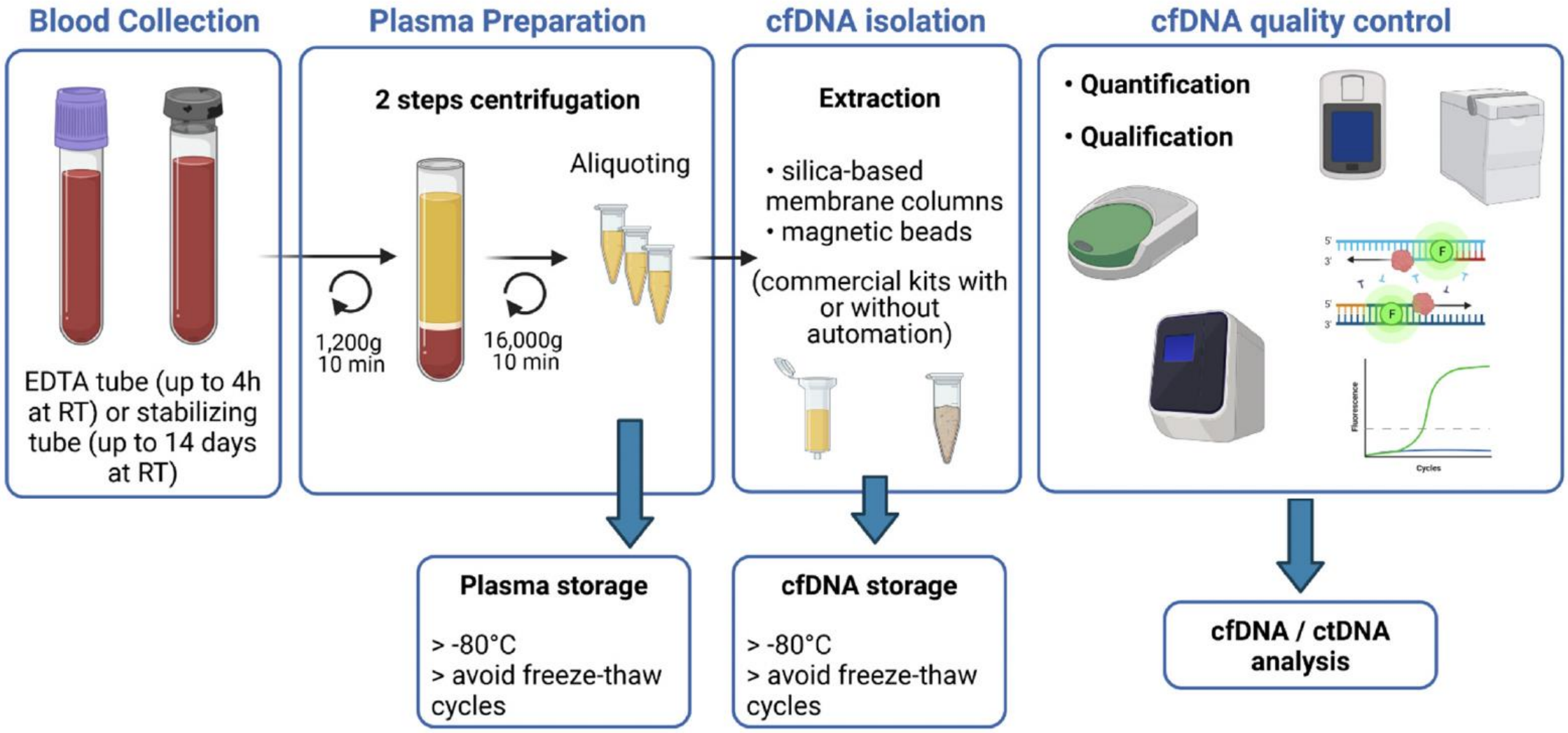
Where's Waldo? Paperback – Picture Book, November 12, 2019
by Martin Handford



https://waldo.candlewick.com/

Pantel K et al. *Nat Rev Clin Oncol*. 2019;16(7):409-424.

Preprocessing for ctDNA analysis



Bohers et al. *Pharmaceuticals*. 2021;14, 596.

Technology to optimize preanalytical conditions: Collection tubes

Plasma Collection

Tubes for plasma collection	Vendor 1	Vendor 2	Vendor 3	Vendor 4	Vendor 5
					
Cost	$	$$	$$$	$$$$	$$$$$
Blood draw volume (mL)	4, 9	10	2, 10	8.3	8.5
Stability	4-6 h at RT or 4 °C	7 days at RT (15-25 °C) or 24 h at 35 °C	14 days at RT (6-37 °C)	30 days at RT (15-25 °C) or 8 days at 37°C	7 days at RT (18-25 °C) or 16 h at RT (15-30 °C)

RT, room temperature; h, hours

Dominguez-Vigil et al. *Oncotarget*. 2018;9 (2):2912-2933.

Technology to optimize preanalytical conditions: cfDNA extraction kit

Extraction and Purification

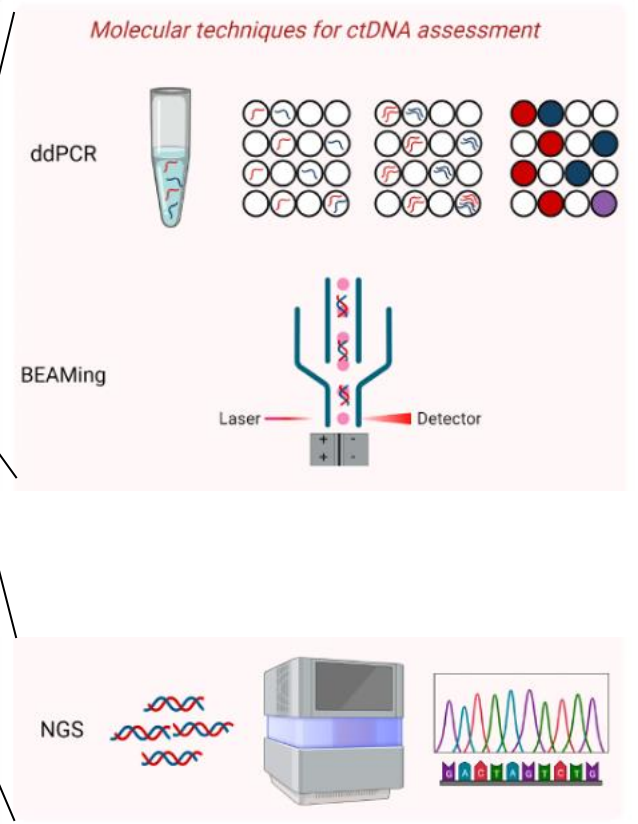
Kits for extraction and purification	Vendor 1	Vendor 2	Vendor 3	Vendor 4	Vendor 5	Vendor 6
Type of separation						
Cost	$	$$	$$$	$$$$	$$$$$	$$$$$$
Reactions per kit	10, 250	50	25, 50	50	50	10, 20, 50
Input volume of plasma (mL)	0.2–0.72	0.1–1	0.5–10	1-5	0.2–10	0.010-10
Elution volume (µL)	5-30	20	15-50	20-150	≥50	25-100

