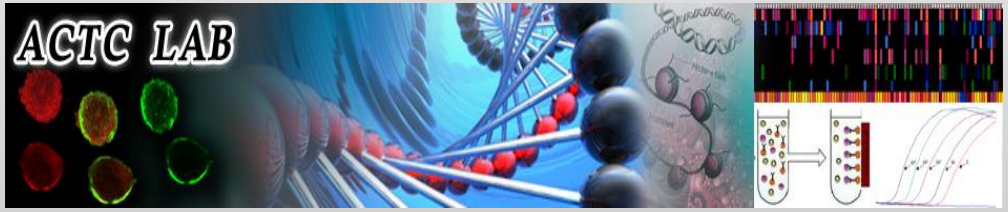
STEPUPIORS SUMMER SCHOOL
"LIQUID BIOPSY TECHNOLOGIES"
ATHENS, SEPT 30 – OCT 04, 2024

ctDNA: methylation analysis/ MSP, cfMeDIP

Aliki Ntzifa, PhD

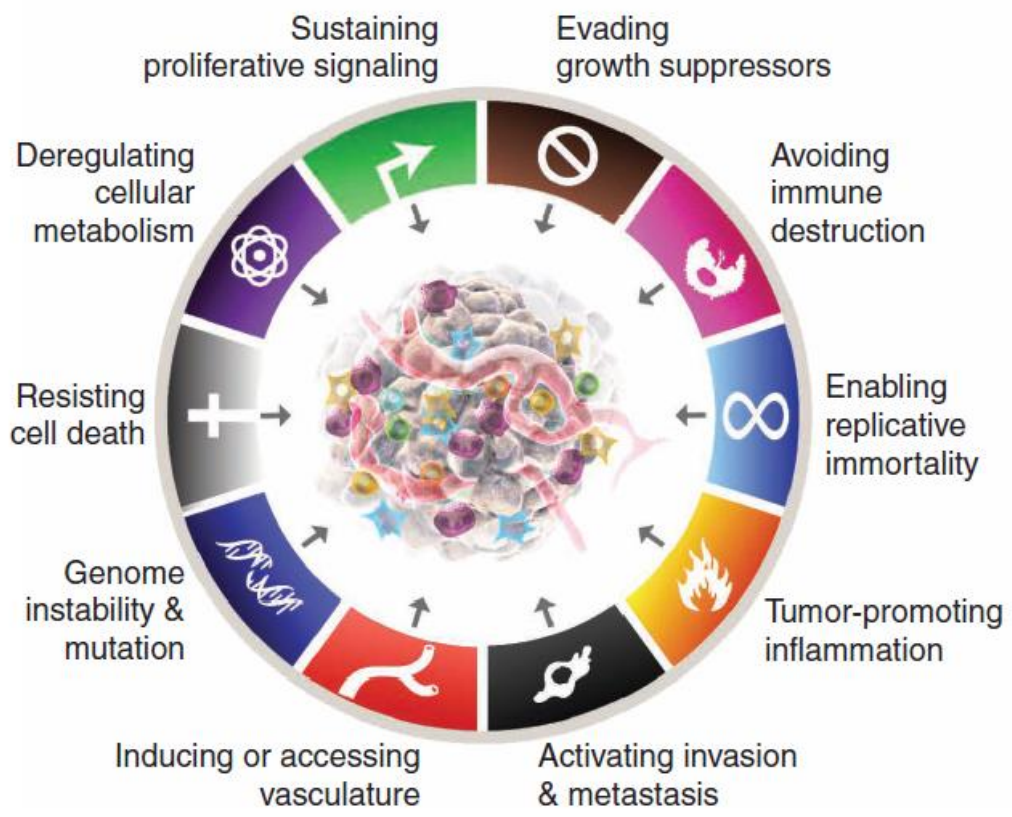
Analysis of Circulating Tumor Cells (ACTC) Lab, Dpt of Chemistry, National and Kapodistrian University of Athens

HELLENIC REPUBLIC
National and Kapodistrian University of Athens
EST. 1837



"HALLMARKS OF CANCER: NEW DIMENSIONS"

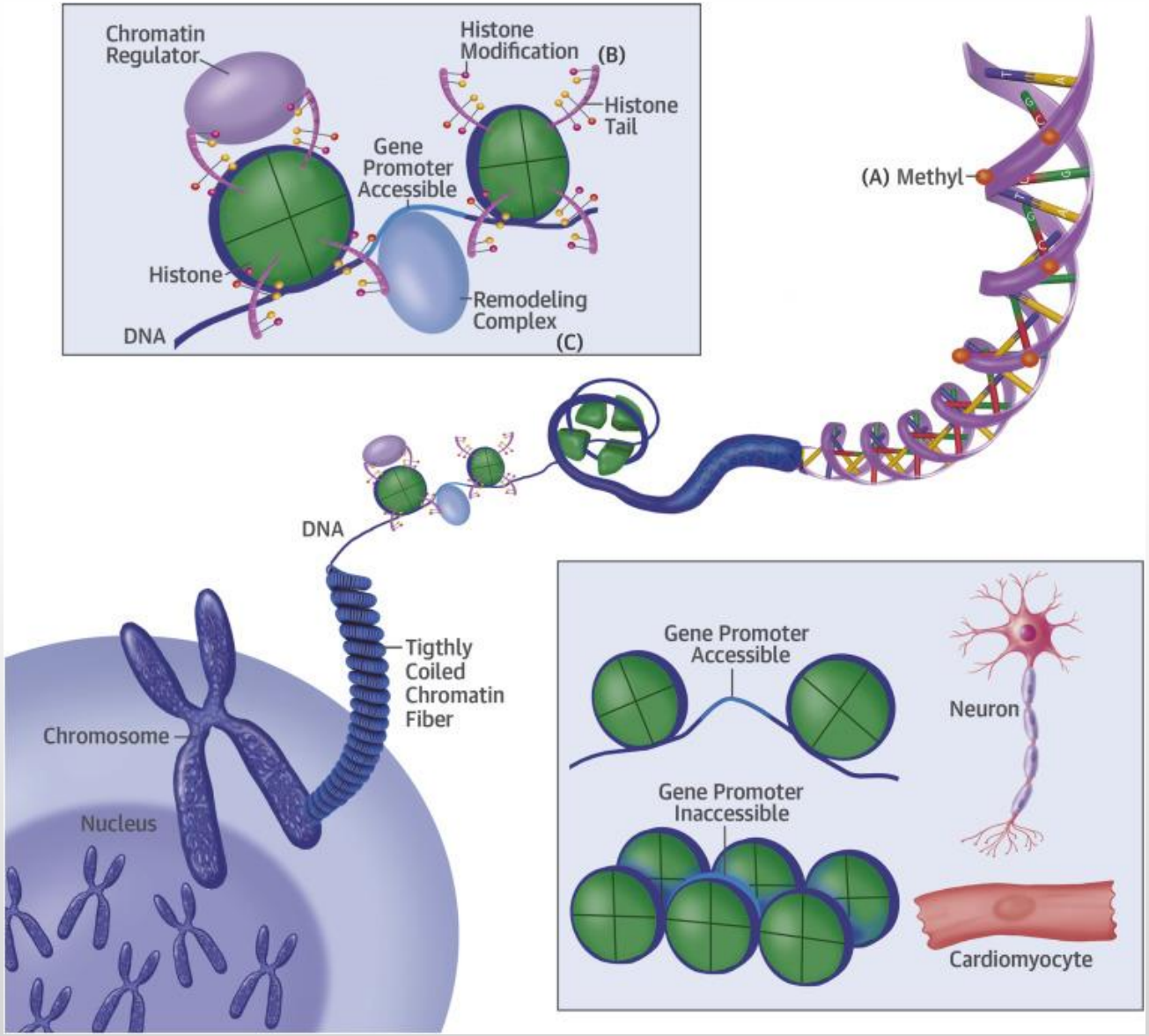
2011



2022

Hanahan D. Cancer Discov. 2022 Jan;12(1):31-46

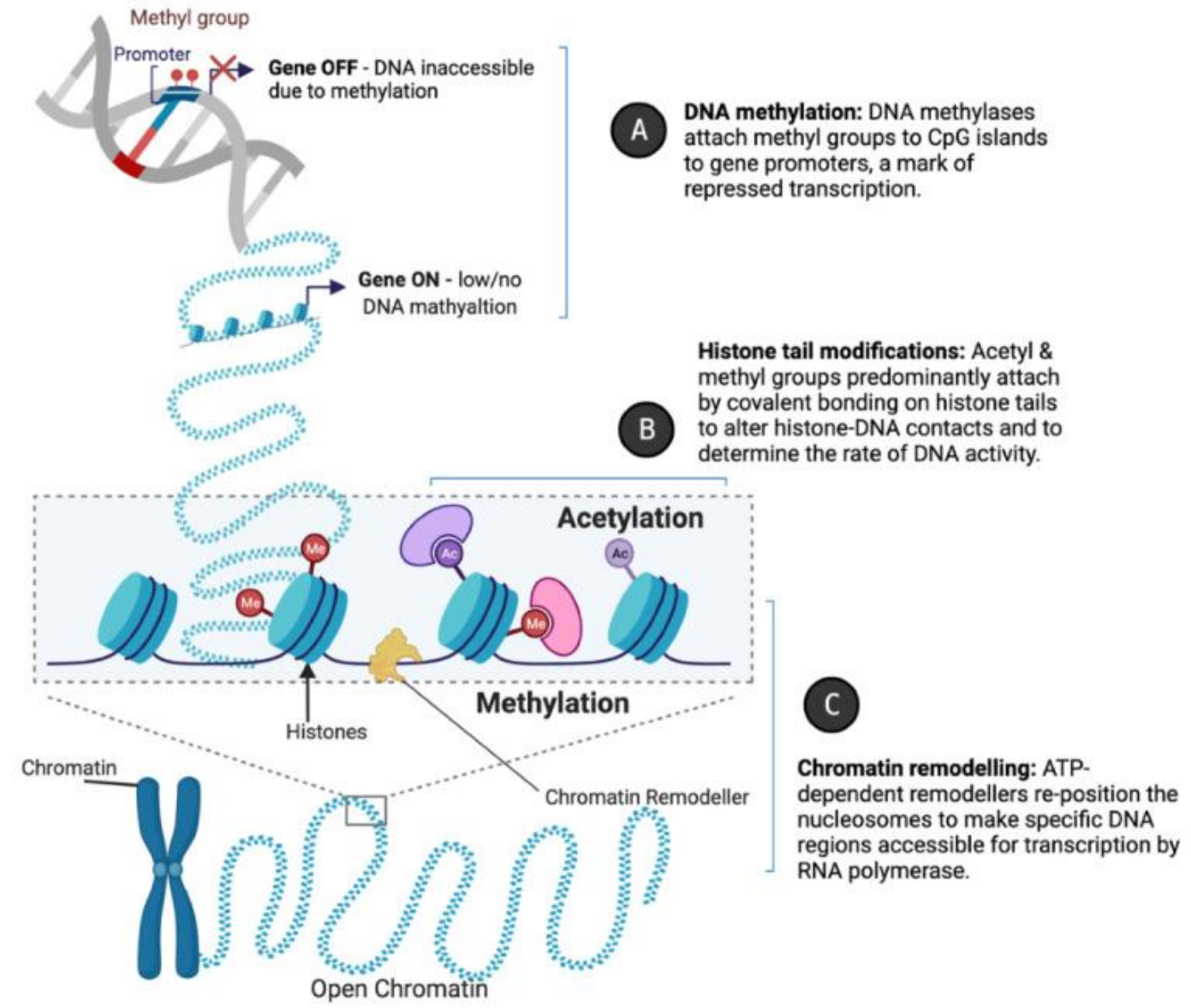
THE EPIGENOME



The epigenome is an umbrella term for a myriad of chemical compounds that influence gene expression by latching on to DNA and silencing or activating genes without altering the DNA sequence

van der Harst et al. J Am Coll Cardiol. 2017 ;70(5):590-606

EPIGENETIC MECHANISMS

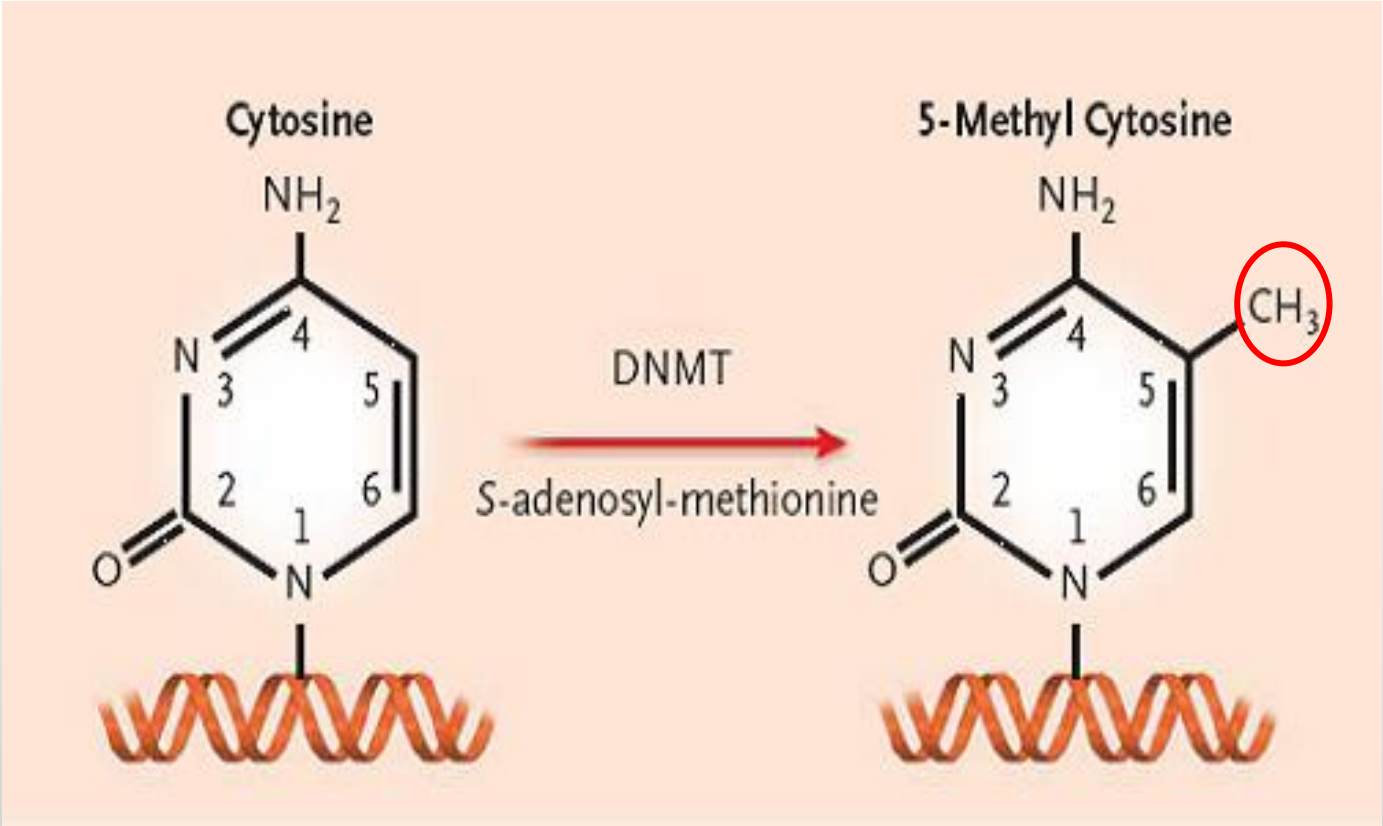


1. DNA methylation
2. post-translational histone modifications
3. ncRNA-based mechanisms

Garratt H, et al. Noncoding RNA. 2021 Sep 23;7(4):62

DNA METHYLATION

- **DNA molecules can be altered covalently by the attachment of methyl groups to cytosine bases.**
- **This modification of genomic DNA is as important as mutation in shutting down tumor suppresor genes.**
- **In mammalian cells this methylation is found only when these bases are located in a position that is 5’ to guanosines CpG**



