Circulating tumor DNA-based analyses of solid tumors

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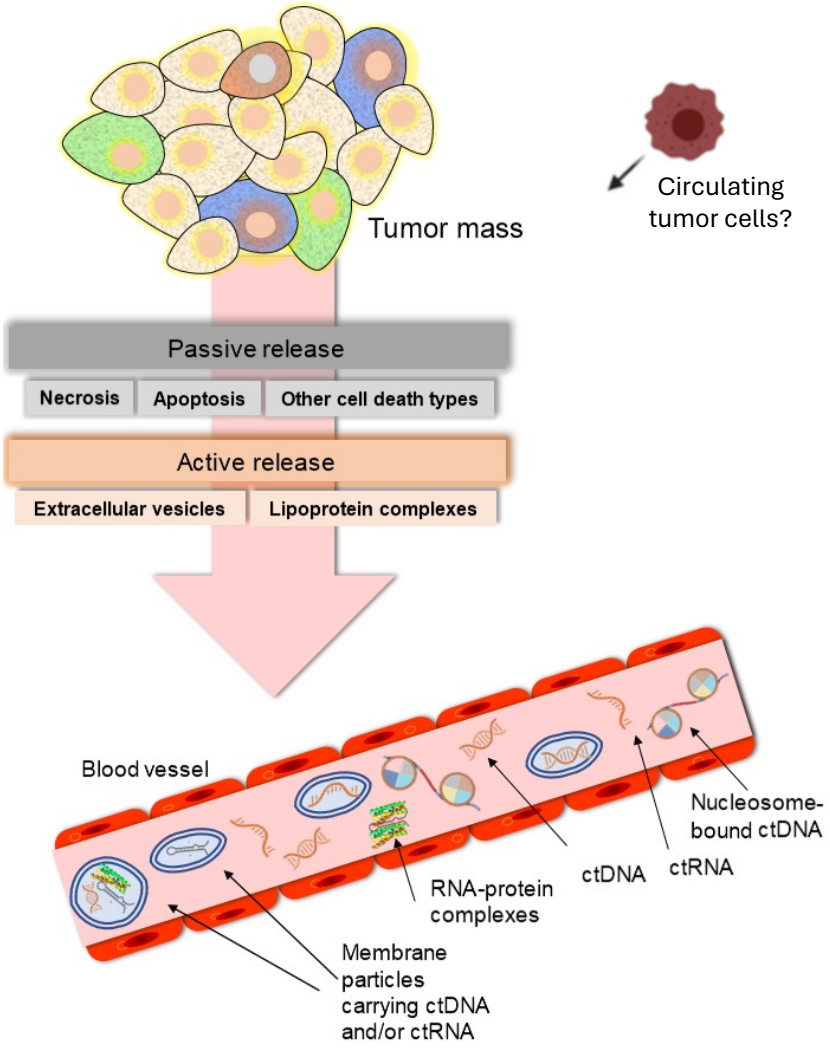
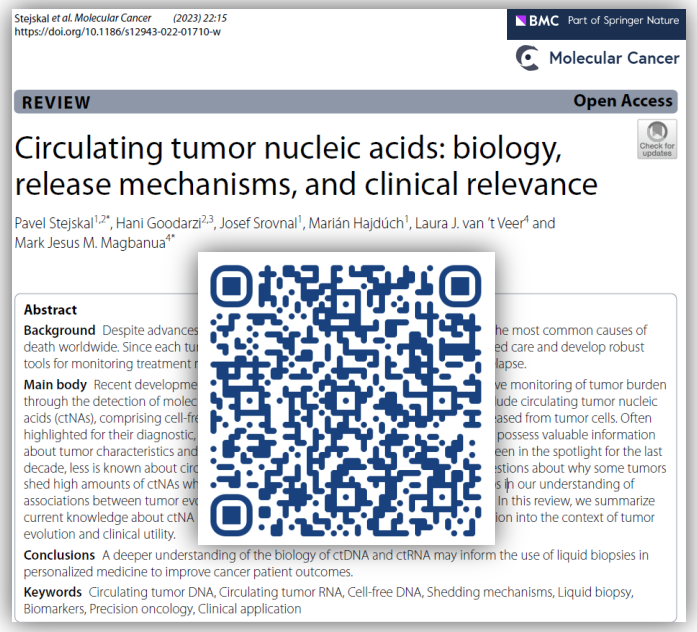
Circulating Cell-free/Tumor DNA (cfDNA/ctDNA)



Circulating tumor nucleic acid release mechanisms



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Circulating tumor nucleic acids: biology, release mechanisms, and clinical relevance