Dominguez-Vigil et al. *Oncotarget*. 2018;9 (2):2912-2933.

Methods for ctDNA detection

Method	Technology	Sensitivity	Type of Alteration
qPCR	ARMS-Scorpions PCR	0.05–0.1%	Known point mutation
	Clamping PCR	0.1–1%	
	TaqMan	0.1–1%	
Digital PCR	Beaming	0.01%	
	ddPCR	0.001%	
Target sequencing	TAm-Seq	>2%	Point mutations in gene regions; structural alterations in gene regions
	SAFE-SeqS	0.1%	
	CAPP-Seq	0.01%	
Whole genome sequencing	Digital karyotyping	0.001%	Genome-wide copy-number changes; personalized genome-wide rearrangements
	PARE	0.001%	

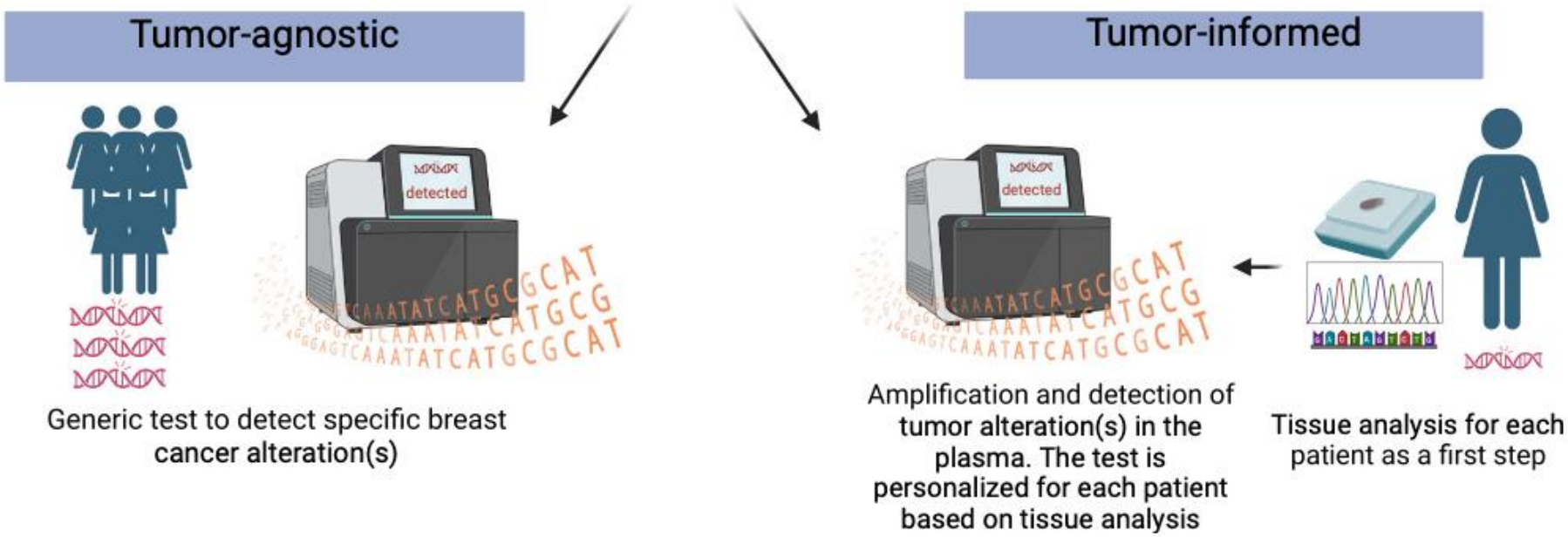
ARMS, amplification refractory mutation system; BEAMing, beads, emulsion, amplification, magnetics; CAPP-Seq, cancer personalized profiling by deep sequencing; ddPCR, droplet digital PCR; PARE, parallel analysis of RNA ends; qPCR, quantitative PCR; SAFE-SeqS, safe-sequencing system; TAm-Seq, tagged-amplicon deep sequencing.