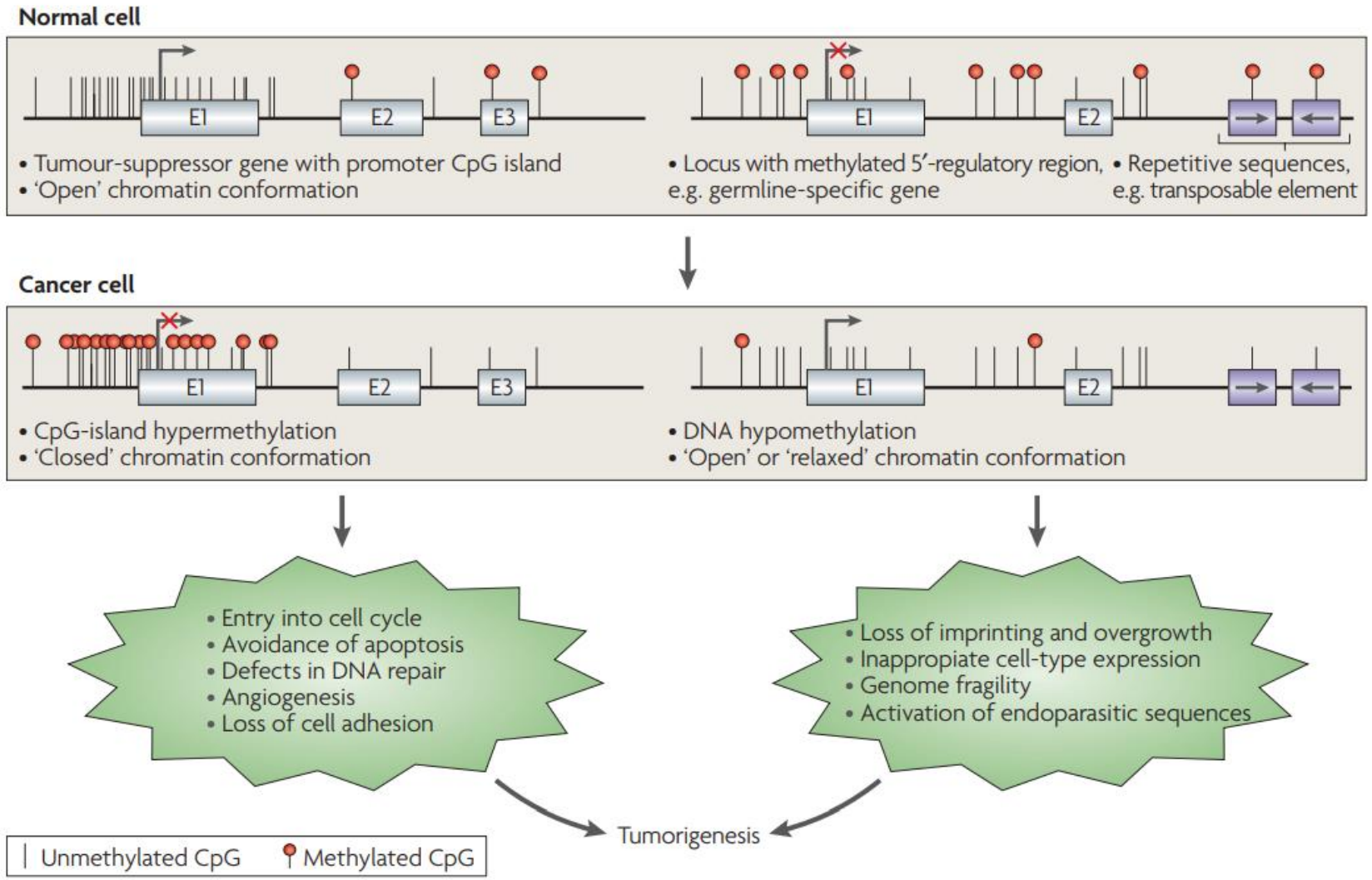
Herman J.G, Baylin S.B. NEJM 2003, 349, 2042-54

DNA METHYLATION & CANCER

As compared with normal cells, the malignant cells show major disruptions in their DNA methylation patterns:

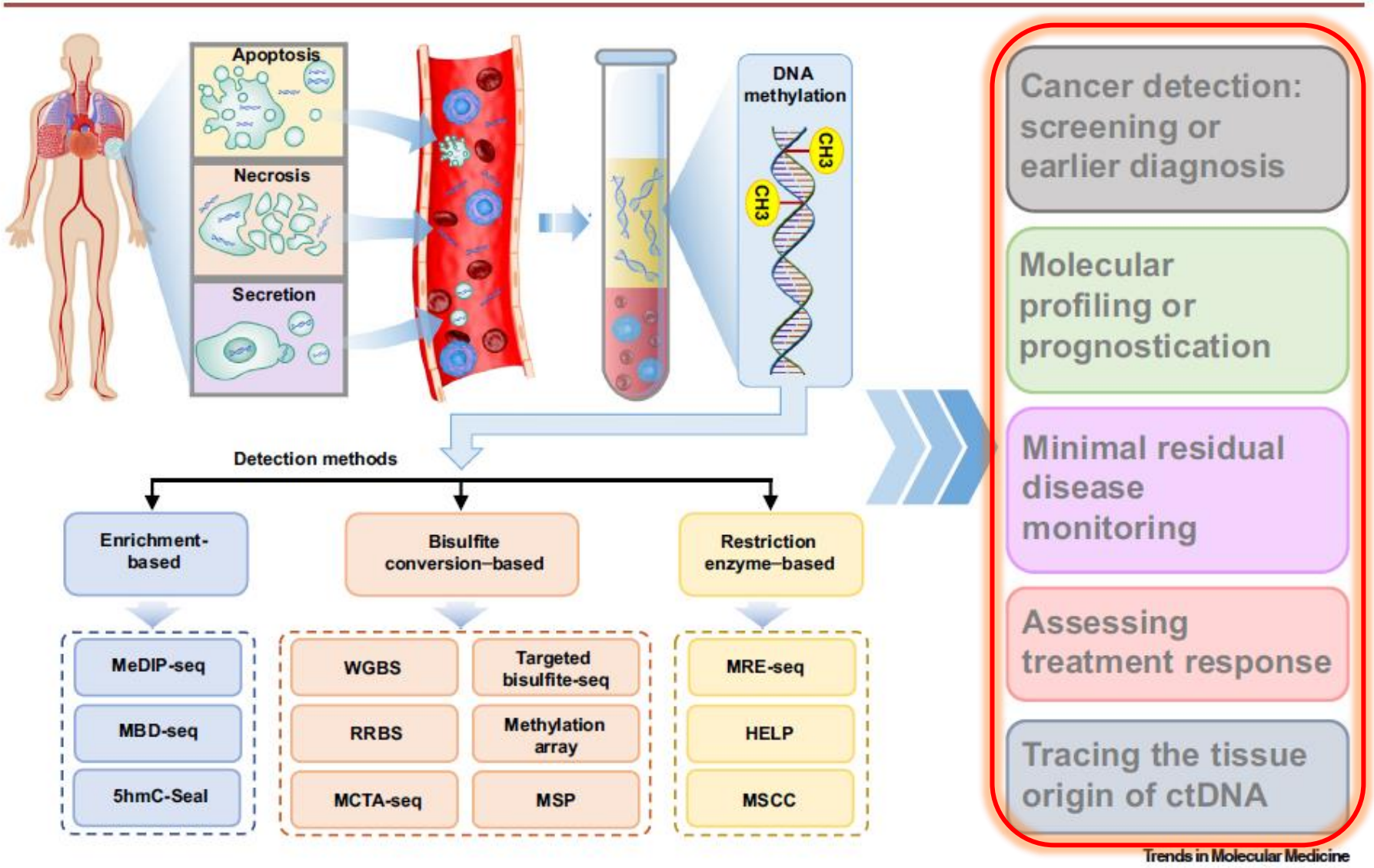
a) <u>Global hypomethylation</u> that leads to oncogene activation and chromosomal instability

b) <u>Hypermethylation</u> in promoter CpG islands that leads in transcriptional silencing of tumor suppressor and cancer related genes



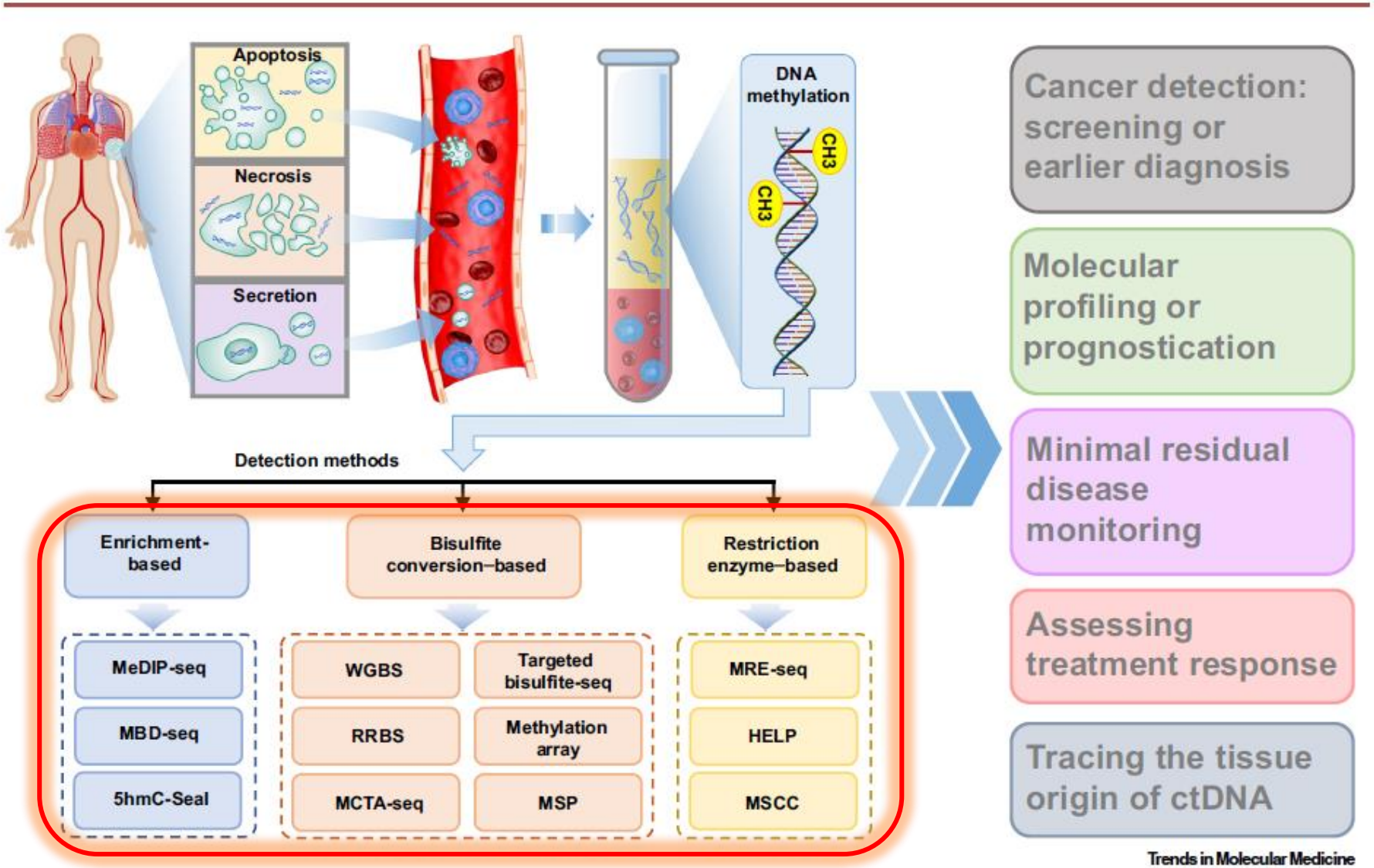
Esteller M. Nat Rev Genet 2007 Vol 8, 286-298

DNA METHYLATION & LIQUID BIOPSY



Luo H, et al. Trends Mol Med. 2021 May;27(5):482-500.

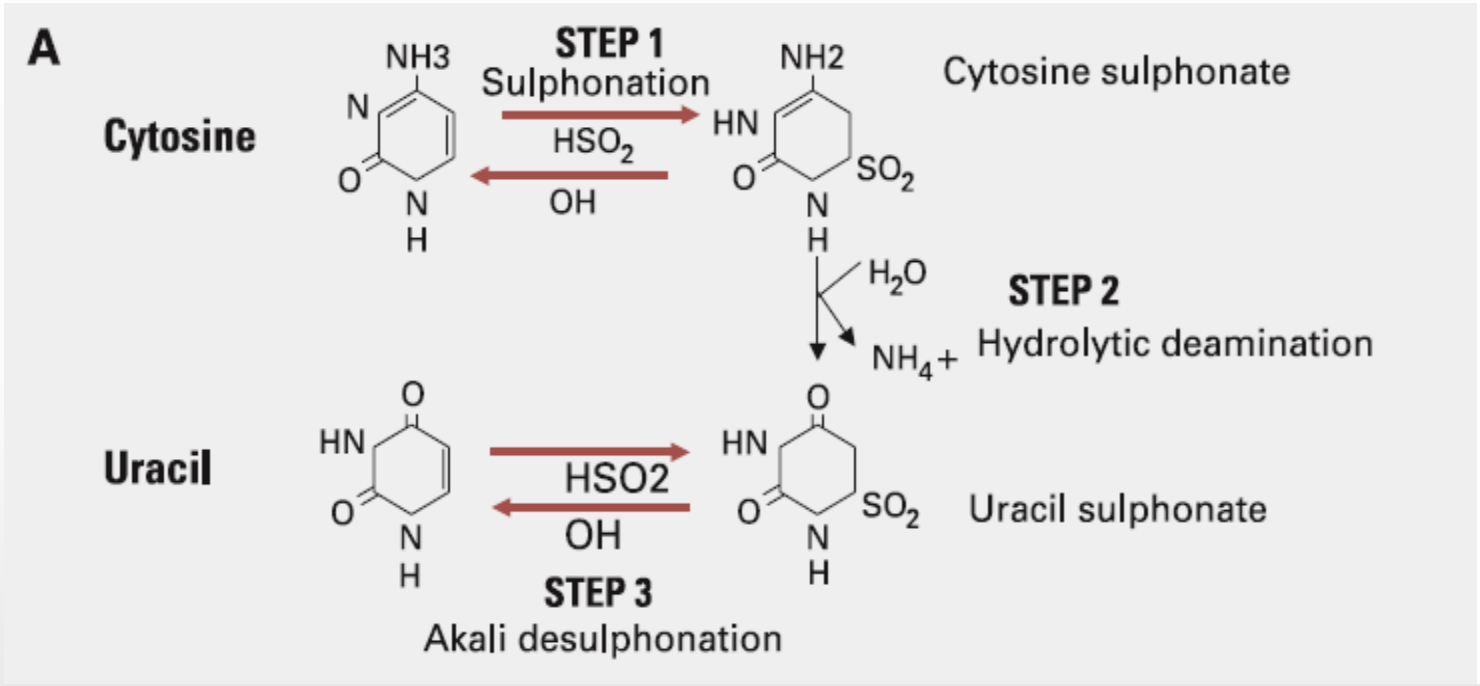
DNA METHYLATION & LIQUID BIOPSY

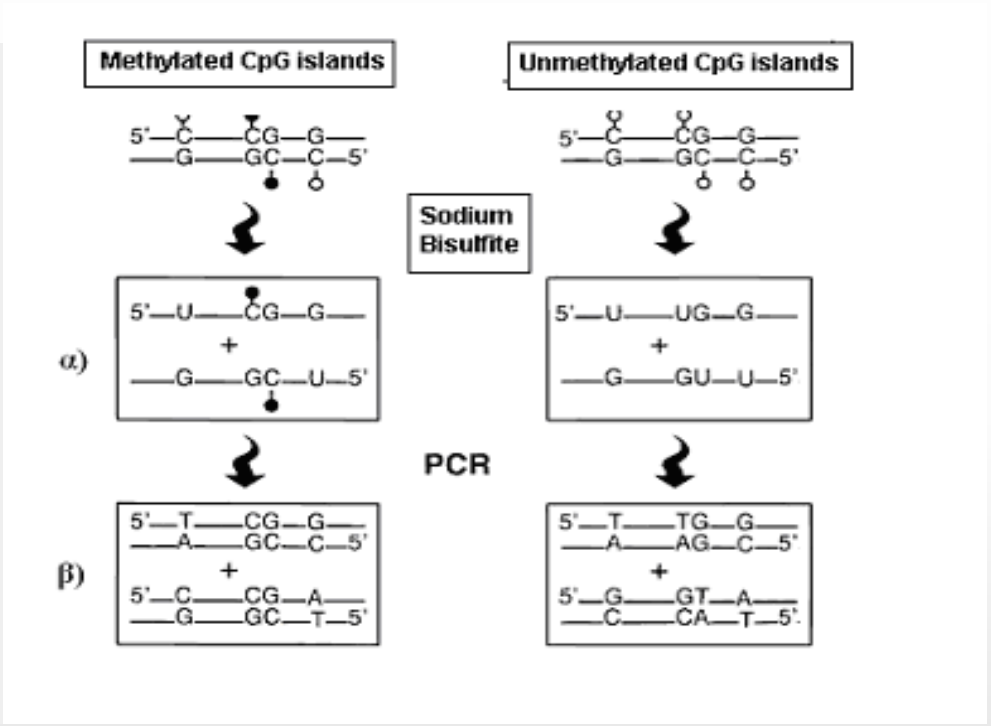


Luo H, et al. Trends Mol Med. 2021 May;27(5):482-500.