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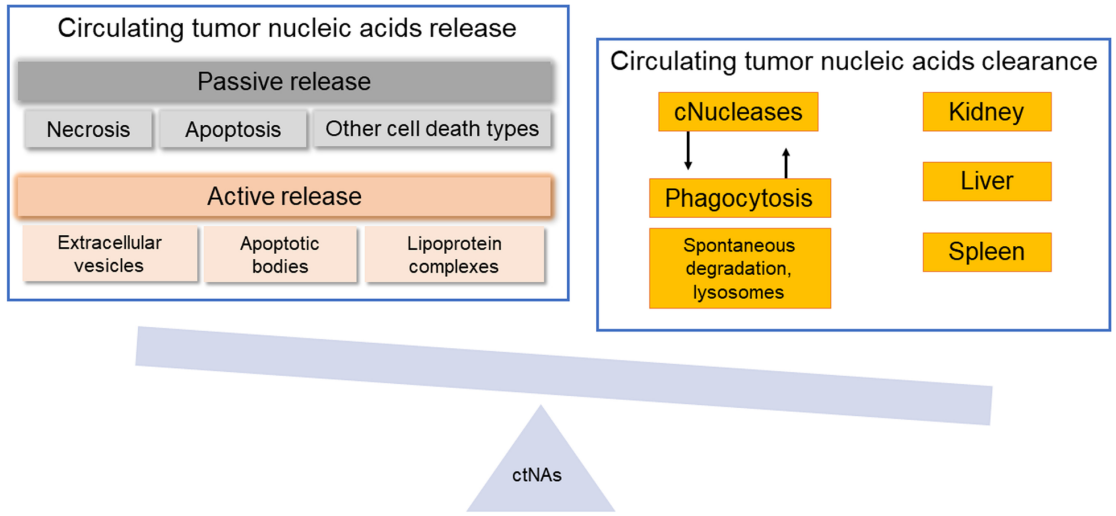
Abstract

Background Despite advances … he most common causes of death worldwide. Since each tu… ed care and develop robust tools for monitoring treatment r… lapse.

Main body Recent developme… ve monitoring of tumor burden through the detection of molec… ude circulating tumor nucleic acids (ctNAs), comprising cell-fre… ased from tumor cells. Often highlighted for their diagnostic,… possess valuable information about tumor characteristics and… een in the spotlight for the last decade, less is known about circ… stions about why some tumors shed high amounts of ctNAs wh… s in our understanding of associations between tumor evo… In this review, we summarize current knowledge about ctNA… ion into the context of tumor evolution and clinical utility.

Conclusions A deeper understanding of the biology of ctDNA and ctRNA may inform the use of liquid biopsies in personalized medicine to improve cancer patient outcomes.

Keywords Circulating tumor DNA, Circulating tumor RNA, Cell-free DNA, Shedding mechanisms, Liquid biopsy, Biomarkers, Precision oncology, Clinical application



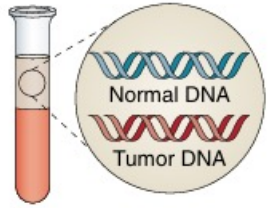
Factors determining the levels of circulating tumor nucleic acids

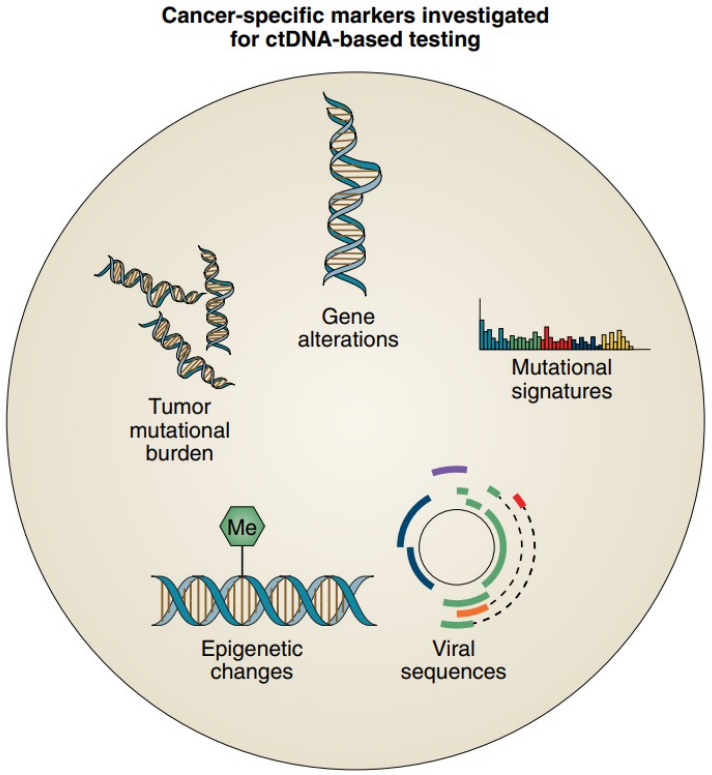
The Potential of ctDNA and Liquid Biopsies in Cancer

Diagnosis
Early detection
Identification of actionable alterations
Measuring tumor heterogeneity

Monitoring for Residual Disease
Determining treatment efficacy
Testing for new actionable alterations
Drug resistance

Monitoring for Therapeutic Response
Assessing remission or progression





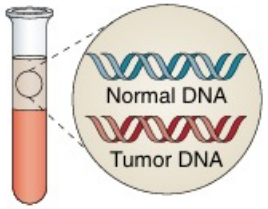
Cescon, D.W., *et al. Nat Cancer* **1**, 276–290 (2020).