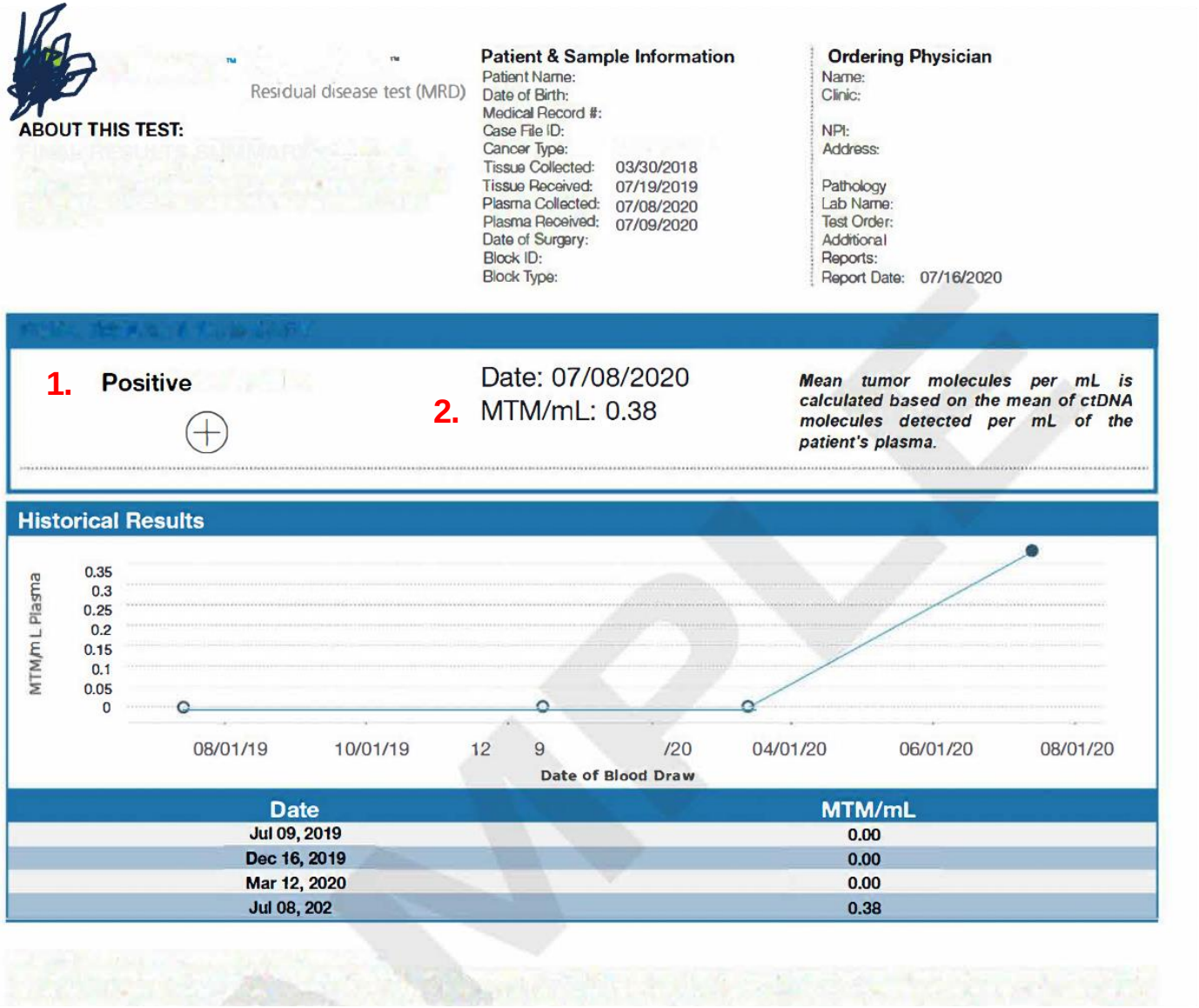
Dominguez-Vigil et al. *Oncotarget*. 2018;9 (2):2912-2933.
Vlataki et al. *Cells*. 2023, 12, 1573.

Two types of NGS-based ctDNA detection platform

Tumor-agnostic	Tumor-informed
• No need for primary tumor analysis • Fragmentomics and methylation analysis possible • Available for screening • Detection of emergent mutation(s)	• More sensitive and specific • Better validation during neadjuvant chemotherapy and minimal residual disease monitoring
• Usually less sensitive and specific	• Time consuming to sequence the tumor and generate an assay



Sample Patient Report



ABOUT THIS TEST:

Residual disease test (MRD)

Patient & Sample Information
Patient Name:
Date of Birth:
Medical Record #:
Case File ID:
Cancer Type:
Tissue Collected: 03/30/2018
Tissue Received: 07/19/2019
Plasma Collected: 07/08/2020
Plasma Received: 07/09/2020
Date of Surgery:
Block ID:
Block Type:

Ordering Physician
Name:
Clinic:
NPI:
Address:
Pathology
Lab Name:
Test Order:
Additional
Reports:
Report Date: 07/16/2020

1. **Positive** ⊕

Date: 07/08/2020
2. MTM/mL: 0.38

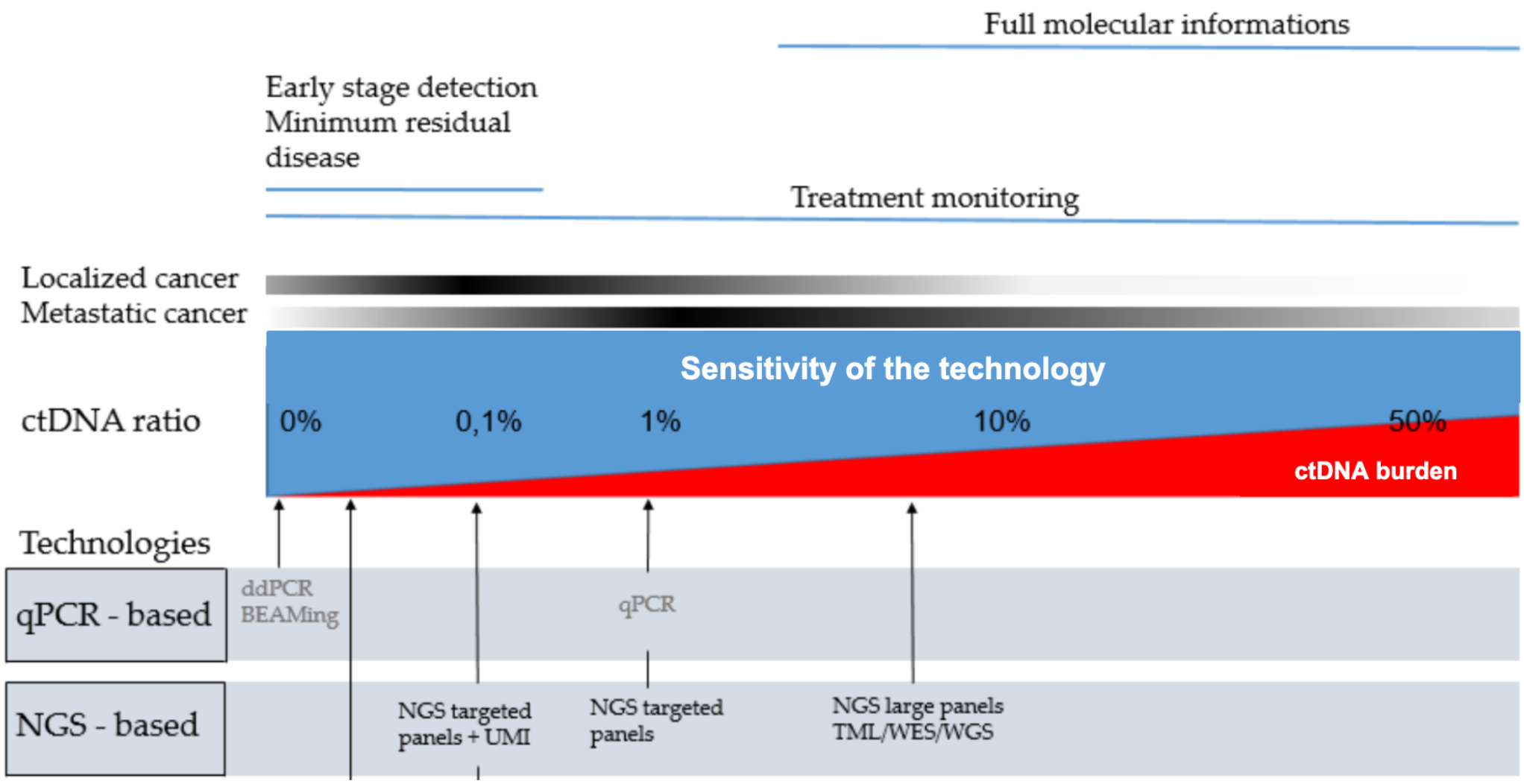
Mean tumor molecules per mL is calculated based on the mean of ctDNA molecules detected per mL of the patient's plasma.