BISULFITE CONVERSION-BASED

- DNA sodium bisulfite treatment



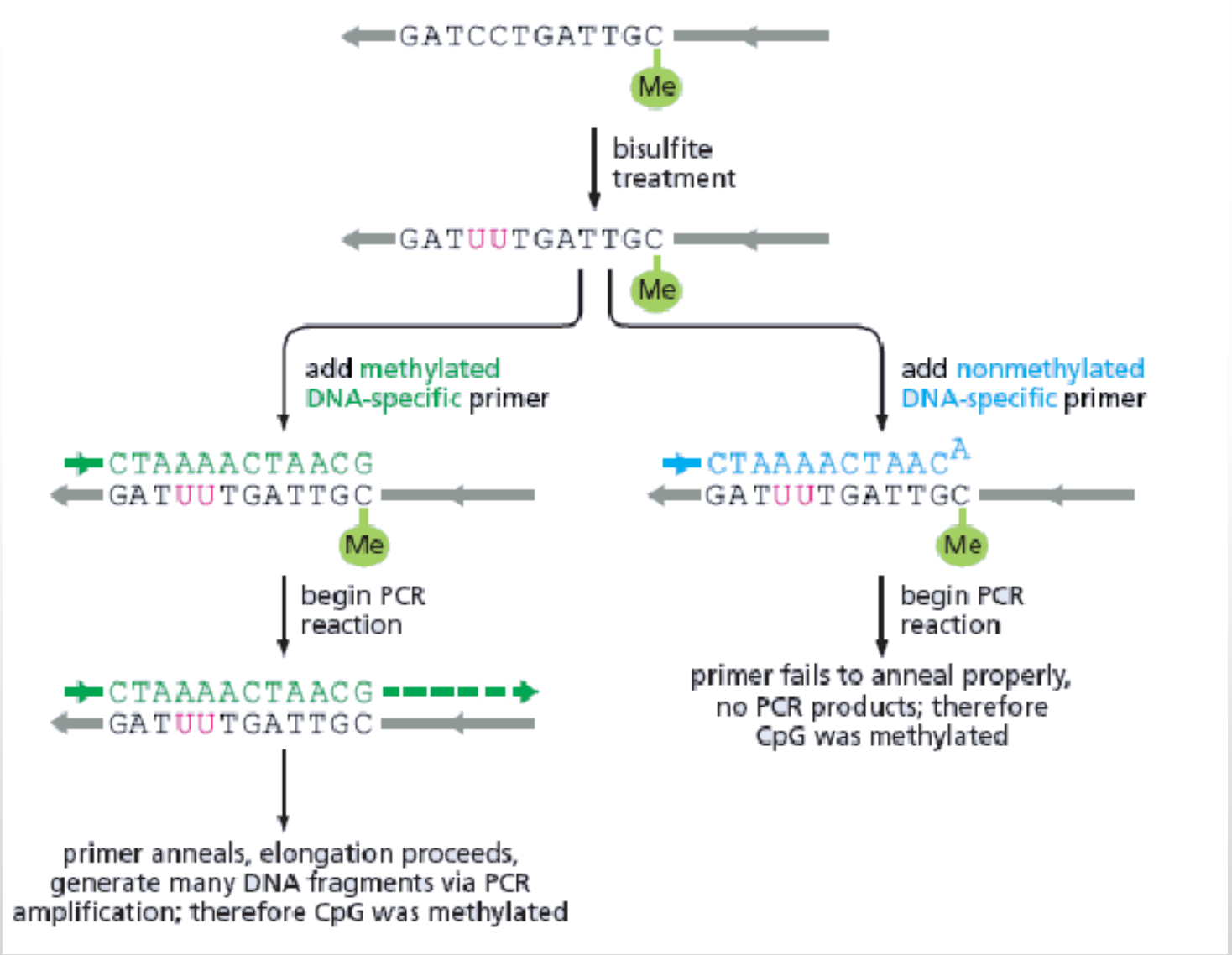


❖ deamination of non-methylated cytosine into uracil after sodium bisulfite treatment while methylated cytosines remain unchanged in CpG dinucleotides, allowing the discrimination between methylated and unmethylated DNA

METHYLATION SPECIFIC PCR (MSP)

SB treatment converts all unmethylated, but not methylated, cytosine to uracil

converted DNA is no longer self-complementary and amplification of either the top or bottom DNA strand requires different primers



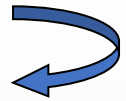
MSP REACTION PRIMER DESIGN

ATGTCG GGGGAG CCTGAG CTCATT GAGCTG CGGGAG CTGGCA CCCGCT GGG CGCGCT GGG

ATGTNG GGGGAG CCTGAG CTCATT GAGCTG NGGGAG CTGGCA CCNGCT GGG NGNGCT GGG

ATGTNG GGGGAG TTTGAG TTTATT GAGTTG NGGGAG TTGGTA TTNGTT GGG NGNGTT GGG

ATGTCG GGGGAG TTTGAG TTTATT GAGTTG CGGGAG TTGGTA TTCGTT GGG CGCGTT GGG



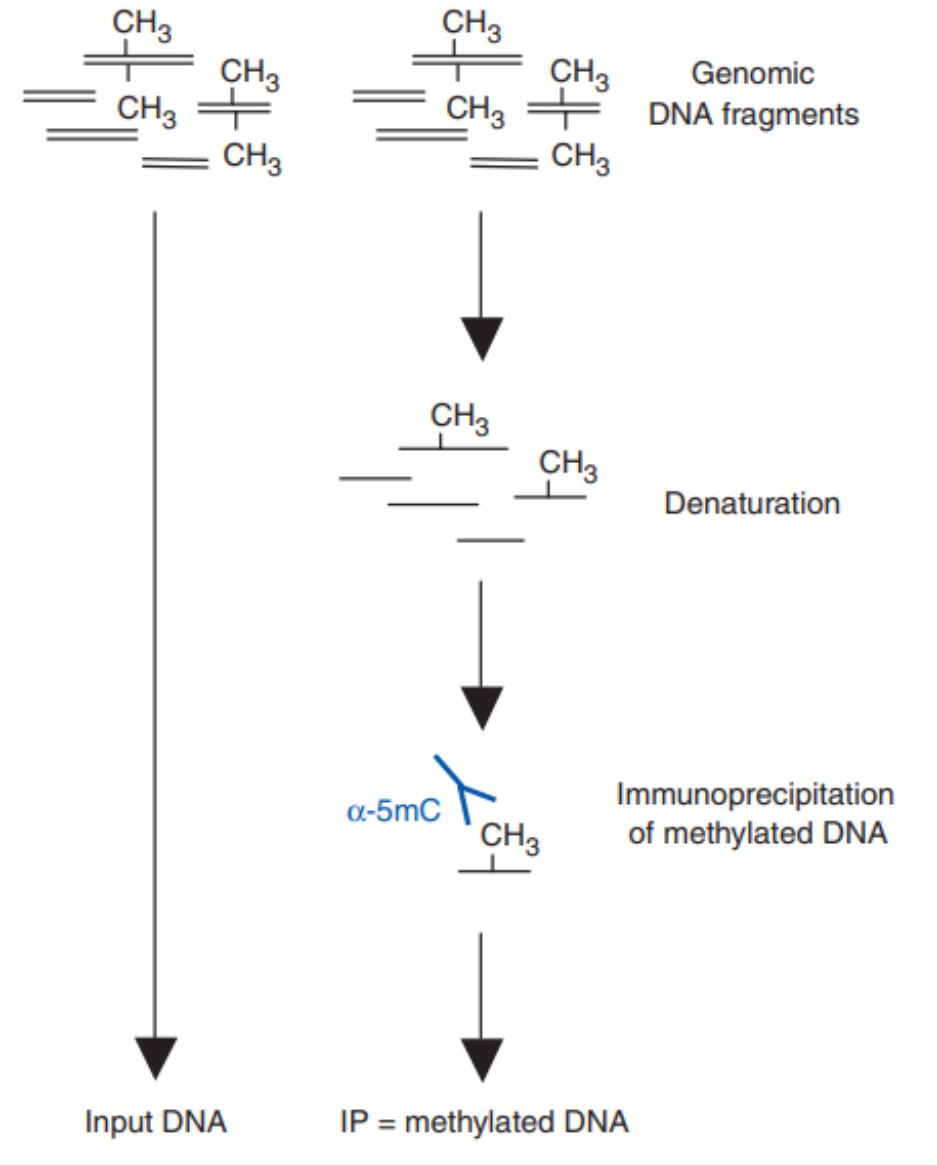
CRITERIA

1. methylated and unmethylated primers should anneal to the same CpG containing region
2. primers should be around 30 bp in length, to ensure specificity
3. primers sequence should contain at least 4–5 thymines derived from non CpG cytosines to assure specificity for converted DNA
4. each primer should contain from one to three CpGs located at its 3end
5. the annealing temperature of both primers should be similar (difference should be less than 2 ◦C and should range from60◦C to 65 ◦C

BISULFITE CONVERSION-FREE

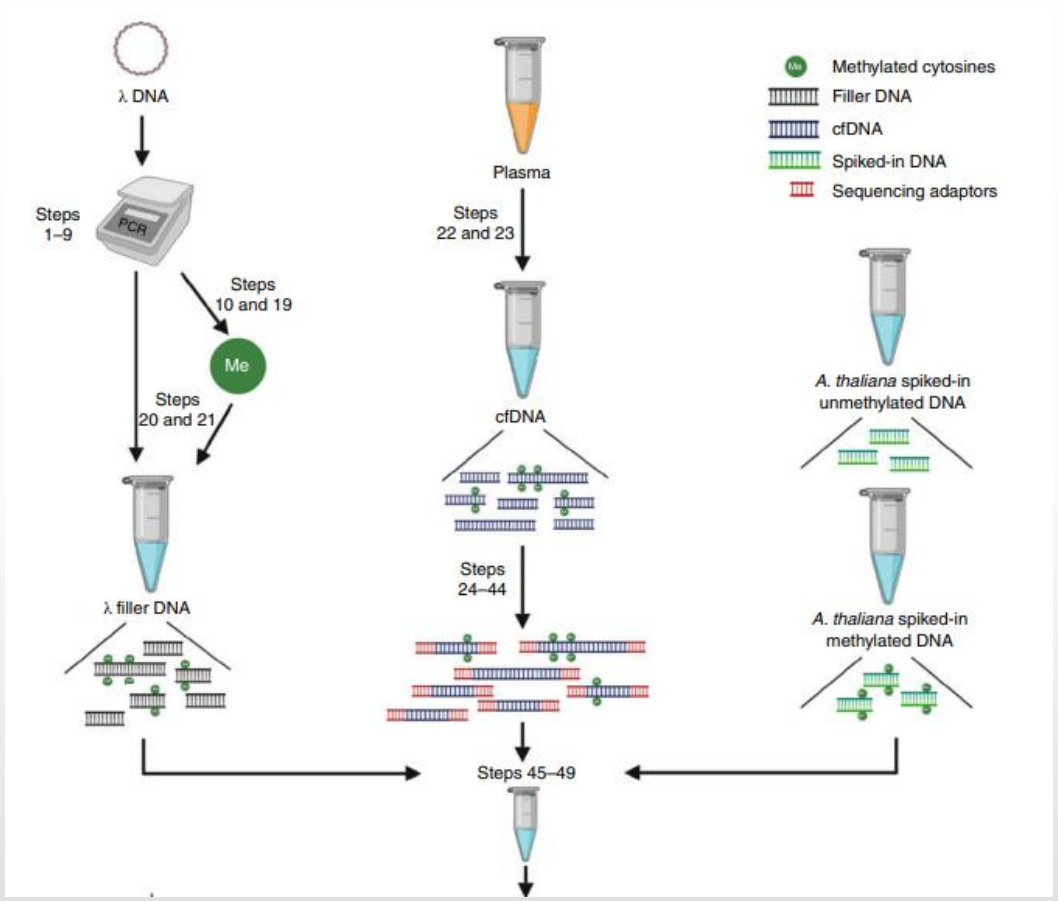
Methylation analysis by DNA immunoprecipitation (MeDIP)

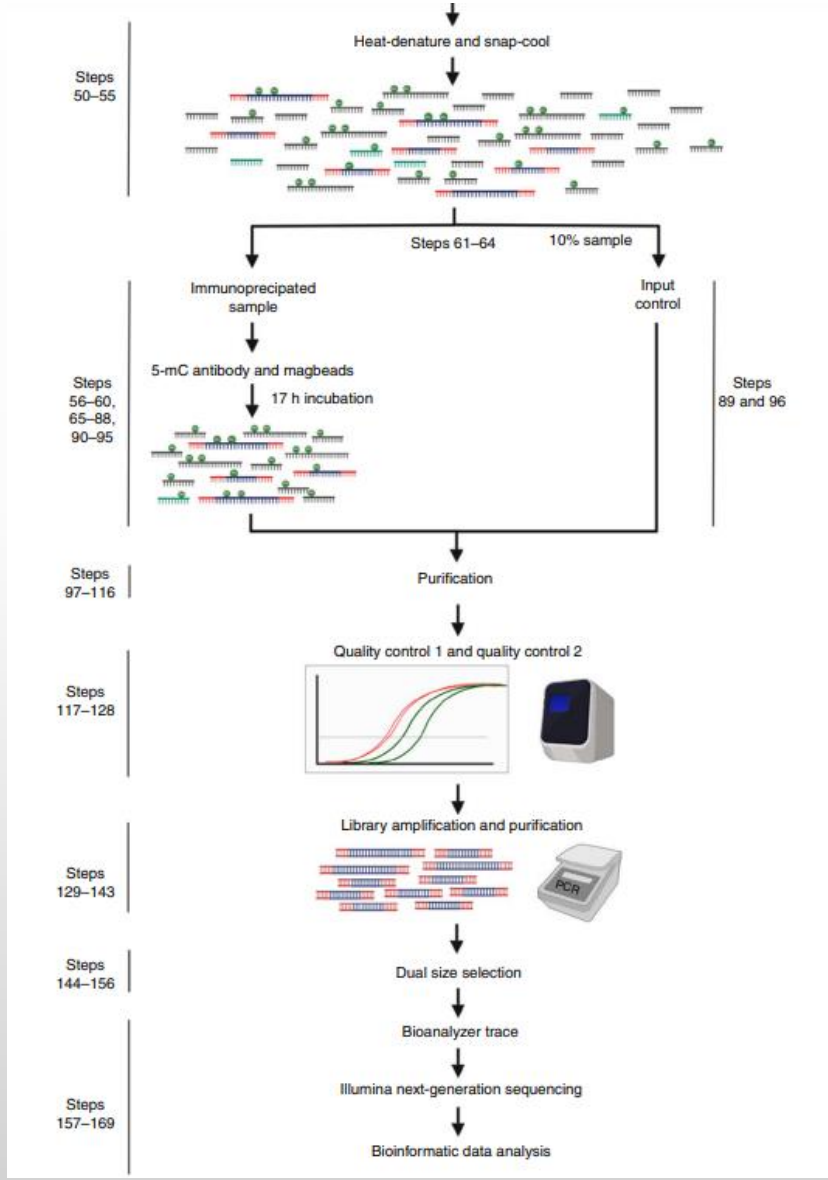
- **specifically enriches the methylated DNA fragments by using 5’-methylcytosine antibody and then performs highthroughput sequencing.**



Weber M, et al. Nat Genet. 2005 Aug;37(8):853-62

cf-MeDIP seq





Shen SY, et al. Nat Protoc. 2019 Oct;14(10):2749-2780

ctDNA application of methylation analysis

EARLY DIAGNOSIS-SCREENING

Observational Study › Ann Oncol. 2021 Sep;32(9):1167-1177. doi: 10.1016/j.annonc.2021.05.806.
Epub 2021 Jun 24.

Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set

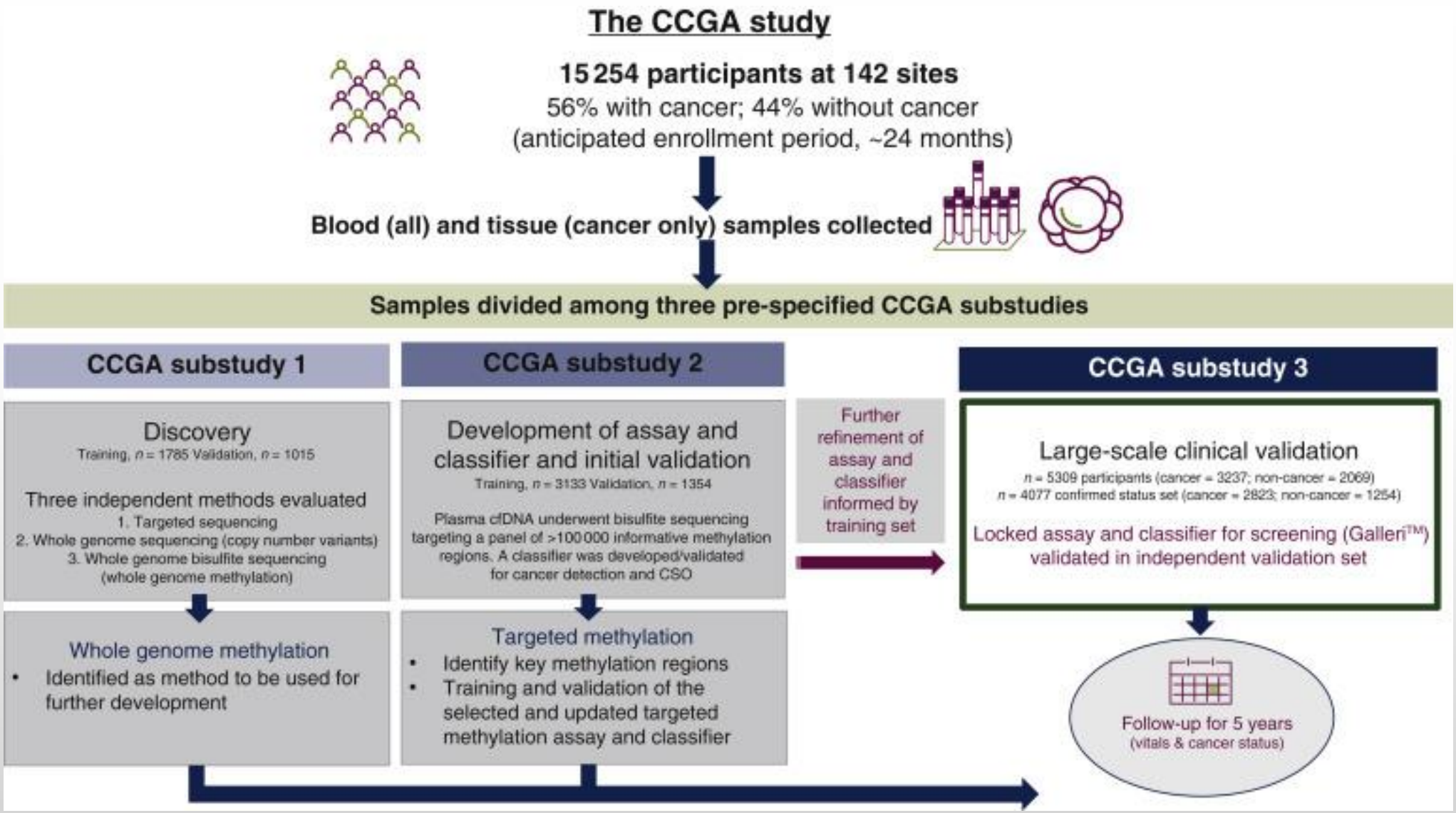
E A Klein [1], D Richards [2], A Cohn [3], M Tummala [4], R Lapham [5], D Cosgrove [6], G Chung [7], J Clement [8], J Gao [9], N Hunkapiller [9], A Jamshidi [9], K N Kurtzman [9], M V Seiden [10], C Swanton [11], M C Liu [12]

A multi-cancer early detection (MCED) test used to complement existing screening could increase the number of cancers detected through population screening, potentially improving clinical outcomes.