Approved diagnostic tests for detecting altered genes in cfDNA

Test	Approval	Cancer type	Analyte	Technology	Target
Cobas® EGFR Mutation Test v2	FDA, EMA	Non-small cell lung	Blood (cfDNA), tissue DNA (FFPE)	RT-PCR	*EGFR* gene
FoundationOne® Liquid CDx	FDA	Multiple	Blood (cfDNA)	NGS	Panel (300 genes)
Guardant360 CDx	FDA	Multiple	Blood (cfDNA)	NGS	Panel (55 genes)
Qiagen *therascreen* PIK3CA RGQ PCR Kit	FDA	Breast (selection of patients eligible for treatment with alpha-selective PIK3-inhibitor alpelisib)	Blood (cfDNA), tissue DNA (FFPE)	RT-PCR	*PIK3CA* gene
Epi proColon®	FDA	Colorectal	Blood (cfDNA)	RT-PCR	Methylated *SEPT9* gene

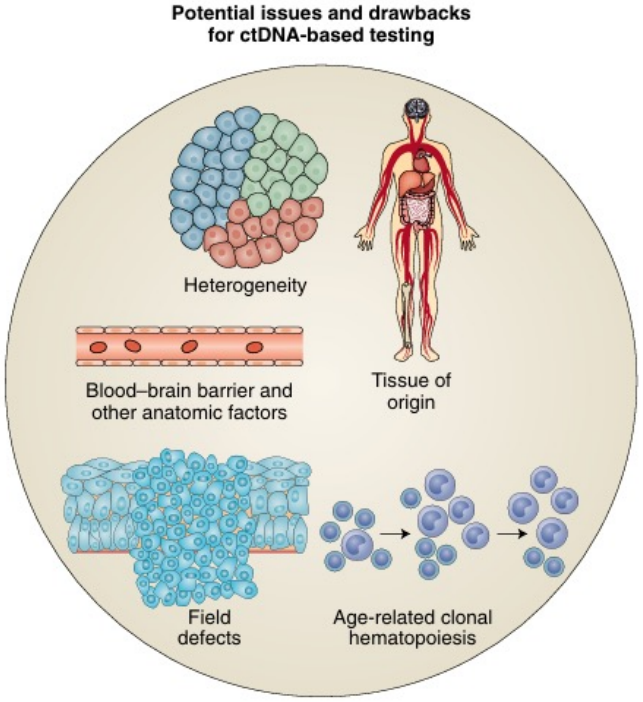
FDA Food and Drug Administration, *EMA* European Medicines Agency, *RT-PCR* real-time PCR, *NGS* Next generation sequencing

The Challenges of ctDNA and Liquid Biopsies in Cancer



Limitations		Implications
Low sensitivity	Low ctDNA to cfDNA ratio owing to the predominance of clonal haematopoiesis	Negative results may be non-informative
Non-uniform ctDNA shedding	Heterogeneity in ctDNA shedding from lesions (eg, brain metastases), anatomic barriers	Potentially low ctDNA levels for detection, thus limiting the clinical application
Subclone origination/specificity	Difficult to identify the origin of ctDNA from multiple lesions or anatomically restricted tumors (early-stage NSCLC and sarcomas)	Identification of subclone origination by ctDNA may require tumor biopsy assessment
Lack of standardization	Preanalytical as well as analytical approaches vary across sample types and laboratories	Limited use in the clinial settings

Tivey, A., *et al. Nat Rev Clin Oncol* **19**, 600–612 (2022), Domínguez-Vigil IG, *et al. Oncotarget*.9:2912-2922 (2017), Stejskal, P., *et al. Mol Cancer* **22**, 15 (2023).