Historical Results

Date	MTM/mL
Jul 09, 2019	0.00
Dec 16, 2019	0.00
Mar 12, 2020	0.00
Jul 08, 202	0.38

1. Binary test result: ctDNA+ or ctDNA-
2. ctDNA concentration:
   - Mean tumor molecules per mL (MTM/mL)
   - Variant allele frequency (VAF) or Mutant allele frequency (MAF)
3. List of mutations detected

Sensitivity and information from ctDNA detection methods



Moati et al. *Cancers* 2021;13:4743.

Challenges faced: Heterogeneity in the sensitivity of ctDNA assays

Review of 57 studies, including 5779 patients

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Castaneda et al. 2022[47]	9	40	8	95	0.53 (0.28-0.77)	0.70 (0.62-0.78)
Chen et al. 2017[42]	4	0	9	25	0.31 (0.09-0.61)	1.00 (0.86-1.00)
Daidone et al. 2018[48]	7	1	3	16	0.70 (0.35-0.93)	0.94 (0.71-1.00)
Garcia-Murillas et al. 2019[10]	23	0	6	115	0.79 (0.60-0.92)	1.00 (0.97-1.00)
Garcia-Murillas et al. 2022[43]	11	4	2	45	0.85 (0.55-0.98)	0.92 (0.80-0.98)
Liu et al. 2022[44]	11	97	4	222	0.73 (0.45-0.92)	0.70 (0.64-0.75)
Medford et al. 2022[49]	2	0	0	40	1.00 (0.16-1.00)	1.00 (0.91-1.00)
Olsson et al. 2015[45]	12	0	2	6	0.86 (0.57-0.98)	1.00 (0.54-1.00)
Shaw et al. 2022[35]	30	5	4	117	0.88 (0.73-0.97)	0.96 (0.91-0.99)
Shimazaki et al. 2022[50]	2	3	2	24	0.50 (0.07-0.93)	0.89 (0.71-0.98)
Turner et al. 2017[39]	14	0	4	25	0.78 (0.52-0.94)	1.00 (0.86-1.00)