The Circulating Cell-free Genome Atlas study (CCGA; NCT02889978) was a prospective, case-controlled, observational study and demonstrated that a blood-based MCED test utilizing cell-free DNA (cfDNA) sequencing in combination with machine learning could detect cancer signals across multiple cancer types and predict cancer signal origin (CSO) with high accuracy.

The objective of this third and final CCGA substudy was to validate an MCED test version further refined for use as a screening tool.

EARLY DIAGNOSIS-SCREENING



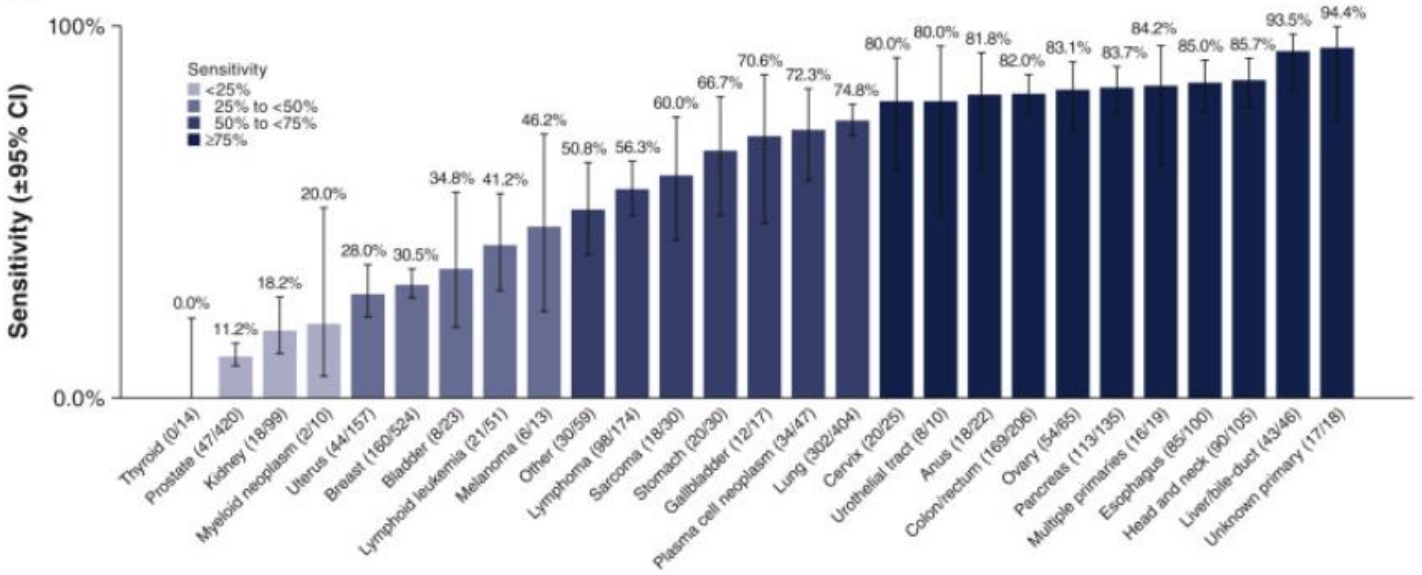
EARLY DIAGNOSIS-SCREENING

A

	Cancer	Non-cancer	Total
	2823	1254	4077
Test positive	1453	6	1459
Test negative	1370	1248	2618
	Sensitivity = 1453/2823 51.5% (49.6%-53.3%)	Specificity = 1248/1254 99.5% (99.0%-99.8%)	

Two-sided 95% Wilson confidence intervals were calculated.

B



<u>Specificity</u> for cancer signal detection was 99.5% [95% confidence interval (CI): 99.0% to 99.8%].
<u>Overall sensitivity</u> for cancer signal detection was 51.5% (49.6% to 53.3%); <u>sensitivity increased with stage</u> [stage I: 16.8% (14.5% to 19.5%), stage II: 40.4% (36.8% to 44.1%), stage III: 77.0% (73.4% to 80.3%), stage IV: 90.1% (87.5% to 92.2%)].
Stage I-III sensitivity was 67.6% (64.4% to 70.6%) in 12 pre-specified cancers that account for approximately two-thirds of annual USA cancer deaths and was 40.7% (38.7% to 42.9%) in all cancers. <u>Cancer signals were detected across >50 cancer types</u>. Overall accuracy of CSO prediction in true positives was 88.7% (87.0% to 90.2%).

EARLY DIAGNOSIS-SCREENING

epi pro colon®

Aberrant methylation in the promoter region of the *Sept9* gene v2 region has been associated with the occurrence of CRC- cellular transition from adenoma to carcinoma

PRESEPT clinical trial
(NCT00696345)
In the prospective clinical trial, sensitivity for all stages of CRC was 68% (95% CI 53%–80%) and for stage I–III CRC, 64% (48%–77%). Adjusted specificity, on the basis of negative colonoscopy findings, was 80.0% (78%–82%).

NCT01580540
At a sensitivity of 72%, the Epi proColon test is non- inferior to FIT for CRC detection, although at a lower specificity. With negative predictive values of 99.8%, both methods are identical in confirming the absence of CRC.

EARLY DIAGNOSIS-SCREENING

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 14, 2024 VOL. 390 NO. 11

A Cell-free DNA Blood-Based Test for Colorectal Cancer Screening

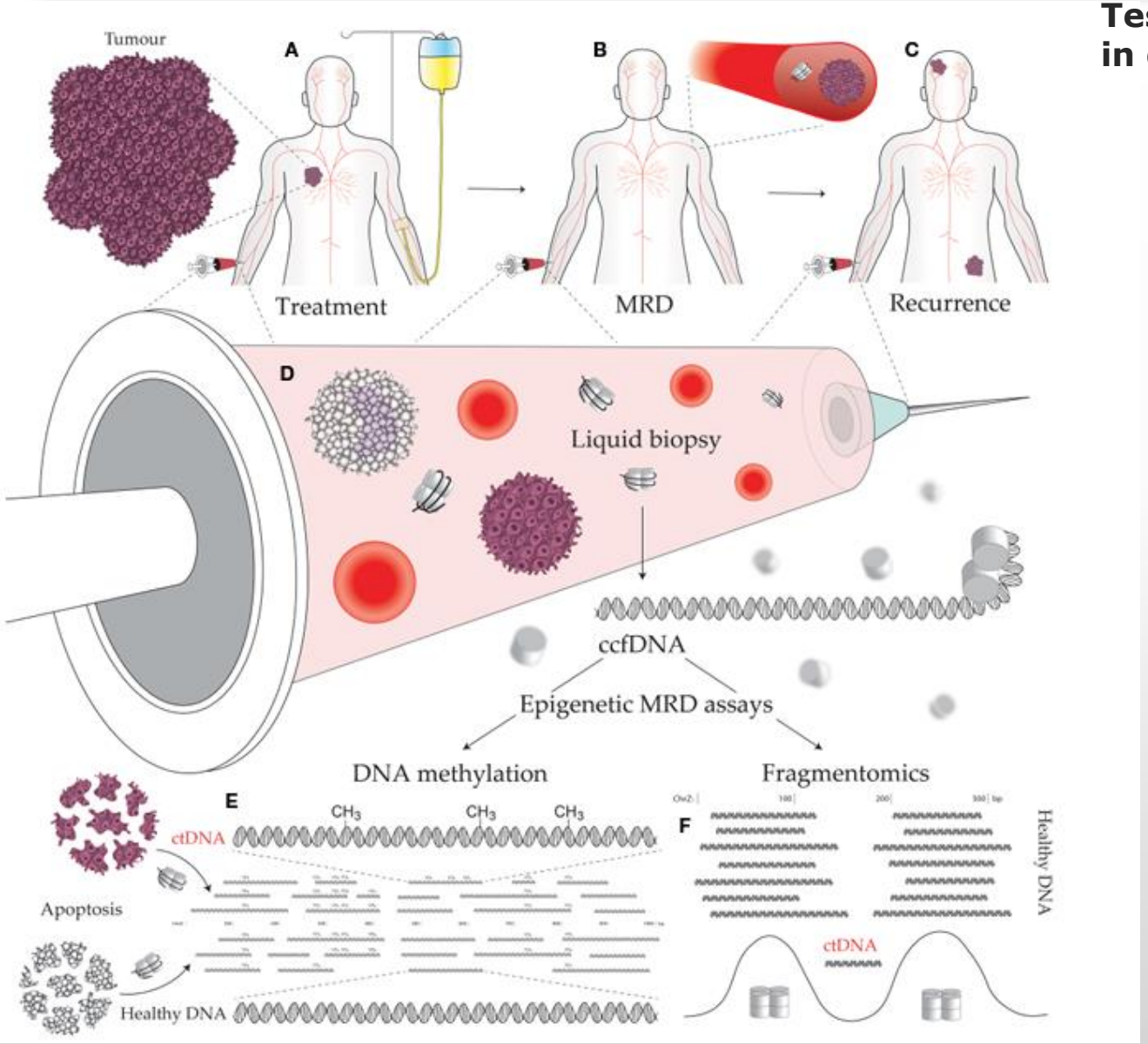
Daniel C. Chung, M.D., Darrell M. Gray II, M.D., M.P.H., Harminder Singh, M.D., Rachel B. Issaka, M.D., M.A.S., Victoria M. Raymond, M.S., Craig Eagle, M.D., Sylvia Hu, Ph.D., Darya I. Chudova, Ph.D., AmirAli Talasaz, Ph.D., Joel K. Greenson, M.D., Frank A. Sinicrope, M.D., Samir Gupta, M.D., M.S.C.S., and William M. Grady, M.D.

Table 2. Sensitivity and Specificity of the Cell-free DNA (cfDNA) Blood-Based Test for the Most Advanced Findings on Colonoscopy.*

Variable	Most Advanced Finding on Colonoscopy	cfDNA Blood-Based Test	
		Positive Test	Sensitivity (95% CI)
	no.	*no.*	%
Colorectal cancer			
Any	65	54	83.1 (72.2–90.3)
Stage I, II, or III*	48	42	87.5 (75.3–94.1)
Advanced precancerous lesions†	1116	147	13.2 (11.3–15.3)
			Specificity (95% CI)
Nonadvanced adenomas, nonneoplastic findings, and negative colonoscopy	6680	698	89.6 (88.8–90.3)
Nonneoplastic findings and negative colonoscopy	4514	457	89.9 (89.0–90.7)

- The panel interrogates cfDNA genomic alterations, aberrant methylation status, and fragmentomic patterns
- evaluate the performance of the cfDNA blood-based test (Shield, Guardant Health) to detect asymptomatic and early-stage colorectal cancer in a screening-relevant population.
- In an average-risk screening population, this cfDNA blood-based test had 83% sensitivity for colorectal cancer, 90% specificity for advanced neoplasia, and 13% sensitivity for advanced precancerous lesions.

MINIMAL RESIDUAL DISEASE



Johnston AD, et al, Front Oncol. 2023

Tests for mininmal residual disease currently avaliable or in development.

Test/Company	Technology/method	Clinical Applications	Development Stage/Regulatory Approval	Citations
Signatera Company: Natera	Somatic mutation detection in ctDNA using multiplex-PCR NGS assays Personalised targets from whole-exome sequencing of primary tumors	MRD and recurrence in: - Colorectal cancer (Stage I-IV) - Epithelial ovarian cancer (Stage I-IV)	BDD status (USA)	(39, 40)
RaDaR Company: Inivata	Somatic mutation detection in ctDNA using multiplex-PCR NGS assays Personalised targets from whole-exome sequencing of primary tumors	MRD and recurrence in: - Breast cancer	CE mark (Europe) BDD status (USA)	(41, 42)
FoundationOne Tracker Company: Foundation Medicine	Somatic mutation detection in ctDNA using bespoke multiplex-PCR NGS assays Personalised targets from whole-exome sequencing of primary tumors	MRD, treatment response and recurrence in: - Colorectal cancer - Bladder cancer	BDD status (USA)	(43)
Sentinel Trail Company: Strata Oncology	Somatic mutation detection in ctDNA using bespoke multiplex-PCR NGS assays Personalised targets from whole-exome sequencing of primary tumors	Treatment response and recurrence in: - Stage 1-3 solid tumors	Clinical Trial (NCT05082701)	(44)
brPROPHET Company: Burning Rock Biotech	Somatic mutation detection in ctDNA using bespoke hybridzation capture poanels and NGS. Personalised targets from whole-exome sequencing of primary tumors	MRD and recurrence in: - Colorectal cancer	Ungoing clinical studies	(45)
PhasED-seq Company: Foresight Diagnostics	Detection of phased varients (PVs), where two or more mutations on the same sequenced fragment of ctDNA PV panel assembled from whole-genome sequences of 2,538 tumors	MRD and recurrence in: - B-cell lymphomas	Developing as LDT	(46)
CORRECT-MRD II Company: Exact Sciences	Highly sensitive variaent detection using targeted linear pre-amplification of ccfDNA, UMI ligation, then somatic mutation detection using bespoke multiplex-PCR NGS assays. Personalised targets from whole-genome sequencing of primary tumors.	MRD and recurrence in: - Colorectal cancer (Stage II & III)	Clinical Trial (NCT05210283)	(47)
ECLIPSE Company: Guardant Health	Somatic mutation, DNA methylation and fragmentomic profiling of ccfDNA. 500 kB hybridization capture panel (LUNAR) for cancer detection using NGS.	Early detection, MRD and recurrence in: - Colorectal cancer	Clinical Trial (NCT04136002)	(48)
ctDNA Methylation Sequencing for Myeloma Company: MethylGene Tech	ctDNA methylation sequencing	MRD and recurrence in: - Multiple myeloma	Clinical Trial (NCT05578625)	(49)
Targeted methylation platform Company: GRAIL	ctDNA targeted methylation detection	MRD and recurrence	In development	(50)
Colvera Company: Clinical Genomics	real-time PCR test for detecing DNA methylation of BCAT1 and IKZF1 genes	MRD and recurrence in: - Colorectal cancer	Currently offered as an LDT	(51, 52)
Bladder EpiCheck Company: Nucleix	Multiplex DNA methylation-based PCR assay	Tumor recurrence in: - Bladder cancer	CE mark (Europe)	(53, 54)
ColonAiQ Company: Singlera Genomics	Six-plex methylation PCR test	MRD, treatment response and recurrence in: - Colorectal cancer	Clinical Trial (NCT05444491) Clinical Trial (NCT05536089)	(55)

MINIMAL RESIDUAL DISEASE

European Association of Urology

EUO Priority Article – Bladder Cancer

Performance of the Bladder EpiCheck™ Methylation Test for Patients Under Surveillance for Non–muscle-invasive Bladder Cancer: Results of a Multicenter, Prospective, Blinded Clinical Trial

J. Alfred Witjes[a,], Juan Morote[b], Erik B. Cornel[c], Georgios Gakis[d], F. Johannes P. van Valenberg[a], Fernando Lozano[b], Itay A. Sternberg[e], Ellen Willemsen[c], Miriam L. Hegemann[f], Yossi Paitan[g], Ilan Leibovitch[e]*

- *high recurrence rates of up to 50%–70% after 5 years, which means that patients require lifelong postoperative surveillance. The use of urinary markers rather than invasive cystoscopy simplifies surveillance schedules.*

- The Bladder EpiCheck test analyzes 15 methylation biomarkers and determines whether this pattern is consistent with bladder cancer presence or absence. The validation study showed 90% sensitivity, 83% specificity, and NPV of 97% among 222 NMIBC patients undergoing surveillance.
- The primary objective of the current study was to determine the sensitivity and specificity of Bladder EpiCheck for patients undergoing surveillance for recurrent bladder cancer. The sensitivity and specificity of the test were compared to a prespecified reference standard of cystoscopy, cytology, and histology.