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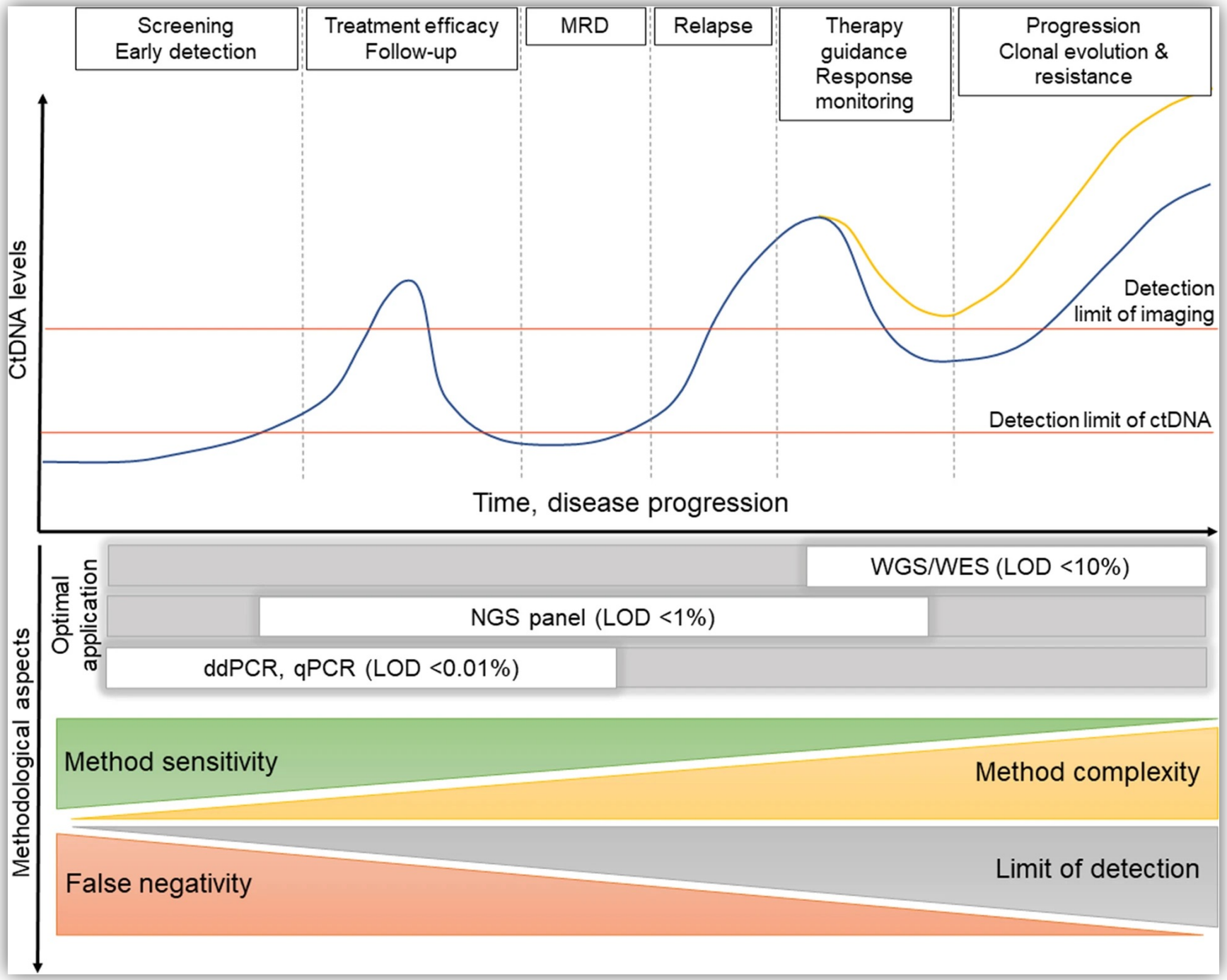
International and interdisciplinary partnerships and consortia

SPIDIA4P consortium - Standardization and improvement of generic Preanalytical tools and procedures for In-vitro DIAgnostics, **Cancer-ID**, **ISLB** - International Society for Liquid Biopsy, **ILSA** - International Liquid Biopsy Standardization Alliance, **ELBS** – European Liquid Biopsy Society, **BLOODPAC** - US Blood Profiling Atlas of Cancer

IMTM

Clinical Utility in Context of Disease Evolution Over Time and Methodological Aspects of ctDNA Testing