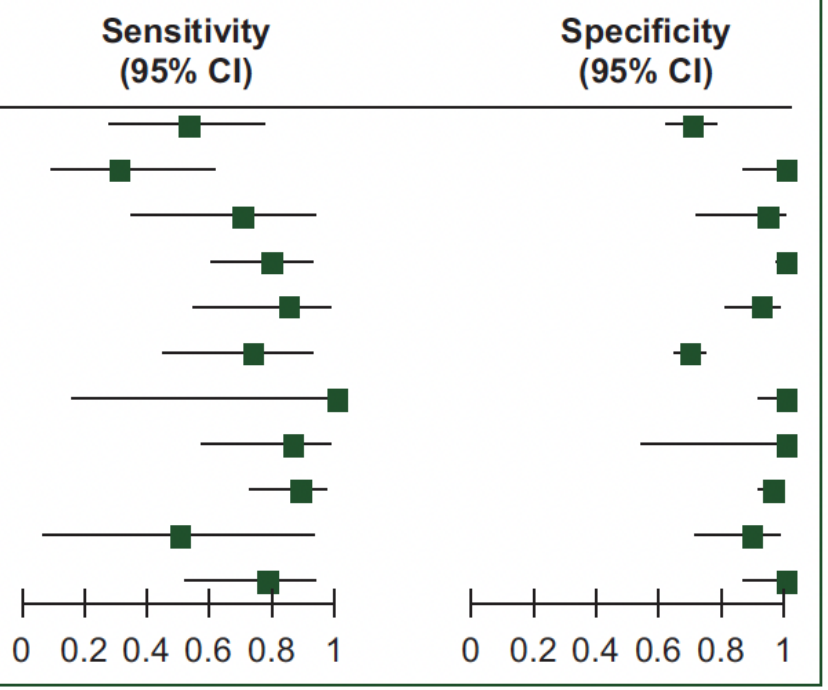
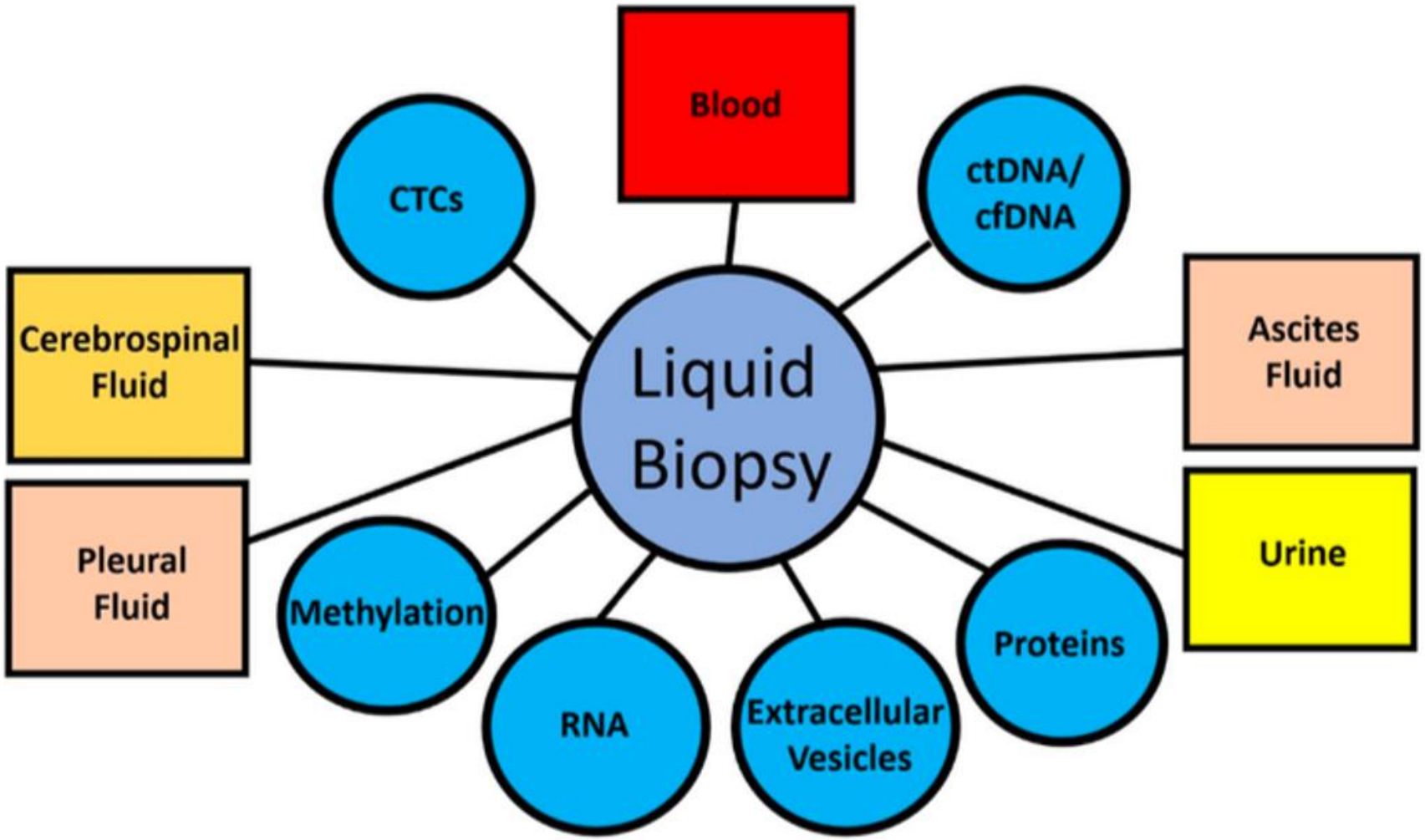


Figure 4. Sensitivity and specificity of circulating tumor DNA (ctDNA) detection for the diagnosis of overt recurrent disease.[47-50]
CI, confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

The sensitivity of ctDNA for diagnosis of overt recurrent disease ranged from 0.31 to 1.00.

Nader-Marta G et al. *ESMO Open*. 2024;9(3):102390.

Integrating ctDNA with other liquid biopsy-based biomarkers from other bodily fluids



Nikanjam et al. *J Hema & Oncol* 2022; 15:13

Challenges faced: ctDNA testing beyond blood

ctDNA analysis using the **cerebrospinal fluid (CSF)** in patients with brain metastasis and/or leptomeningeal disease