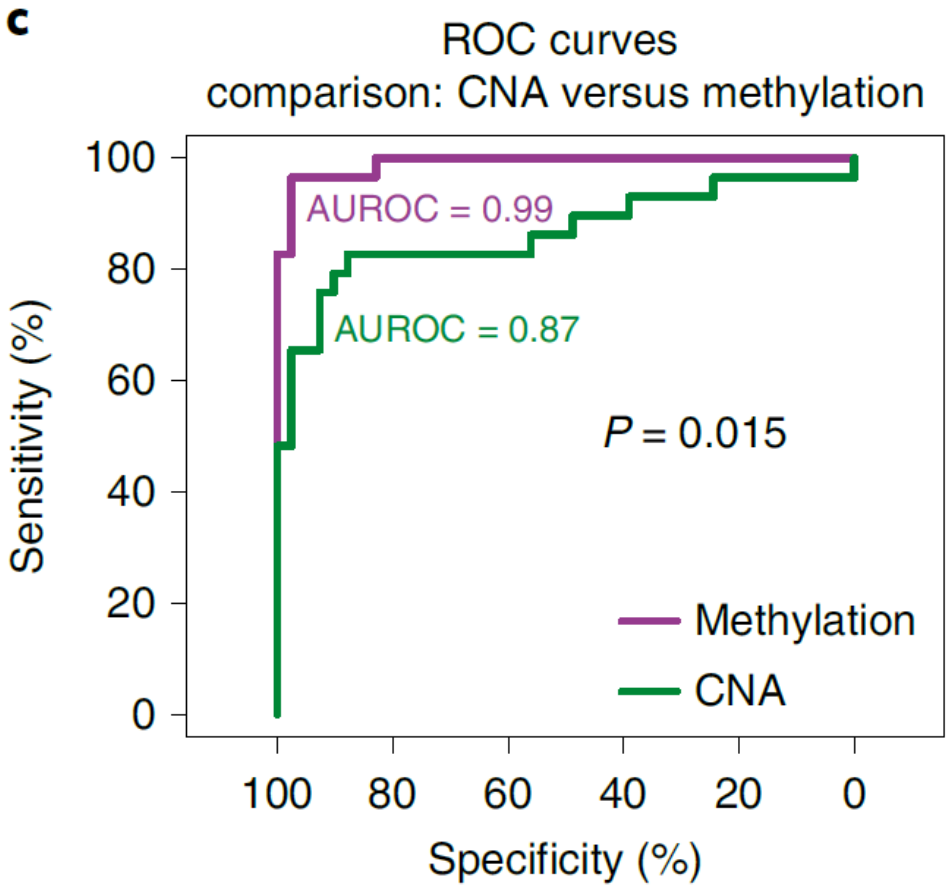
MOLECULAR TUMOUR SUBTYPING

Nat Cancer. 2022 Aug 8. doi: 10.1038/s43018-022-00415-9. Online ahead of print.

cfDNA methylome profiling for detection and subtyping of small cell lung cancers

Francesca Chemi [# 1], Simon P Pearce [# 2], Alexandra Clipson [1], Steven M Hill [2], Alicia-Marie Conway [1 3], Sophie A Richardson [1], Katarzyna Kamieniecka [2], Rebecca Caeser [4], Daniel J White [1], Sumitra Mohan [1], Victoria Foy [1 3], Kathryn L Simpson [5], Melanie Galvin [5], Kristopher K Frese [5], Lynsey Priest [6], Jacklynn Egger [4], Alastair Kerr [2], Pierre P Massion [7], John T Poirier [8], Gerard Brady [1], Fiona Blackhall [3 6], Dominic G Rothwell [9], Charles M Rudin [10], Caroline Dive [11 12 13]

ROC curves and AUROC scores generated by using ichor copy number alterations (CAN, green line) or median classifier tumor prediction score (methylation, purple line) to classify: Limited stage disease (LS-SCLC, stage IA–IIIB, *n* = 29) and non-cancer controls (NCC cfDNA, *n* = 41) samples



ACTC LAB

ACTC lab

breast cancer

Epigenetic alterations in CTC and ctDNA

Clinical Chemistry 57:8
1169–1177 (2011)

DNA Methylation of Tumor Suppressor and Metastasis Suppressor Genes in Circulating Tumor Cells

Maria Chimonidou,[1†] Areti Strati,[1†] Alexandra Tzitzira,[1] Georgia Sotiropoulou,[2] Nikos Malamos,[3] Vasilis Georgoulias,[4] and Evi S. Lianidou[1*]

First study on the epigenetic silencing of tumor suppressor and metastasis suppressor genes in CTCs

"DNA methylation of tumor suppressor and metastasis suppressor genes is a hallmark feature of CTC and confirms their heterogeneity. Our findings add a new dimension to the molecular characterization of CTC and may underlie the acquisition of malignant properties, including their stem-like phenotype"

Chimonidou et al, Clin. Chem., 2011

Epigenetic silencing of tumor suppressor and metastasis suppressor genes in CTCs

Clinical Chemistry

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Volume 57, Issue 8
1 August 2011

DNA Methylation of Tumor Suppressor and Metastasis Suppressor Genes in Circulating Tumor Cells

Maria Chimonidou, Areti Strati, Alexandra Tzitzira, Georgia Sotiropoulou, Nikos Malamos, Vasilis Georgoulias, Evi S Lianidou ✉ Author Notes

Clinical Chemistry, Volume 57, Issue 8, 1 August 2011, Pages 1169–1177,
https://doi.org/10.1373/clinchem.2011.165902
Published: 01 August 2011 **Article history**

2011

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Volume 59, Issue 1
1 January 2013

SOX17 Promoter Methylation in Circulating Tumor Cells and Matched Cell-Free DNA Isolated from Plasma of Patients with Breast Cancer

Maria Chimonidou, Areti Strati, Nikos Malamos, Vasilis Georgoulias, Evi S Lianidou ✉
Author Notes

Clinical Chemistry, Volume 59, Issue 1, 1 January 2013, Pages 270–279,
https://doi.org/10.1373/clinchem.2012.191551

2013

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 42), pp: 72054-72068

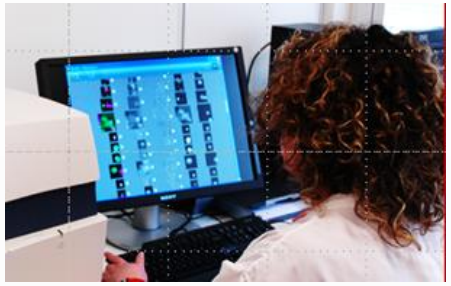
Research Paper

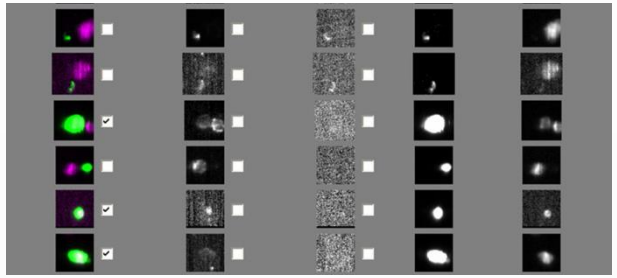
Direct comparison study of DNA methylation markers in EpCAM-positive circulating tumour cells, corresponding circulating tumour DNA, and paired primary tumours in breast cancer

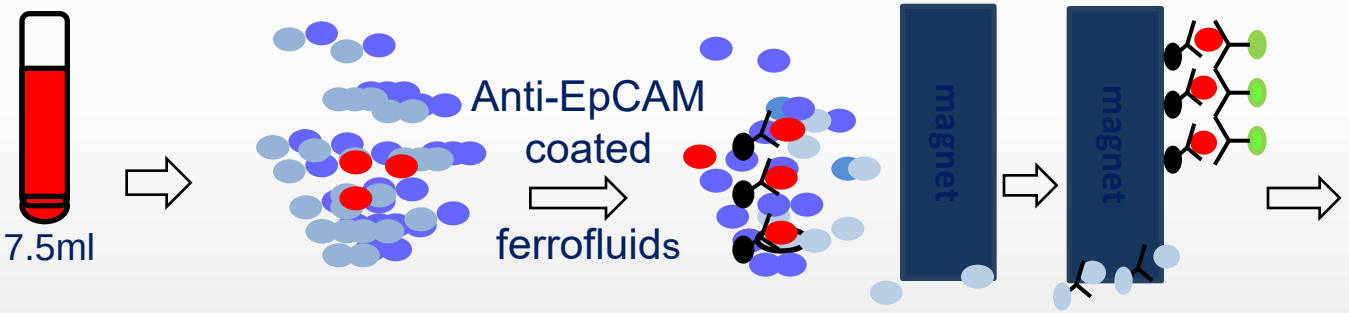
Maria Chimonidou[1], Areti Strati[1], Nikos Malamos[2], Sophia Kouneli[2], Vassilis Georgoulias[3] and Evi Lianidou[1]

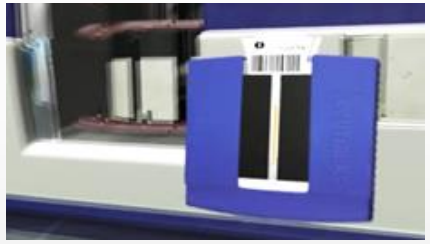
2017

DNA methylation analysis of CTCs isolated from CellSearch cartridges

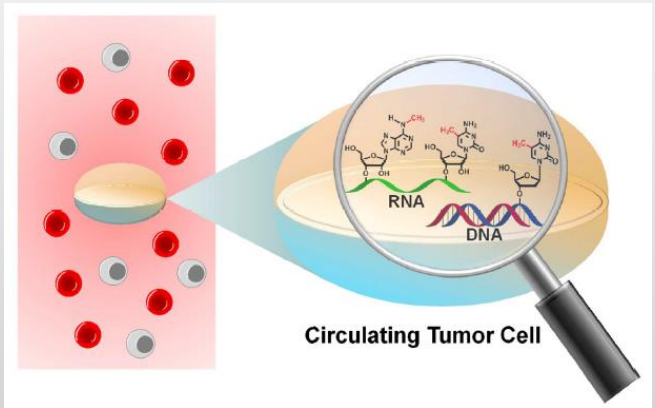








Extract gDNA



Methylation analysis -MSP

Clinical Cancer Research

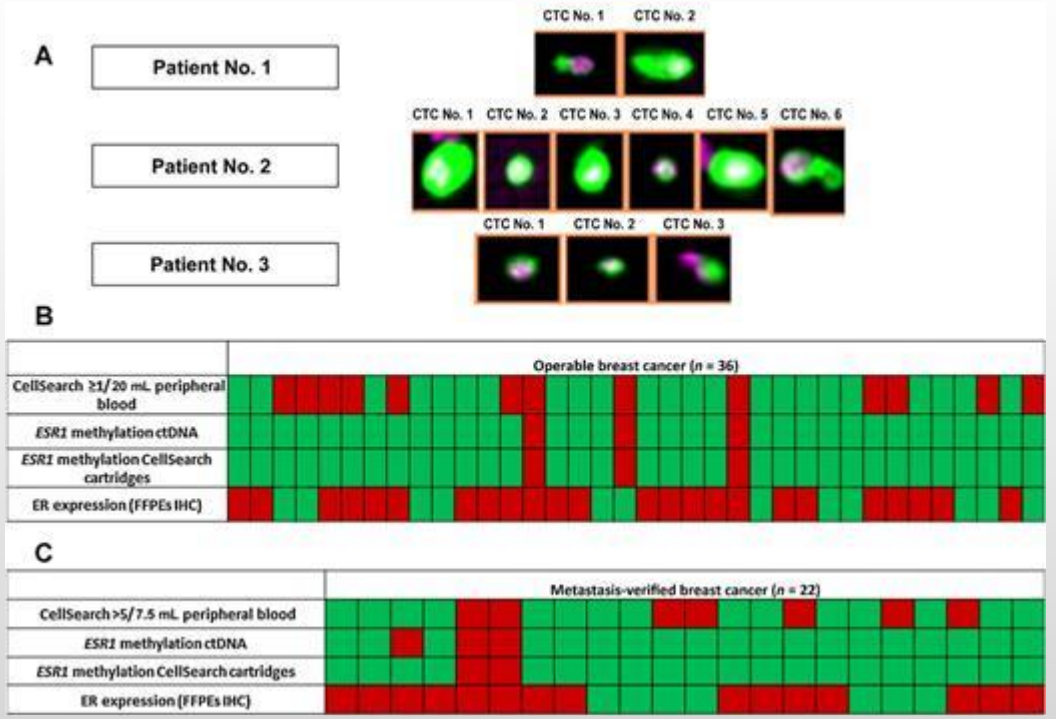
Biology of Human Tumors

ESR1 methylation: a liquid biopsy-based epigenetic assay for the follow up of patients with metastatic breast cancer receiving endocrine treatment

Sofia Mastoraki, Areti Strati, Eleni Tzanikou, Maria Chimonidou, Helen Politaki, Alexandra Voutsina, Amanda Psyrri, Vasilis Georgoulias, and Evi S. Lianidou

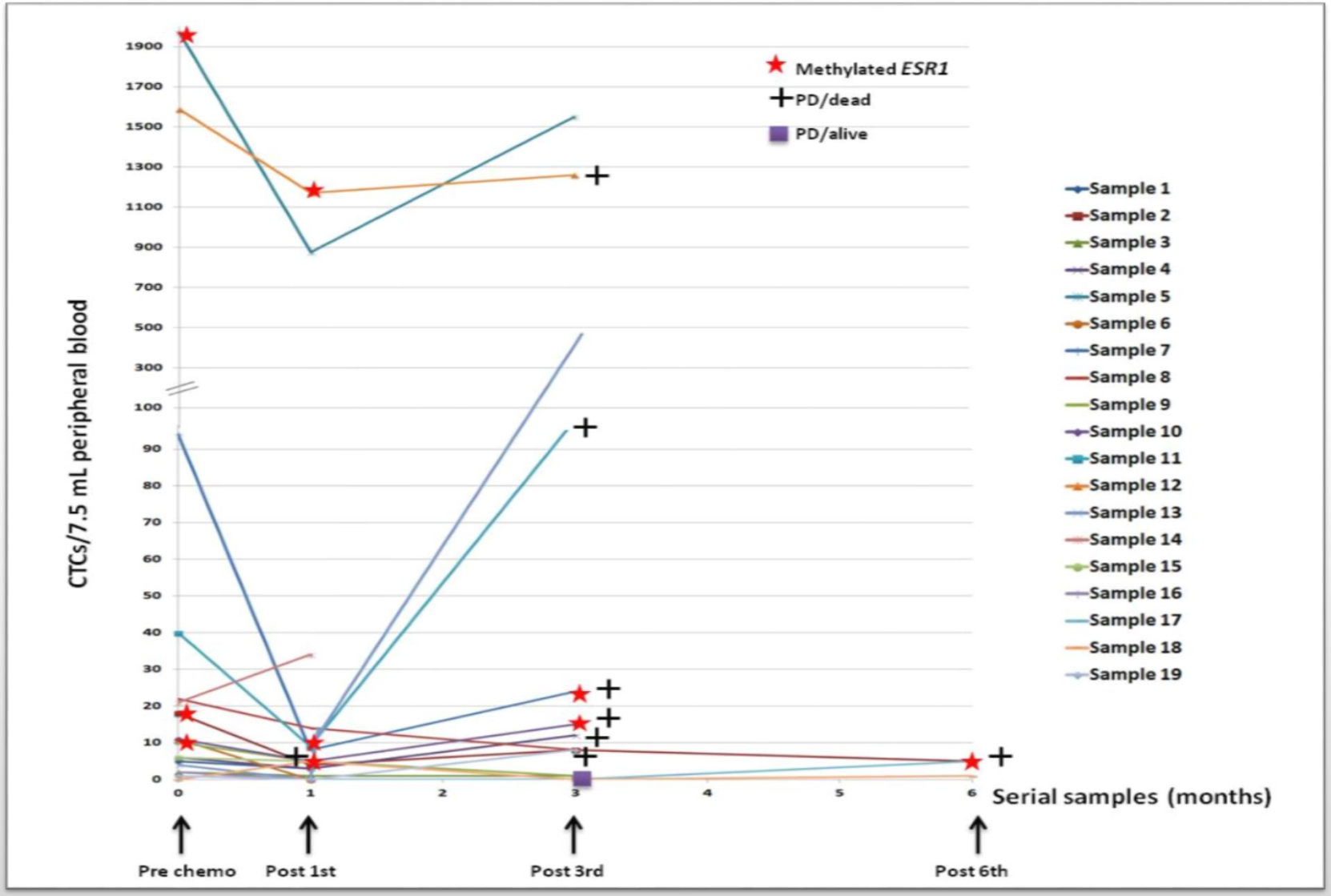
DOI: 10.1158/1078-0432.CCR-17-1181

- ***ESR1*** **epigenetic silencing potentially affects response to endocrine treatment.**
- **We evaluated *ESR1* methylation in CTCs as a potential biomarker for the follow up of BrCa patients receiving everolimus/exemestane combination in the context of standard treatment**



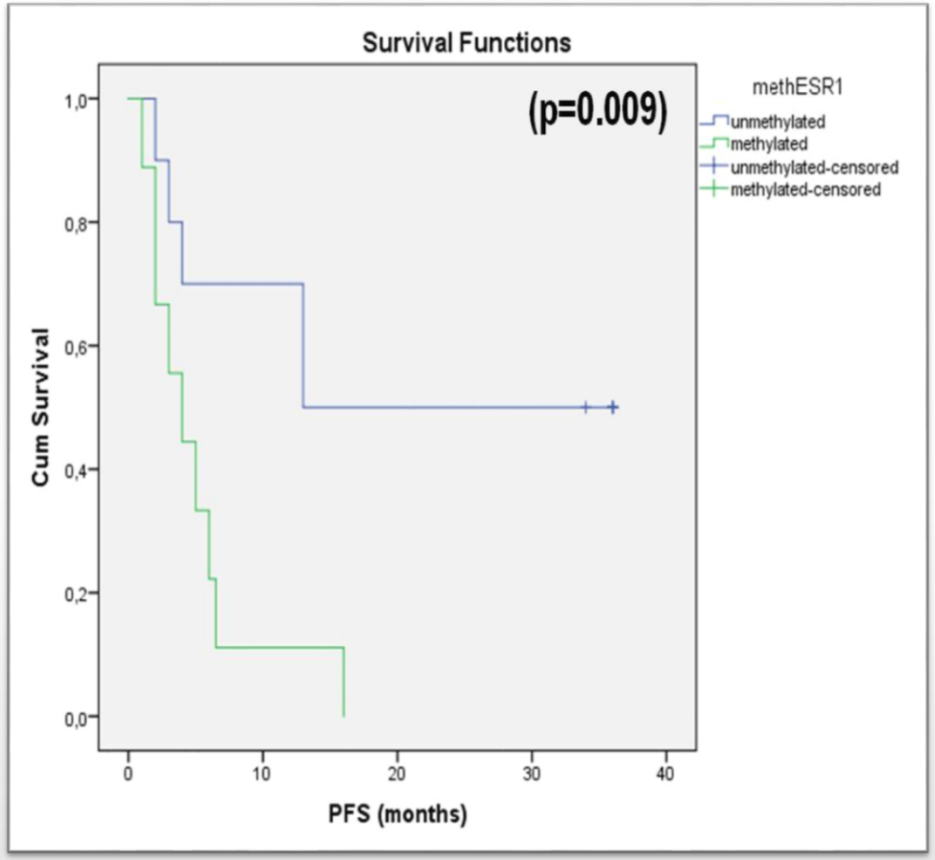
Mastoraki et al. Clin Cancer Res 2018

ESR1 methylation status for each patient in serial CTC samples of BrCa patients receiving everolimus/exemestane treatment at different time points during treatment, in relation to the number of detected CTCs.