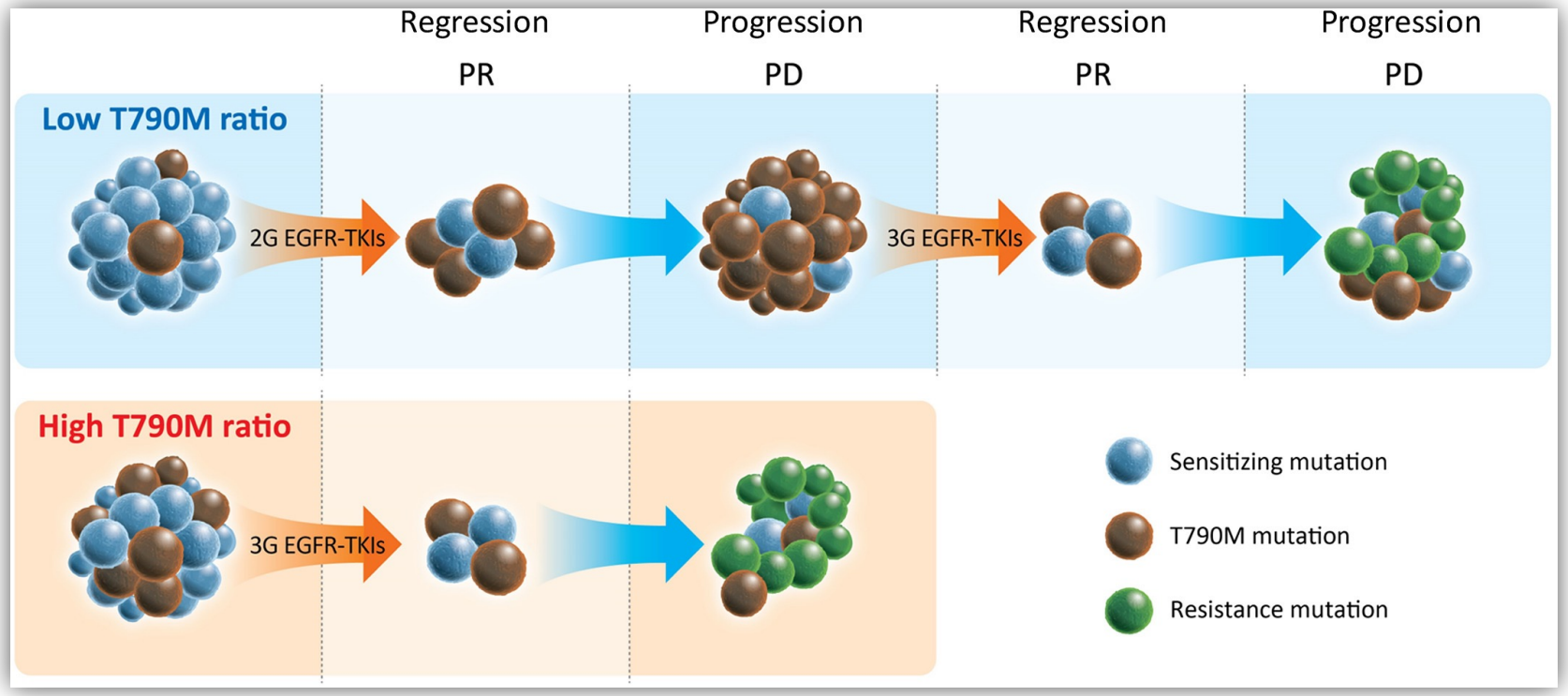


Stejskal, P., *et al. Mol Cancer* **22**, 15 (2023).

EGFR T790M Mutation Detection in cfDNA

Non-small cell lung cancer (NSCLC)

- **Despite the initial response** to anti EGFR receptor-tyrosine kinase inhibitors (TKIs), **most** patients progress within 9–14 months
- **Resistance to EGFR-TKIs -** the occurrence of a secondary *EGFR* kinase domain mutation in exon 20**, the T790M**
- **Third generation of EGFR-TKIs** developed - irreversible blockage of T790M mutant *EGFR*
- Many patients on progression develop lesions in inaccessible locations + poor performance status of the patients difficult to re-biopsy
- Up to 40% of relapsed NSCLC patients unable to provide a tumor tissue sample for molecular analysis



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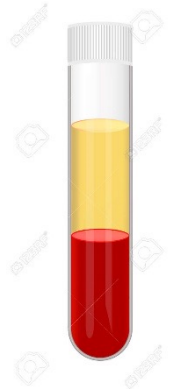
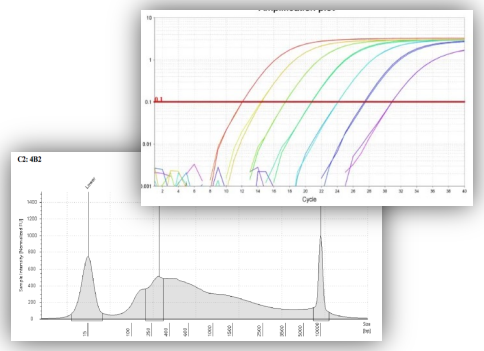
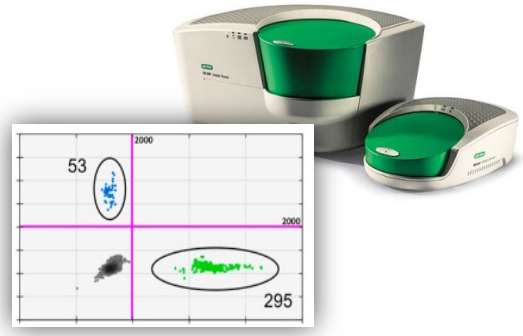
EGFR T790M Mutation Detection in cfDNA