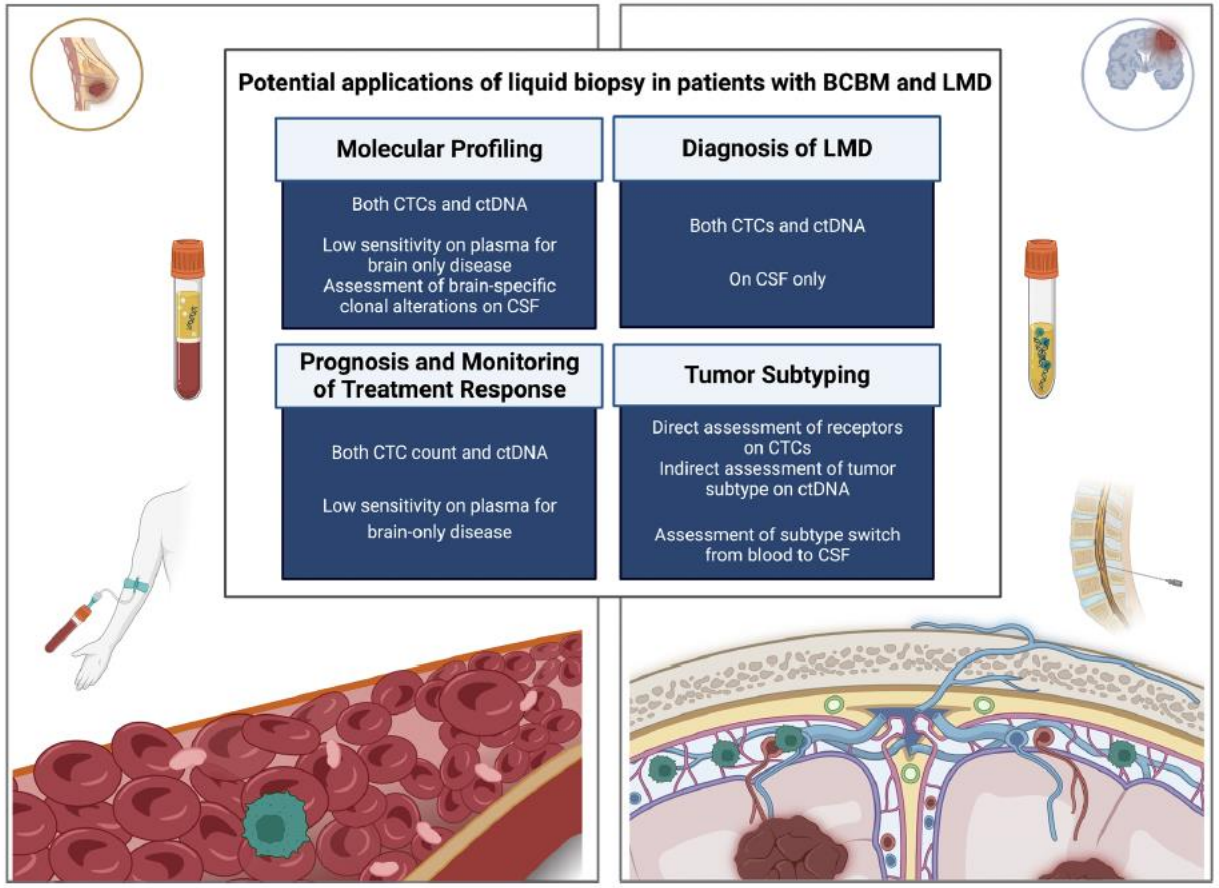


Fig. 1 Potential applications of liquid biopsy in patients with BCBM and LMD. Created with BioRender.com. BCBM breast cancer brain metastasis, CSF cerebrospinal fluid, CTC circulating tumor cells, ctDNA circulating tumor DNA, LMD leptomeningeal disease.

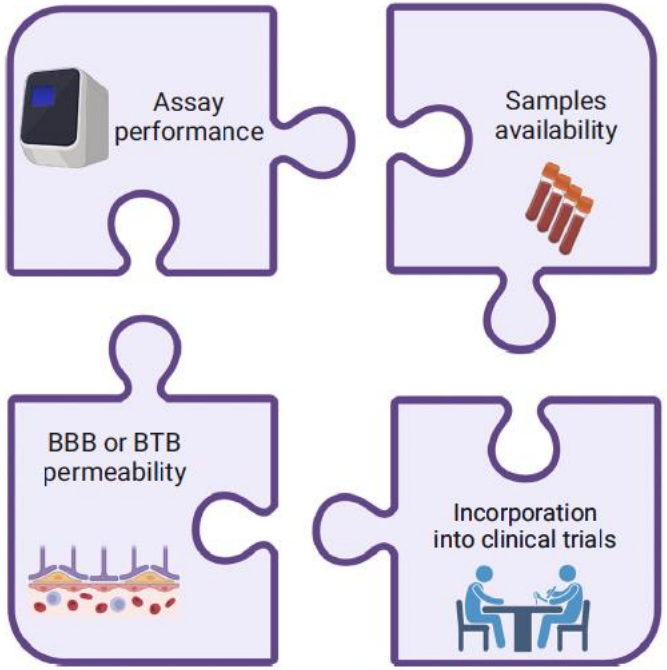
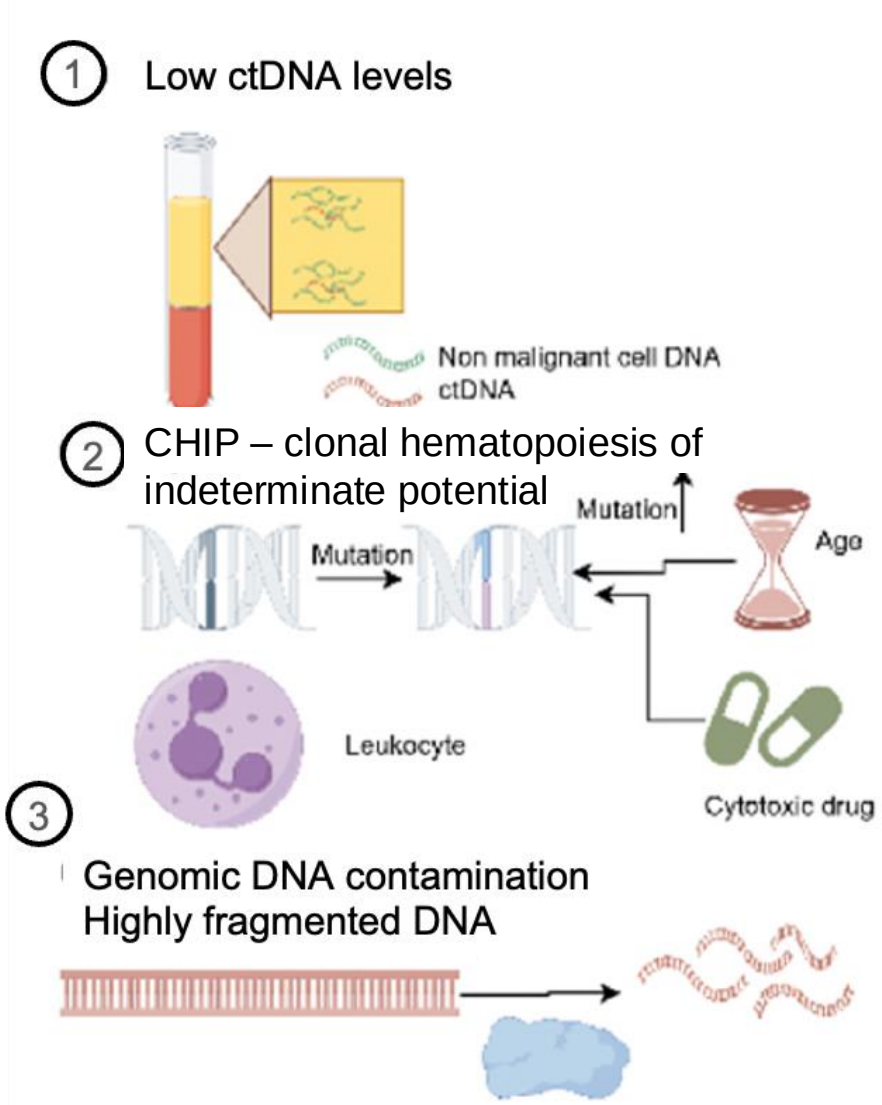
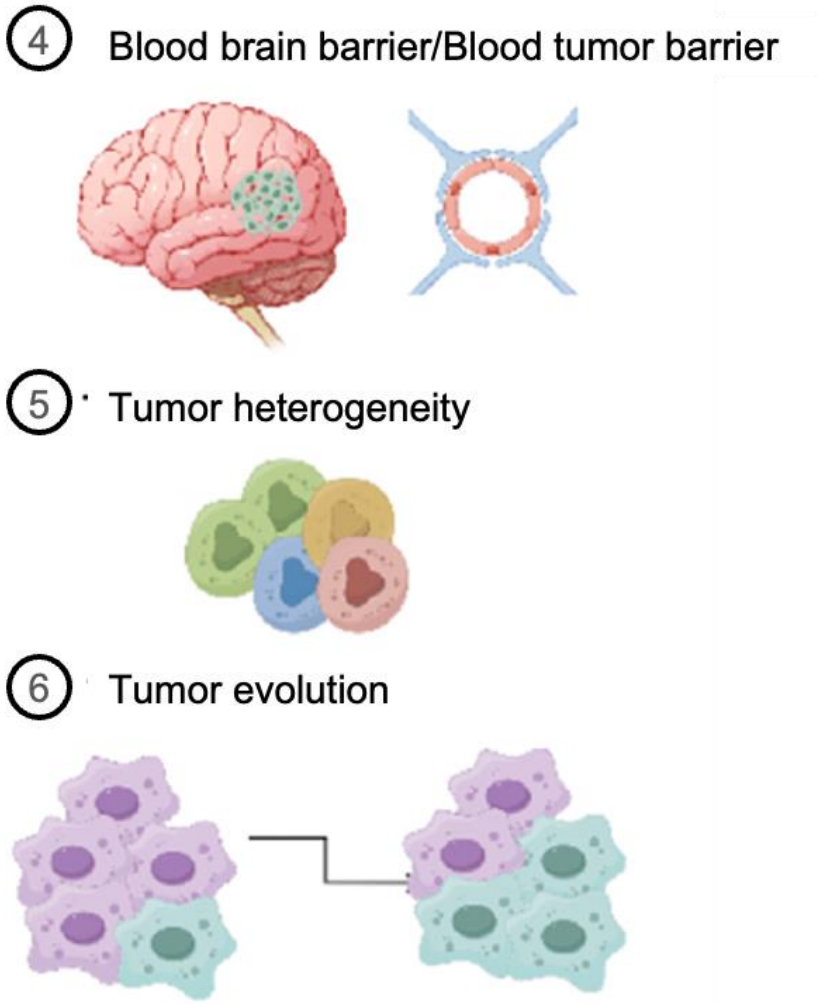