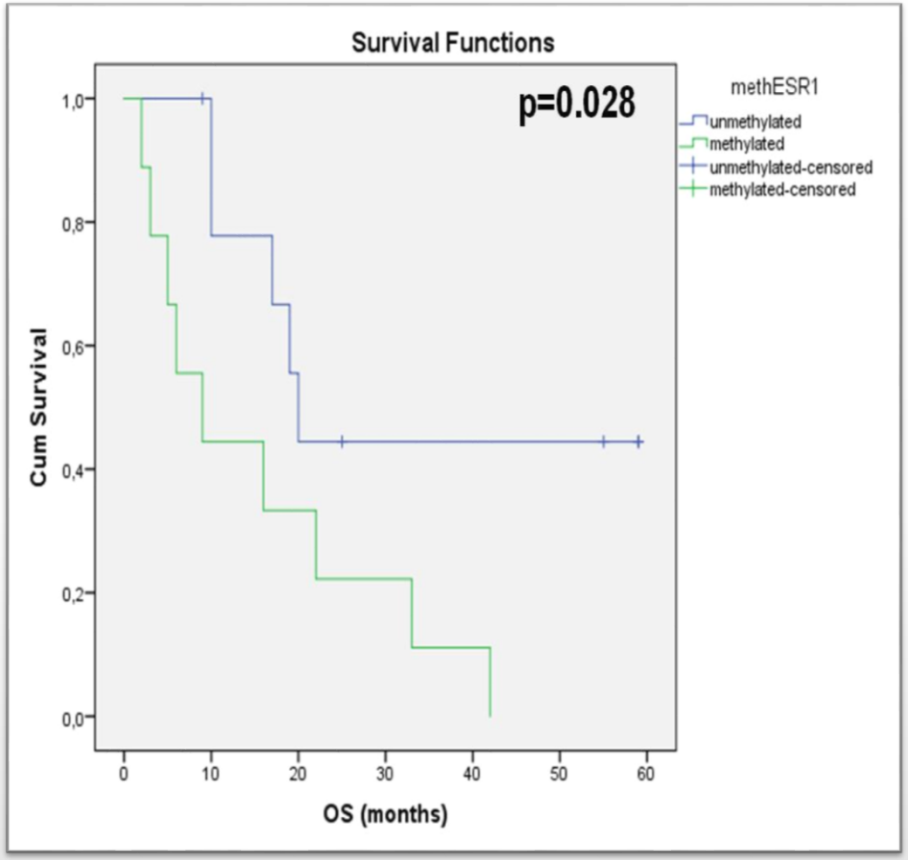


Mastoraki et al. Clin Cancer Research 2018

a) *ESR1* methylation in relation to PFS



b) *ESR1* methylation in relation to OS



Mastoraki et al. Clin Cancer Research 2018

Breast cancer
Integrative LB analysis

Clinical Chemistry

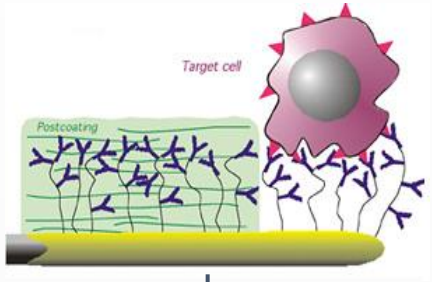
Manuscript Title:	A comprehensive molecular analysis of in-vivo isolated EpCAM-positive circulating tumor cells in Breast Cancer
Manuscript No:	CLINCHEM/2021/333331 [R1]
Manuscript Type:	Article
Date Submitted by the Author:	30 Mar 2021
Complete List of Authors:	Areti Strati, Martha Zavridou, Galatea Kallergi, Eleni Politaki, Andra Kuske, Tobias Gorges, Sabine Riethdorf, Simon Joosse, Claudia Koch, Anna-Lena Bohnen, Volkmar Mueller, George Koutsodontis, Emmanouil Kontopodis, Nikiforita Poulakaki, Amanda Psyrri, Dimitris Mavroudis, Vasilis Georgoulias, Klaus Pantel, and Evi Lianidou
Keywords:	Circulating tumor cells; ESR1 methylation; PIK3CA mutations; breast cancer ; liquid biopsy; molecular assays

***In-vivo* CTC isolation:**
CellCollector, GILUPI
Gene expression: RT-qPCR
DNA mutations: ddPCR
DNA methylation: MSP

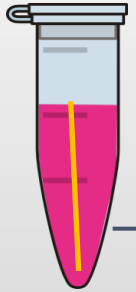
Figure 1

Breast Cancer patients (same blood draw)

***In-vivo* CTC isolation (CellCollector®)**

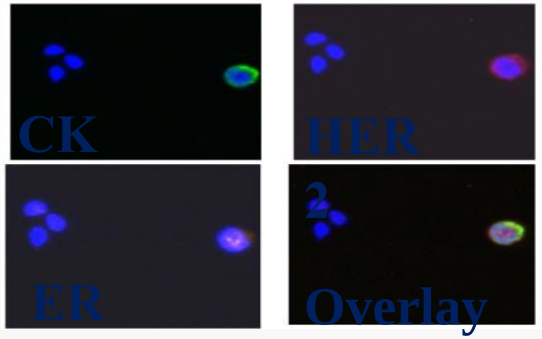


Captured Cell lysis

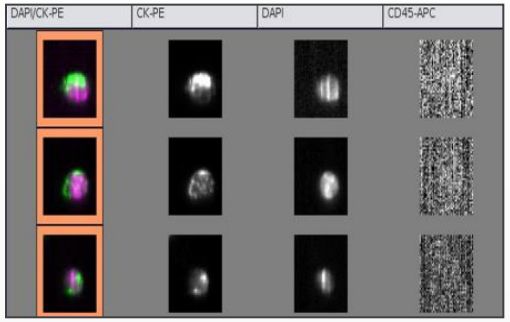


RNA/DNA extraction

Triple IF-staining



CTC enumeration CellSearch®



RNA analysis: RT-qPCR

Epithelial markers : *CK8, CK18, CK19*
Receptors: *ER, PR, HER2*
Growth factors: *EGFR, VEGF*
EMT markers: *TWIST1, VIM, N-CADH*
Stem Cell markers: $CD44^{high}/CD24^{-/low}$, $ALDH1^{high}/CD24^{-/low}$

DNA analysis: WGA and ddPCR

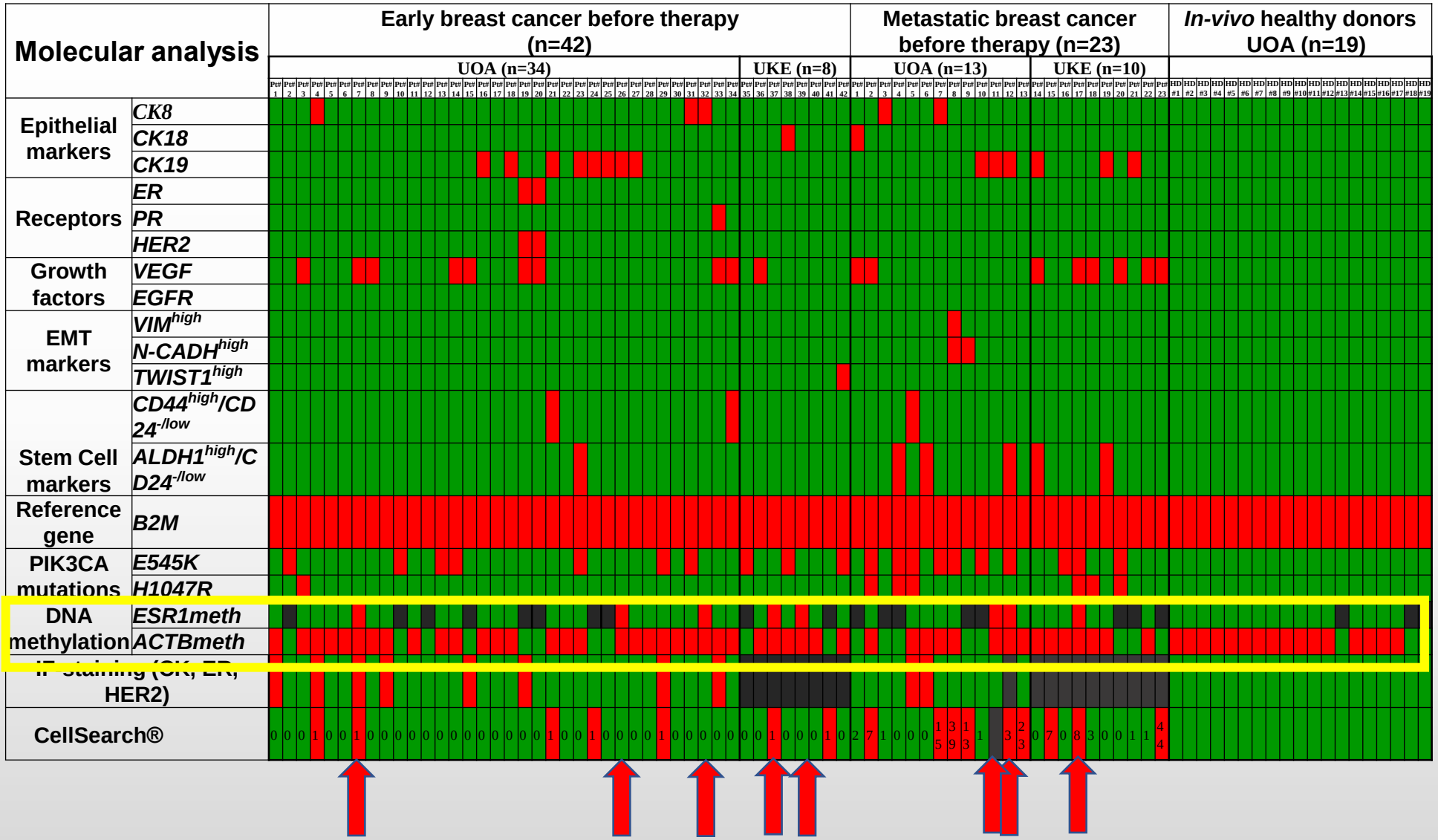
PIK3CA hot spot mutations: *E545K, H1047R*

DNA analysis: real time MSP

DNA methylation: *ESR1*

Strati et al, Clin Chemistry, 2021

CTC in-vivo isolation/molecular analysis, IF-staining and CellSearch® analysis in early BrCa patients without overt metastasis (n=42), MBC BrCa patients before first cycle of therapy (n=23) and healthy individuals (n=19)



Strati et al, Clin Chemistry, 2021

Sci Rep. 2023 Jan 23;13(1):1258. doi: 10.1038/s41598-022-25400-1.

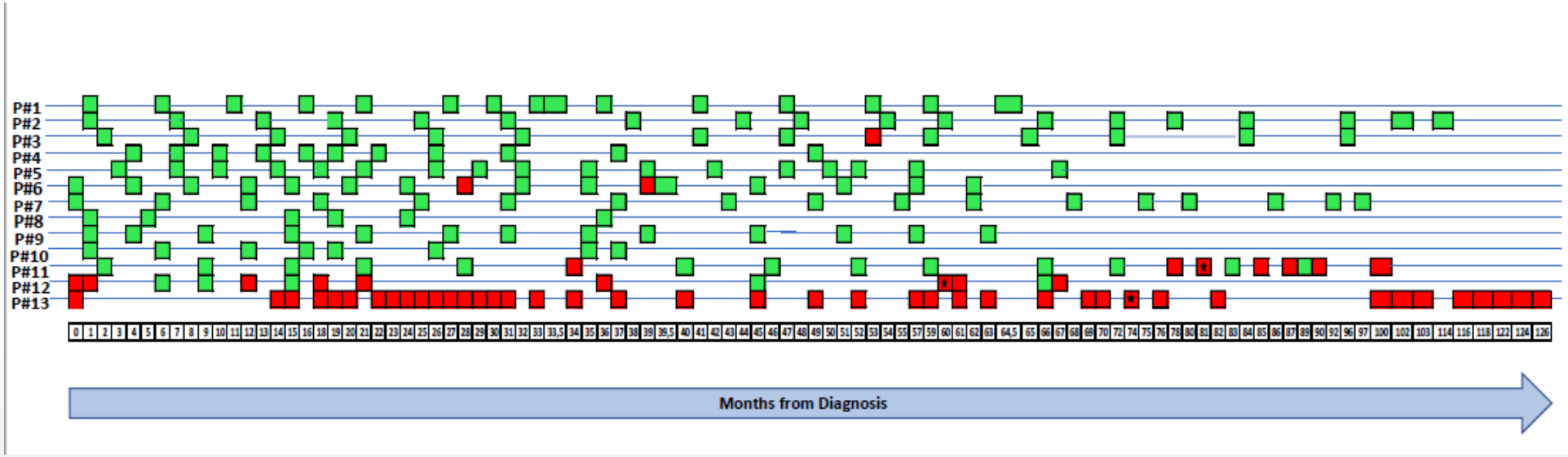
Comprehensive liquid biopsy analysis as a tool for the early detection of minimal residual disease in breast cancer

Dimitra Stergiopoulou [1], Athina Markou [1], Areti Strati [1], Martha Zavridou [1], Eleni Tzanikou [1], Sophia Mastoraki [1], Galatea Kallergi [2], Vassilis Georgoulias [3], Evi Lianidou [4]

13 patients with early-stage operable breast cancer were followed at several time points for a period of ten years, using comprehensive liquid biopsy analysis consisting of:

(a) CTC enumeration using the CellSearch system,
(b) phenotypic analysis of CTCs using Immunofluorescence,
(c) gene expression analysis, in EpCAM(+) CTCs for *CK-19, CD24,CD44, ALDH1*, and *TWIST1* mRNA transcripts (RT-qPCR)
(d) analysis of *PIK3CA* and *ESR1* mutations in EpCAM(+) CTCs and corresponding plasma ctDNA
(e) DNA methylation of *ESR1* in CTCs

Comprehensive liquid biopsy analysis as a tool for the early detection of Minimal Residual Disease in breast cancer



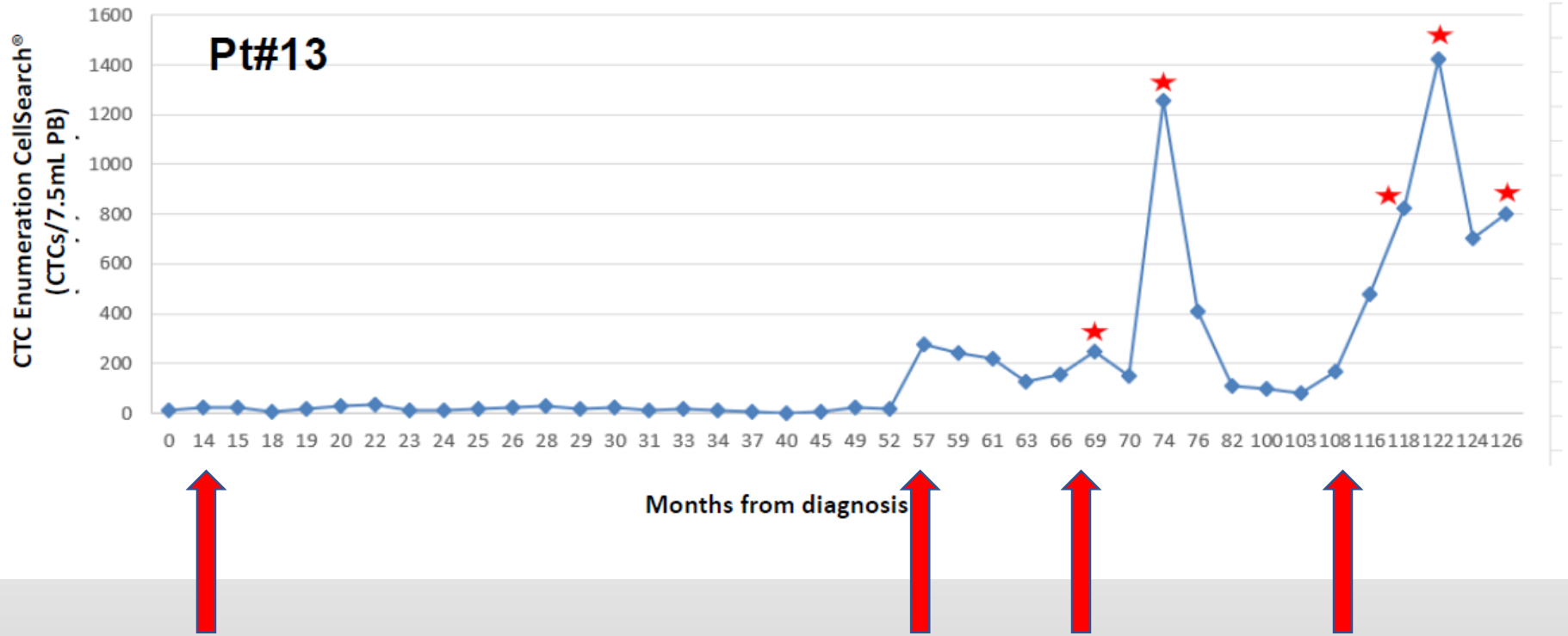
Molecular analysis of EpCAM^{+} CTC fractions for: *CK-19* mRNA expression, DNA mutations (*PIK3CA*, *ESR1*) and CTC enumeration (CellSearch).