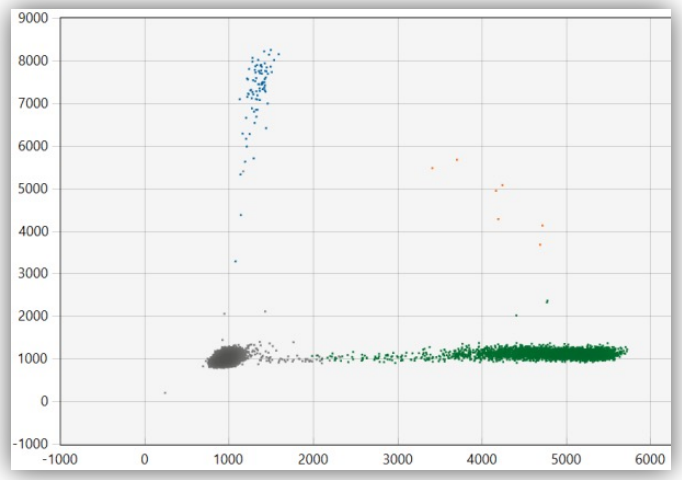
Plasma isolation



Cell-free DNA extraction, quality control



Circulating tumor DNA ddPCR-based detection



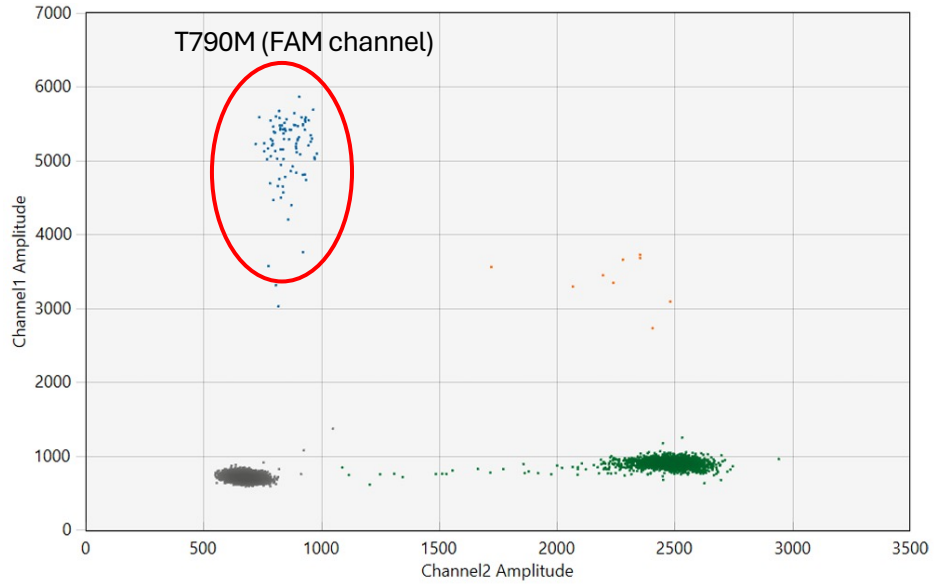
Data analysis

Sample	Channel (target)	Copies/µl **Average** ±StDev
5% T790M	FAM (MUT)	**3,60** ±0,31
	HEX (WT)	48,25 ±6,08
1% T790M	FAM (MUT)	**0,76** ±0,21
	HEX (WT)	40,94 ±5,02
0.1% T790M	FAM (MUT)	**0,08** ±0,01
	HEX (WT)	51,46 ±3,64
0% T790M (WT)	FAM (MUT)	**0,00** ±0,00
	HEX (WT)	54,25 ±1,98

Limit of detection for ddPCR-based EGFR-T790M testing (using Horizon reference cfDNA)

Patient	Channel (target)	Copies/µl **Average** ±StDev
1	FAM (MUT)	**4,81** ±0,81
	HEX (WT)	308 ±11,31
2	FAM (MUT)	**2,13** ±0,36
	HEX (WT)	62,35 ±4,17
3	FAM (MUT)	**4,27** ±0,26
	HEX (WT)	141 ±1,41
4	FAM (MUT)	**6,91** ±0,44
	HEX (WT)	142 ±2,83