Fig. 2 Challenges for liquid biopsy development in patients with central nervous system metastasis from breast cancer. Created with BioRender.com. BBB blood-brain barrier, BTB blood-tumor barrier.

Challenges : Technical and biological barriers to ctDNA detection





The emergence of *ESR1* mutations will be missed using a tumor-informed panel based on the primary tumor.

Zeng et al. *Cancer Letters* 2025; 616: 217574

Challenges : Commercially available ctDNA assays come in many flavors

Lack of standardization and validation across platforms

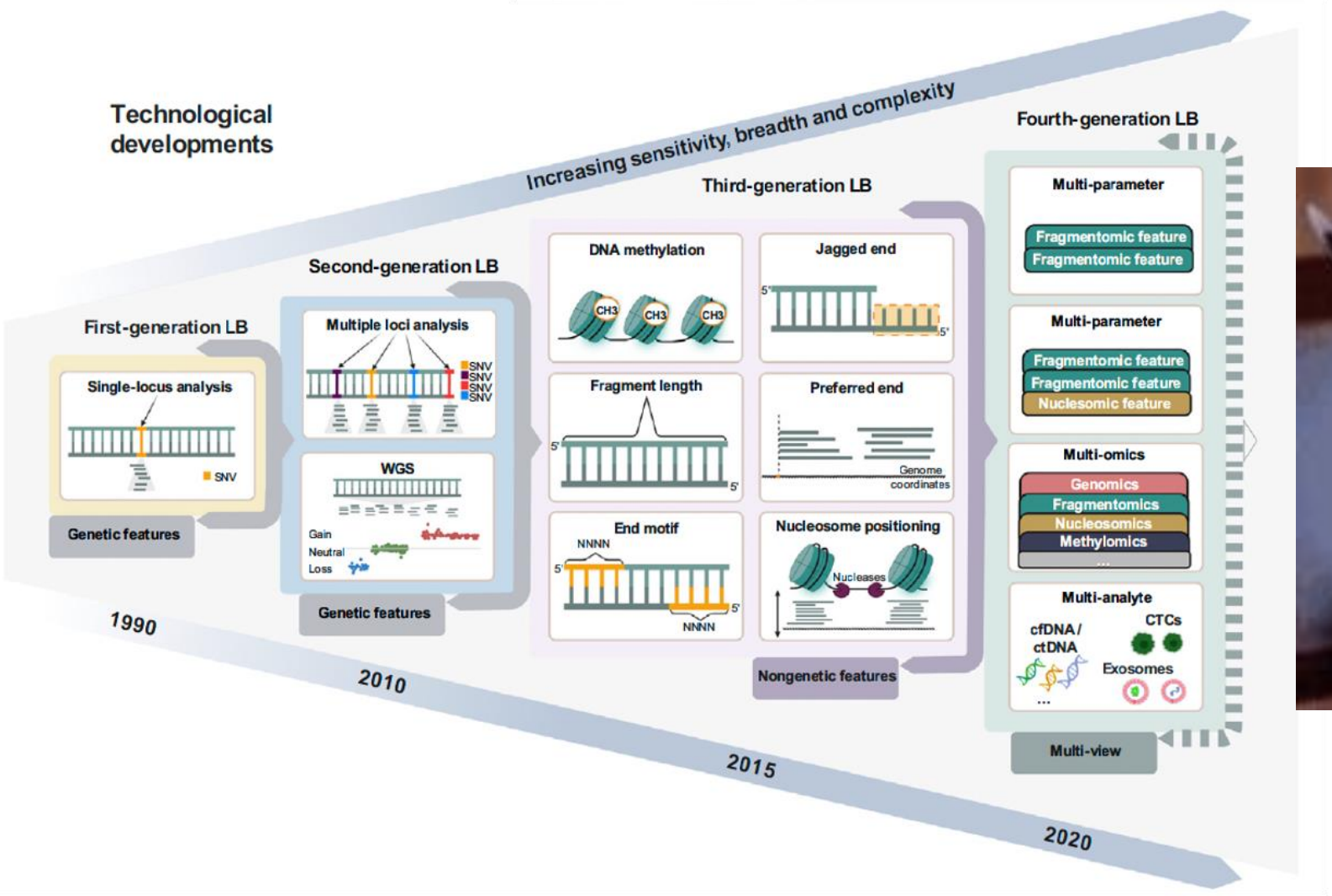
Selected Commercially Available Circulating Tumor DNA Assays