Stars represent the time of relapse/metastasis. (red: positive, green: negative)

Stergiopoulou et al , Sci Rep 2023

Patient #13:
CTC enumeration values (CTCs/7.5mL PB) based on CellSearch®, during the timeline of the disease

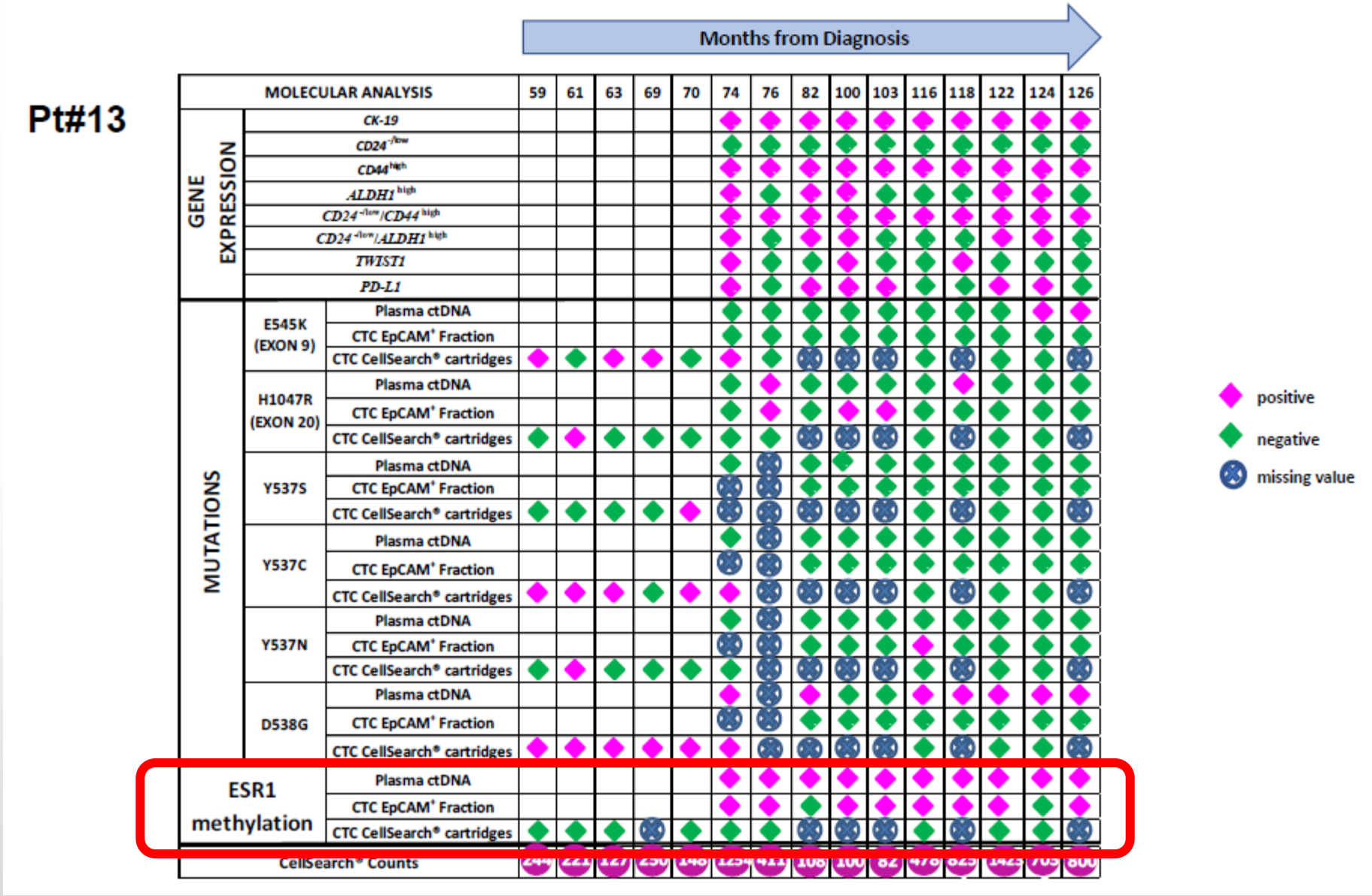
*: Progression of disease (PD)



Early stage: CellSearch counts >10CTCs/22mL PB

Stergiopoulou et al , Sci Rep 2023

Molecular analysis of EpCAM^{+} CTC fractions and CTCs from CellSearch® cartridges



Stergiopoulou et al , Sci Rep 2023

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NSCLC

Epigenetic alterations in CTC and paired ctDNA

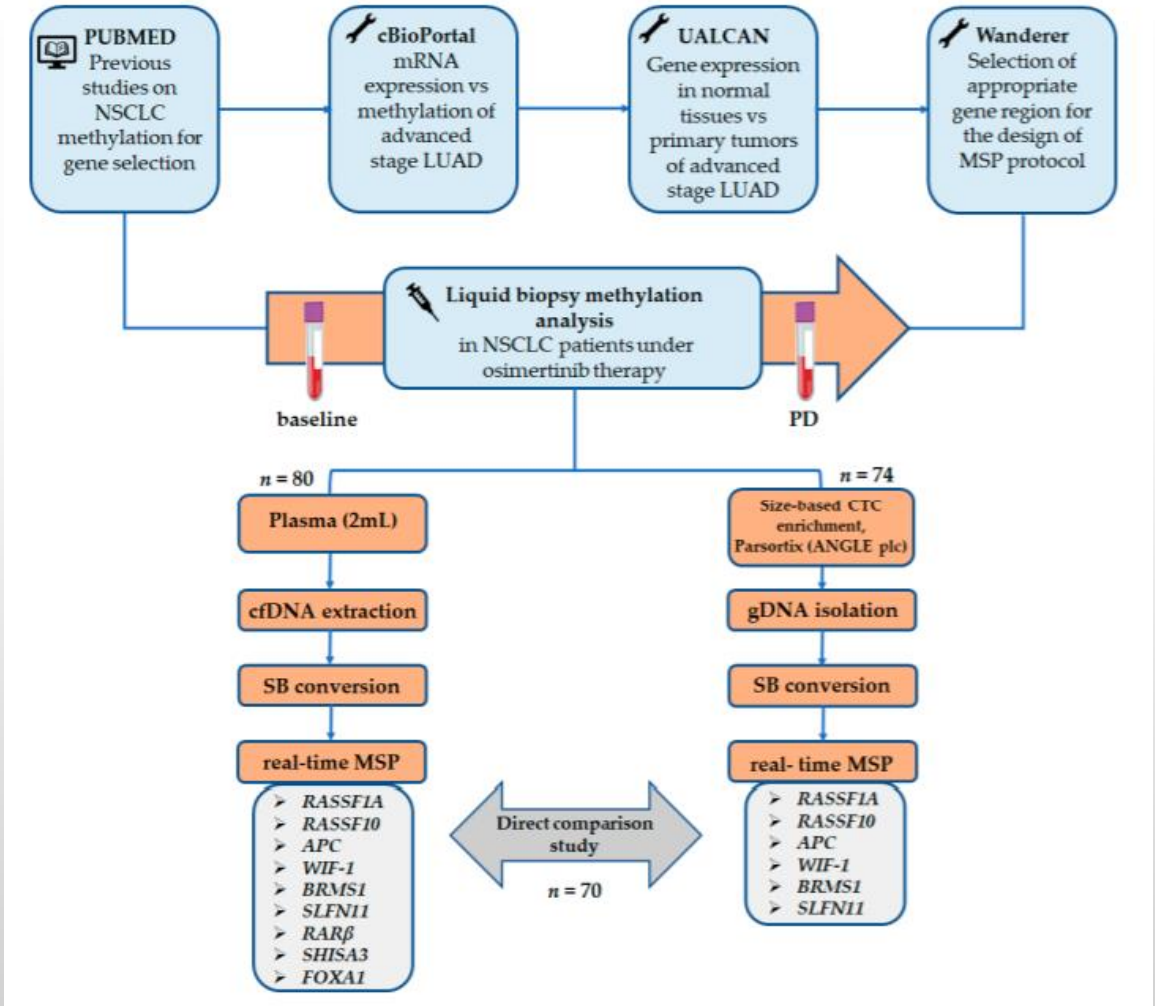
cancers

MDPI

Article

DNA Methylation Analysis in Plasma Cell-Free DNA and Paired CTCs of NSCLC Patients before and after Osimertinib Treatment

Aliki Ntzifa [1], Dora Londra [1], Theodoros Rampias [2], Athanasios Kotsakis [3], Vassilis Georgoulias [4] and Evi Lianidou [1,*]



Direct comparison of DNA methylation markers in plasma-cfDNA and paired CTCs before osimertinib and at PD

patient ID	BASELINE			PD		
	CTC	plasma-cfDNA	combined at least 1 gene methylated	CTC	plasma-cfDNA	combined at least 1 gene methylated
#1	▲▲▲	△	●	⊗	△△	●
#2	▲	⊗	●	⊗	⊗	⊗
#4	⊗	⊗	⊗	⊗	⊗	⊗
#5	⊗	⊗	⊗	▲	▲▲	●
#6	⊗	⊗	⊗	△	△△	●
#7	⊗	⊗	⊗	△	▲	●
#8	⊗	⊗	⊗	⊗	⊗	⊗
#9	⊗	⊗	⊗	△	△△▲	●
#10	⊗	⊗	⊗	⊗	▲	●
#11	⊗	⊗	⊗	⊗	⊗	⊗
#12	⊗	⊗	⊗	▲▲	▲▲	●
#13	△	⊗	●	→		
#14	⊗	⊗	⊗	⊗	⊗	⊗
#16	⊗	⊗	⊗	⊗	▲	●
#17	⊗	△	●	▲	⊗	●
#18	⊗	△	●	⊗	△△▲	●
#19	⊗	⊗	⊗	⊗	△	●
#20	⊗	△	●	▲	△△	●
#21	⊗	⊗	⊗	⊗	⊗	⊗
#23	⊗	⊗	⊗	△	⊗	●
#24	⊗	⊗	⊗	⊗	⊗	⊗
#25	⊗	⊗	⊗	▲	⊗	●
#26	▲	⊗	●	⊗	⊗	⊗
#27	⊗	⊗	⊗	⊗	⊗	⊗
#28	⊗	▲▲▲	●	⊗	▲▲△	●
#29	⊗	⊗	⊗	⊗	▲	●
#30	⊗	▲	●	▲	⊗	●
#31		⊗	⊗		▲	●
#32	⊗	⊗	⊗	⊗	⊗	⊗
#34	▲	⊗	●	⊗	⊗	⊗
#36	⊗	⊗	⊗	⊗	⊗	⊗
#37	⊗	⊗	⊗	⊗	⊗	⊗
#38	⊗	▲	●	⊗	△△	●
#39	⊗	⊗	⊗	→		
#40	△	⊗	●	⊗	⊗	⊗
#41		⊗	⊗		⊗	⊗
#42		⊗	⊗		⊗	⊗
#44	▲	△△	●	⊗	⊗	⊗
#45	⊗	⊗	⊗	⊗	⊗	⊗
#46		▲	●		▲▲	●
#48		⊗	⊗		△	●

▲ :*RASSF1A* methylation
▲ :*RASSF10* methylation
▲ :*BRMS1* methylation
▲ :*SLFN11* methylation
▲ :*WIF-1* methylation
△ :*APC* methylation
⊗ :no methylation
● :at least one gene methylated
→ :no PD
■ :missing

Ntzifa et al, Cancers, 2021

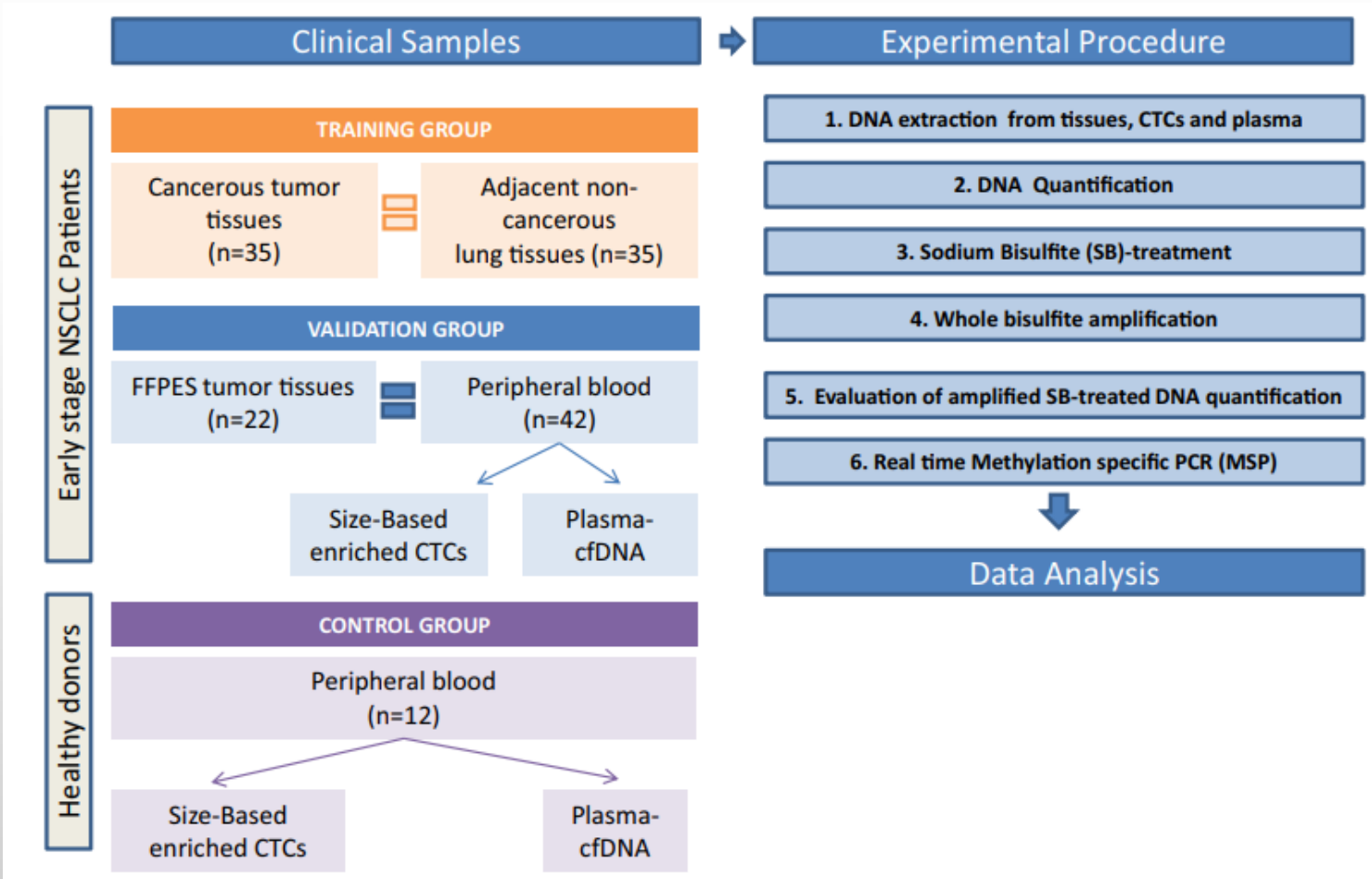
Markou et al. Clinical Epigenetics (2022) 14:61
https://doi.org/10.1186/s13148-022-01283-x

Clinical Epigenetics

RESEARCH Open Access

DNA methylation analysis of tumor suppressor genes in liquid biopsy components of early stage NSCLC: a promising tool for early detection

A. Markou[1*], D. Londra[1], V. Tserpeli[1], I. Kollias[1], E. Tsaroucha[2], I. Vamvakaris[2], K. Potaris[2], I. Pateras[3], A. Kotsakis[4], V. Georgoulias[5] and E. Lianidou[1]