EGFR-T790M detection in plasma of patients with EGFR-T790M positive NSCLC tumors



Amplitude 2D Chart FAM/HEX Channels, patient 4

CfDNA and ctDNA as Linked Reflection of Therapy

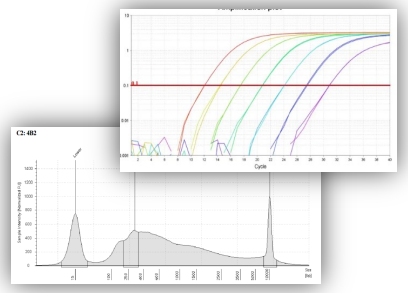
- hLINE-1 sequences (human long interspersed nuclear element-1)
  - ~100,000 of these elements spread across the human genome
  - sensitive approach for quantifying human DNA in small volumes of samples

- Plasma hLINE-1 DNA concentration shown to be related to tumor volume
- Indicator of the response to therapy

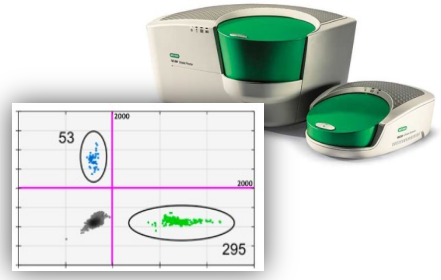
Rago, C., (2007). *Cancer research*, *67*(19), 9364-9370.
Rostami, A., (2020). *STAR protocols*, *1*(3), 100145.

Sample (in time)	**Total cfDNA qPCR** (ng/µl)	**Concentration** (copies/µL) **by ddPCR**			
		E545K MUT	**E545K WT**	**H1047R MUT**	**H1047R WT**
1	26,57	197,32	1364,15	18,65	549,01
2	9,72	200,87	442,39	1,93	291,12
3	4,52	153,20	282,63	2,76	165,93

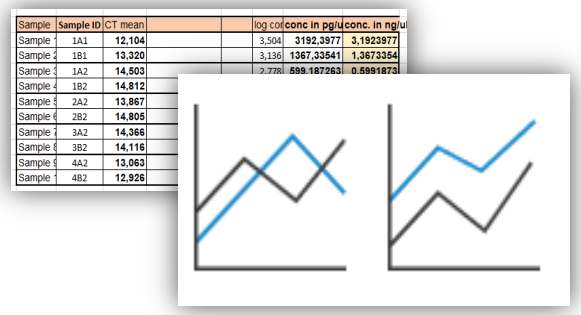
Quantitative analysis of cfDNA and ctDNA extracted from pleural effusion of metastatic breast cancer patient



cfDNA extraction, Qualitative and quantitative analyses



ctDNA quantification



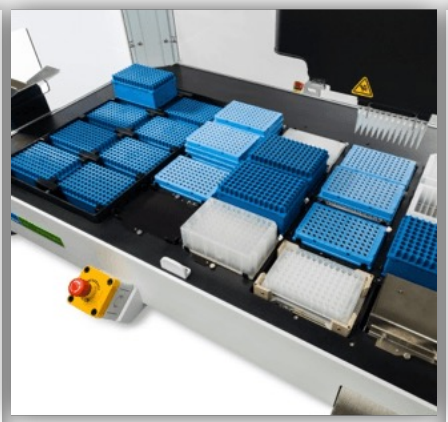
Data analysis, interpretation

- Mutation load as percentage of the total cfDNA

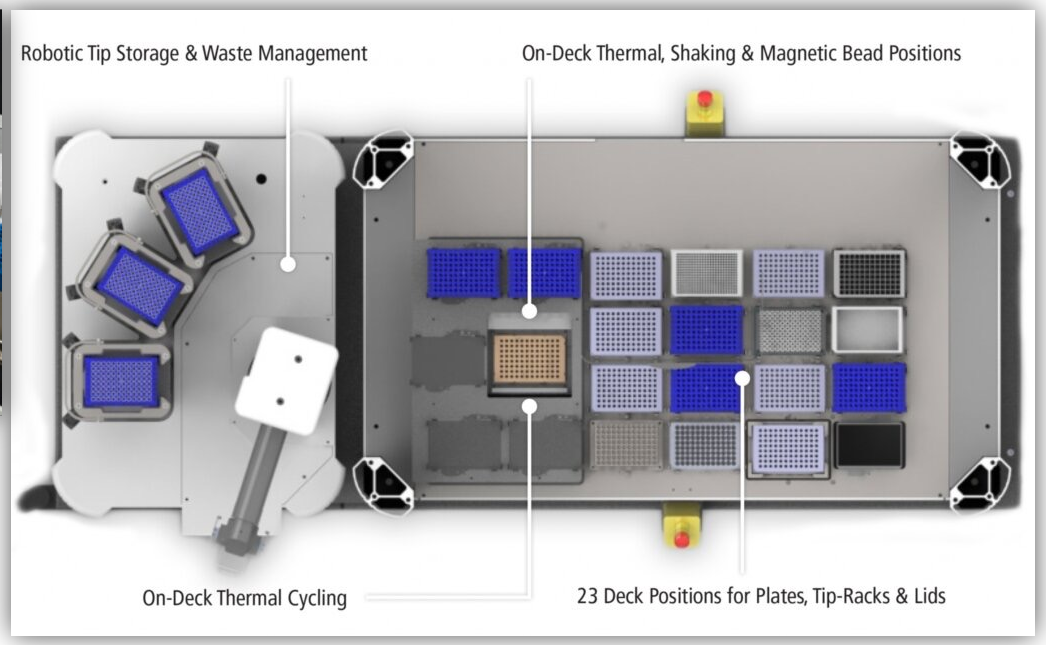
Following Latest Solutions for ctDNA Sequencing