Assay	Assay Type	Clinical Utility	Disease Stage (early *v* metastatic)
Assay 1	Tumor-informed	MRD detection	Early-stage breast cancer
Assay 2			
Assay 3			
Assay 4			
Assay 5			
Assay 6	Tumor-agnostic		
Assay 1	Tumor-agnostic	300-gene liquid biopsy	Metastatic breast cancer
Assay 2		74-gene liquid biopsy	
Assay 3		105-gene liquid biopsy	
Assay 4		44-gene liquid biopsy for solid tumors	
Assay 5	Tumor-informed	Circulating nucleic acid sequencing of up to 23,000+ genes	

Abbreviation: MRD, minimal residual disease.

Xi et al. *JCO Oncol Pract 2024:1460-1470*

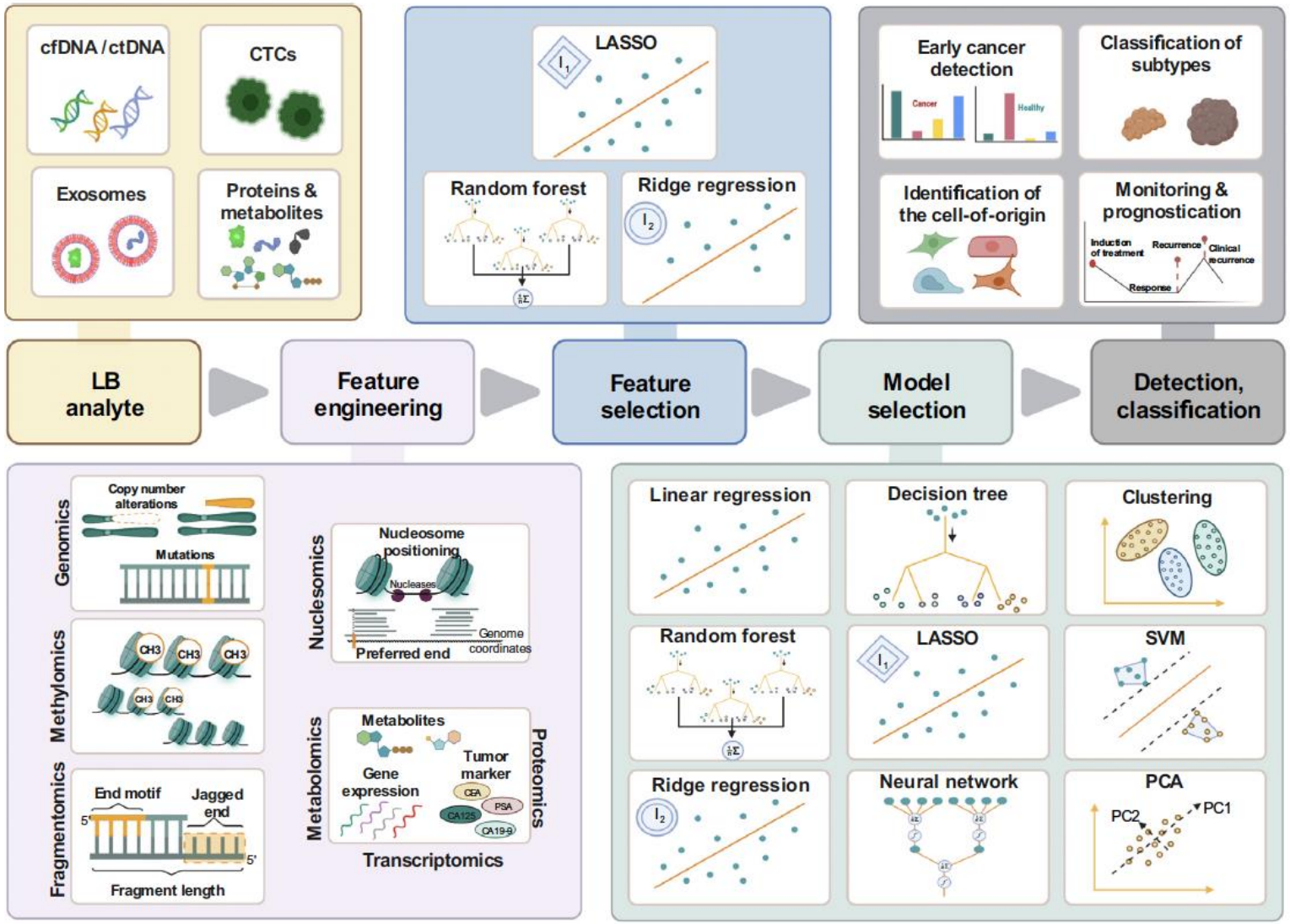
The story of circulating tumor DNA (ctDNA): And the plot thickens!



Moser et al. *Trends in Genetics* 2023; 39:4

v. Future directions

Enter machine learning and artificial intelligence in liquid biopsy research



Summary: The message is in the method

- ctDNA testing is a fast-growing area of research.
- Optimized preanalytical parameters have led to clinical trials using ctDNA as an endpoint or a correlative biomarker.
- ctDNA assays using PCR and/or NGS have allowed higher sensitivity and coverage (number of loci tested).
- Numerous technical and biological challenges need to be overcome.
- Machine learning and AI may help identify optimal liquid biopsy biomarker combinations for predicting outcomes.
- There is a lack of standardization and cross-platform validation for ctDNA testing.