- New generation of Illumina TSO 500 ctDNA NGS panel recently released
  - Faster turnaround time, greater analytical sensitivity with lower cfDNA input requirements, more streamlined workflow
  - Will be implemented at IMTM soon for PDAC and CRC patient plasma samples

- Sciclone G3 NGSx iQ Workstation recently installed to the lab
  - Full automation of PCR preparation and walk-away NGS library preparation with integrated on-deck thermal cycler and Twister III robotic arm to perform library preparation workflows
  - Eliminating variability and risks related to incubation and PCR timing

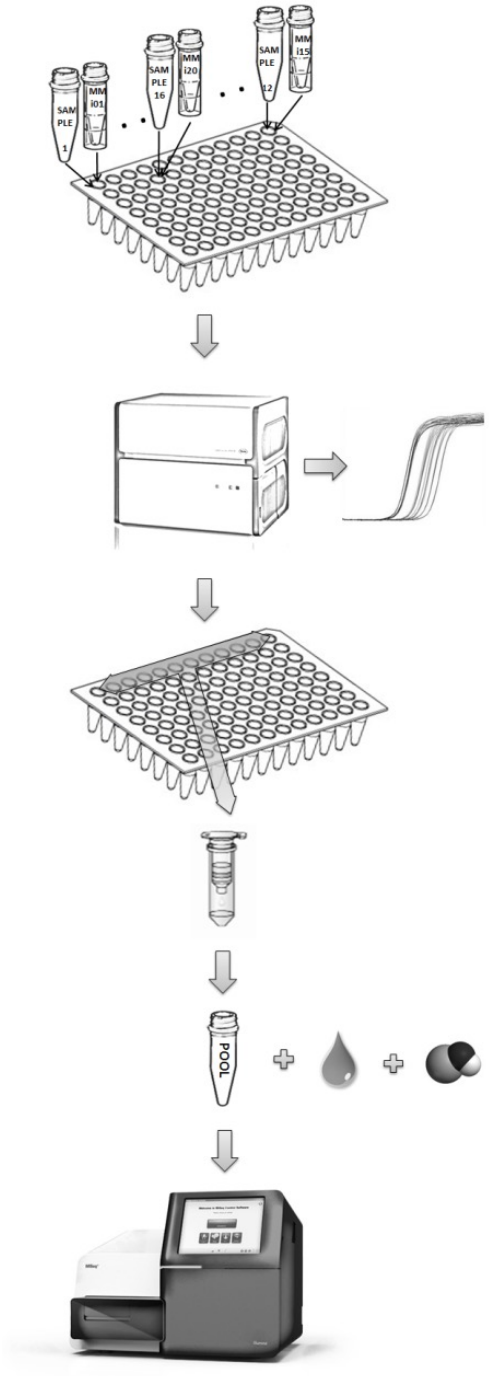


Sciclone G3 NGSx iQ Workstation



Tumor Testing “in a Fast Way” - fastGEN

- User-friendly diagnostic kit with excellent analytical parameters and extremely fast processing developed by IMTM
- Designed to rapidly prepare the sequencing library required for genotyping and indicating the correct therapy
- Robustness achieved by the use of short amplicons obtained by a single polymerase chain reaction with special labelled hybrid primers
- Thanks to the improved sensitivity – applicable also for cfDNA testing



Future Perspectives

- Unprecedented development of liquid biopsy by combining molecular biology, genetics, and computational approaches.
- Generation of vast amounts of data potentially revealing new associations
- Fragmentomic and epigenetic features of cfDNA promising for detecting targets closely reflecting the tissue of origin
- Machine learning algorithms are promising tools facilitating the clinical use of epigenetic and fragmentomic features of ctDNA as well as cancer-related ctRNA signatures
- The clinical implementation of ctNA data can lead to routine preventive screening for predisposition to cancer, monitoring drug efficacy, and predicting the potential for distant recurrence
- CtDNA analysis - potential to transform cancer diagnosis and management

Thank you for your attention!

Thanks to my colleagues

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