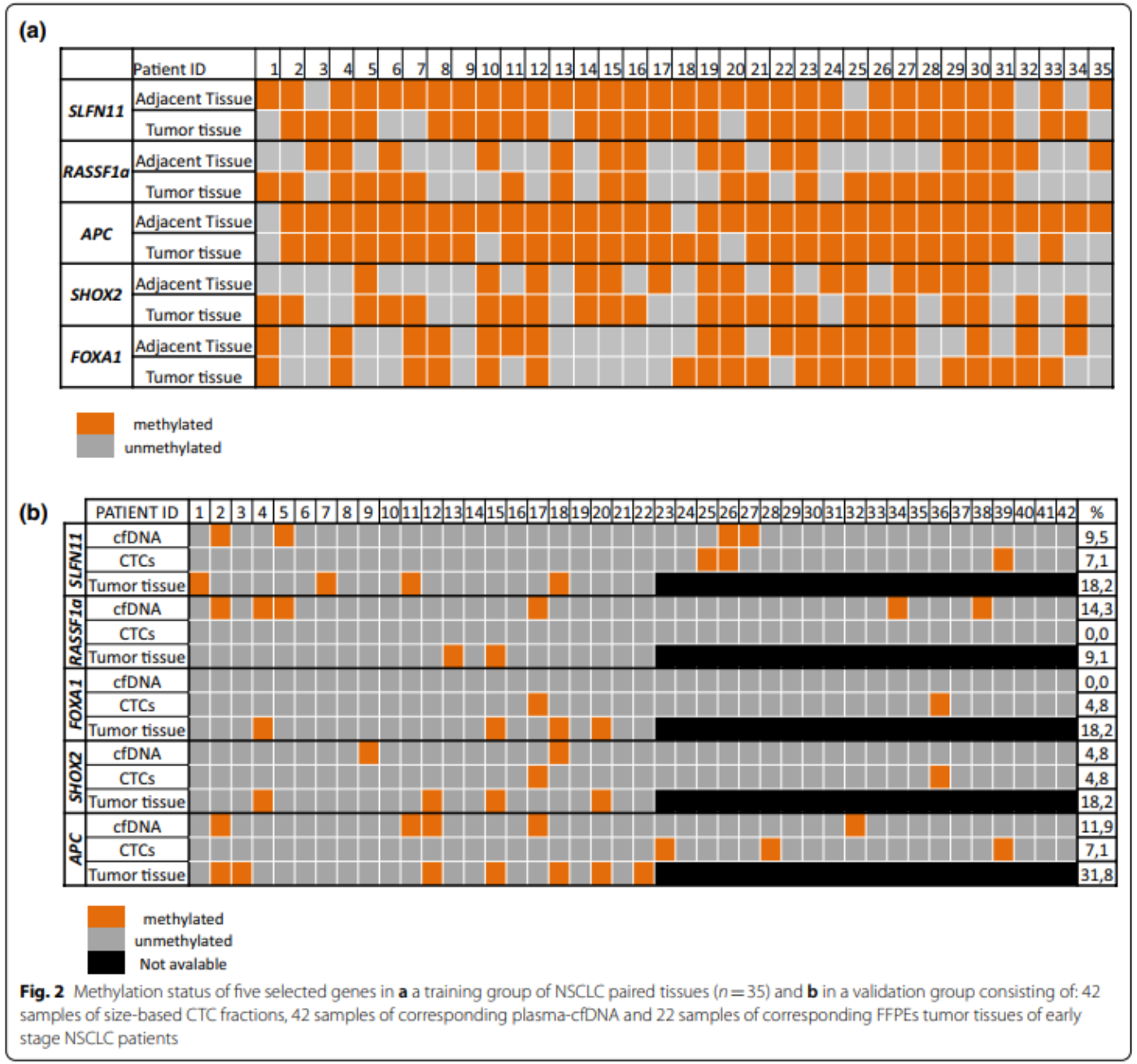
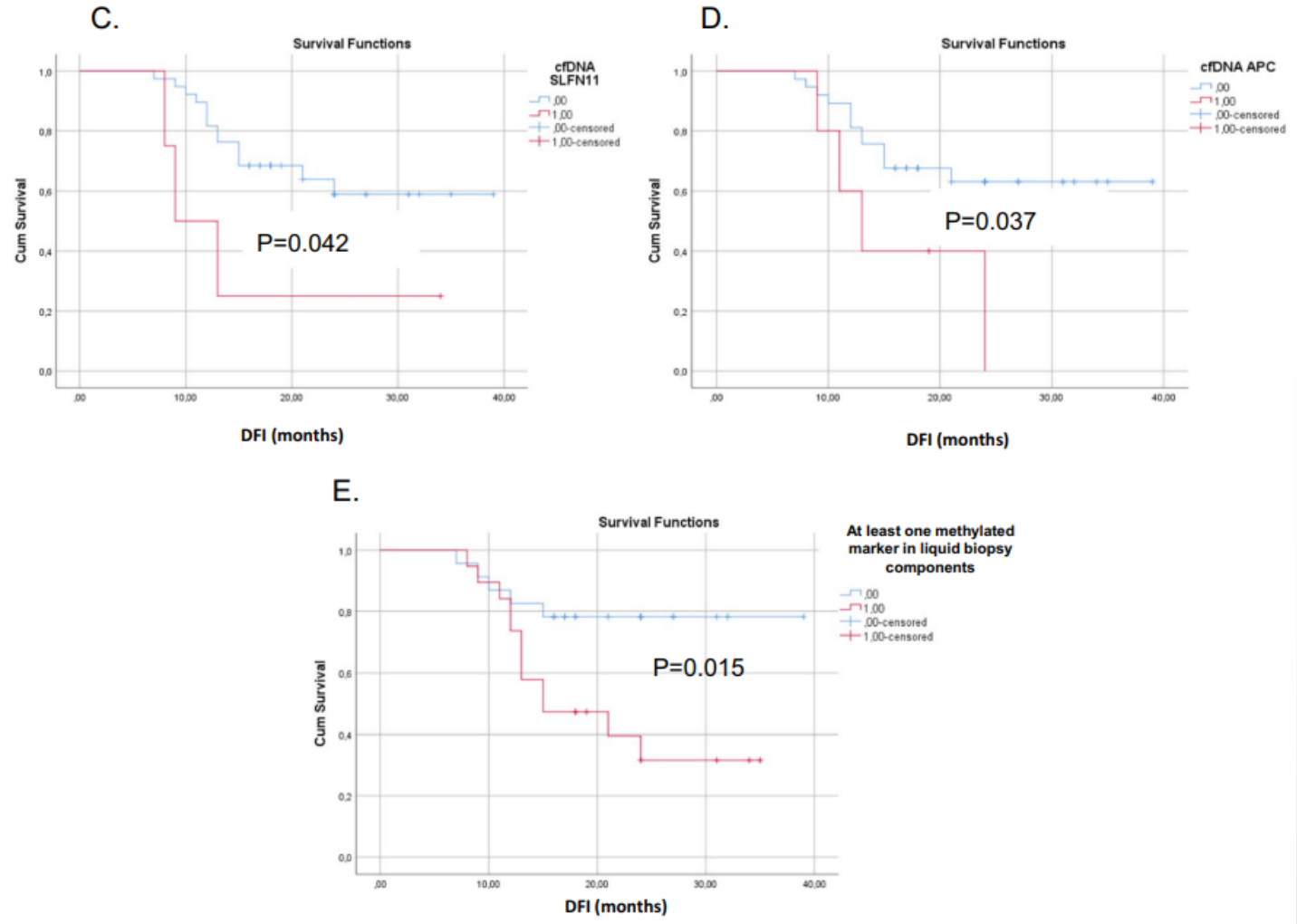


Fig. 2 Methylation status of five selected genes in **a** a training group of NSCLC paired tissues ($n = 35$) and **b** in a validation group consisting of: 42 samples of size-based CTC fractions, 42 samples of corresponding plasma-cfDNA and 22 samples of corresponding FFPEs tumor tissues of early stage NSCLC patients

Markou et al, Cin Epigenetics, 2022



Our fndings indicate that the combination of DNA methylation analysis of tumor suppressor genes in CTCs and matched plasma-cfDNA provides signifcant prognostic information in patients with early-stage NSCLC

Markou et al, Cin Epigenetics, 2022

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prostate cancer

Epigenetic alterations in CTC and ctDNA

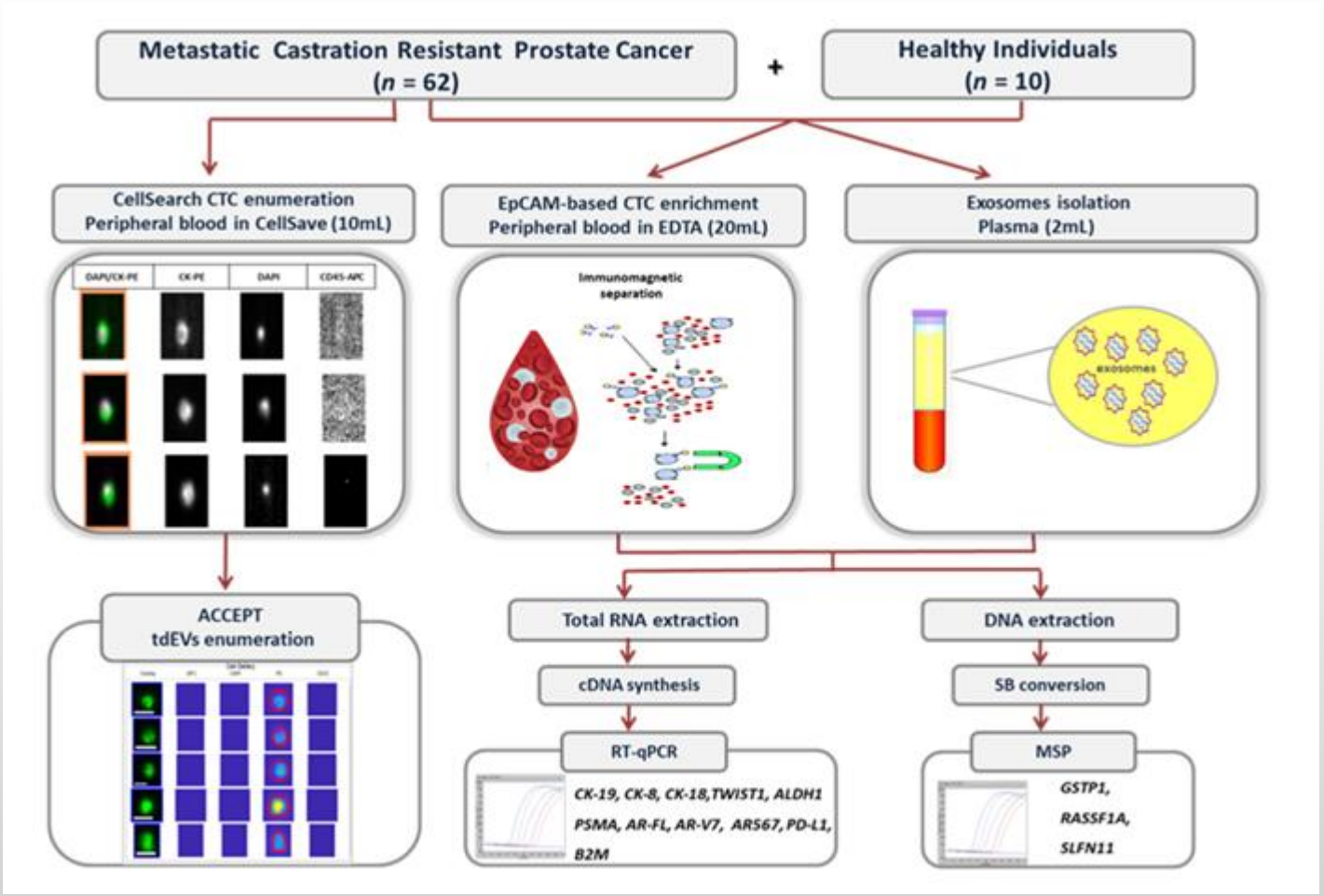
cancers

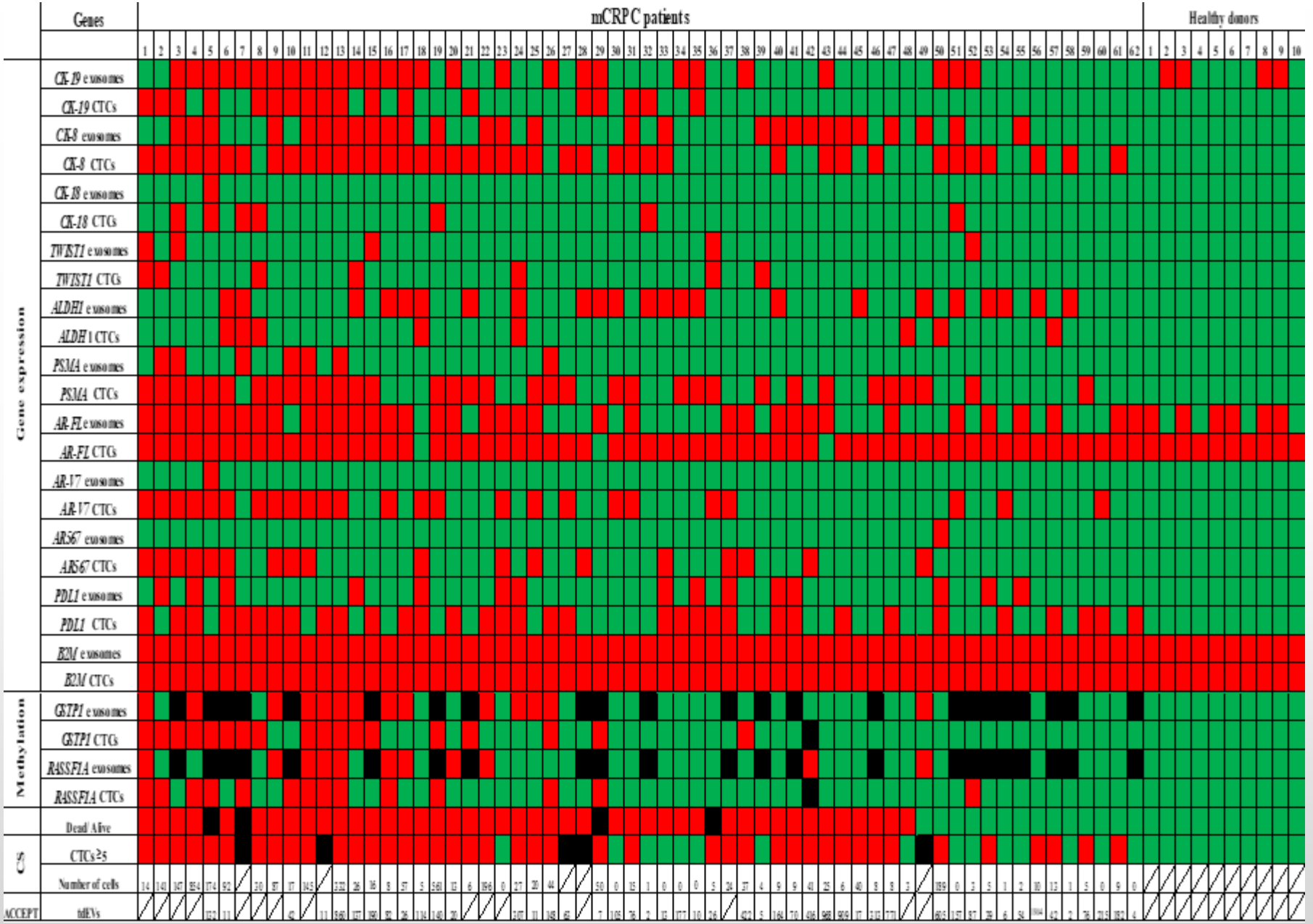
MDPI

Article

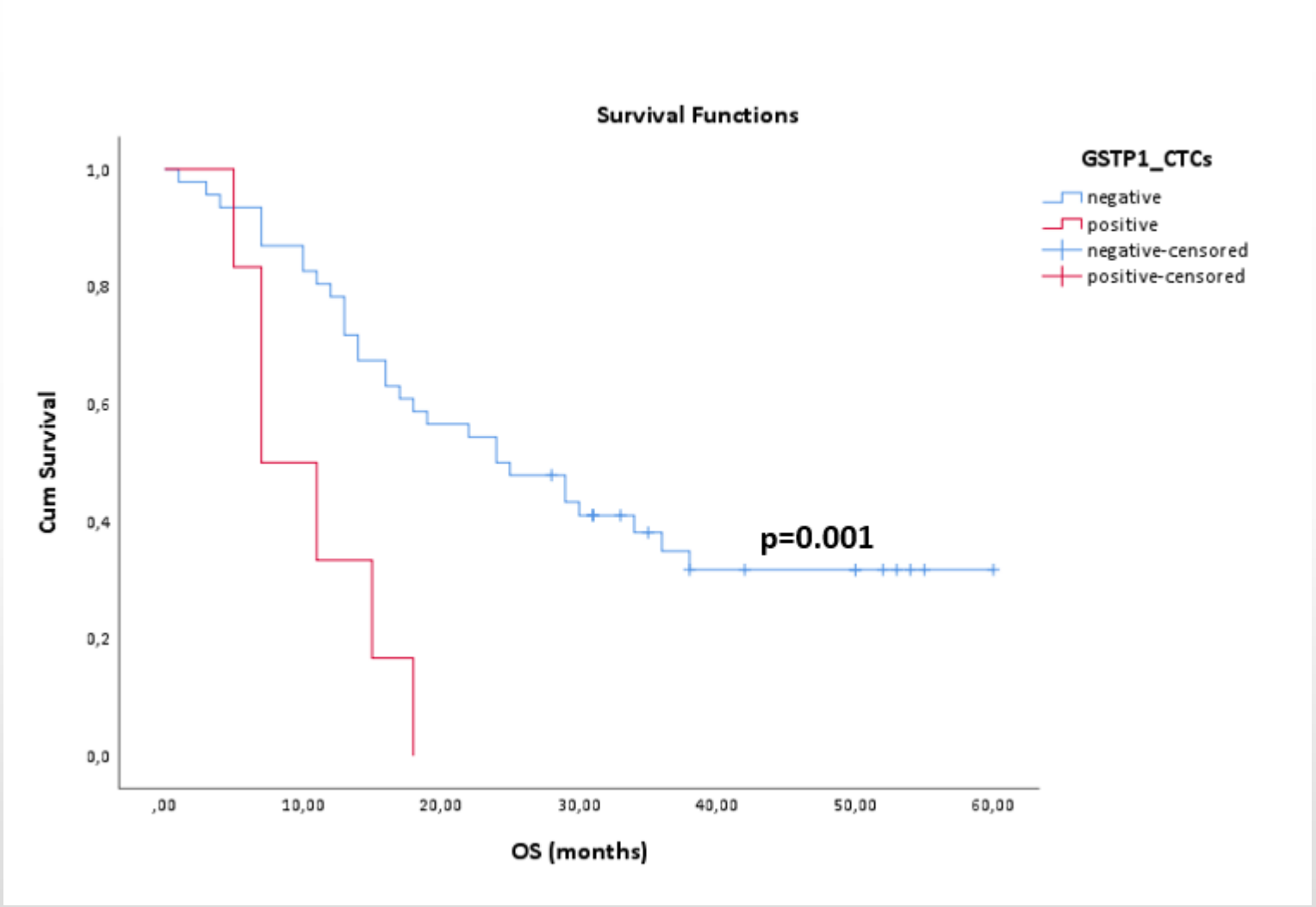
Prognostic Significance of Gene Expression and DNA Methylation Markers in Circulating Tumor Cells and Paired Plasma Derived Exosomes in Metastatic Castration Resistant Prostate Cancer

Martha Zavridou [1], Areti Strati [1], Evangelos Bournakis [2], Stavroula Smilkou [1], Victoria Tserpeli [1] and Evi Lianidou [1,*]





Zavridou et al, CANCERS, 2021



Zavridou et al, CANCERS, 2021

cancers

MDPI

Article

USP44 promoter methylation in plasma cell-free DNA in prostate cancer

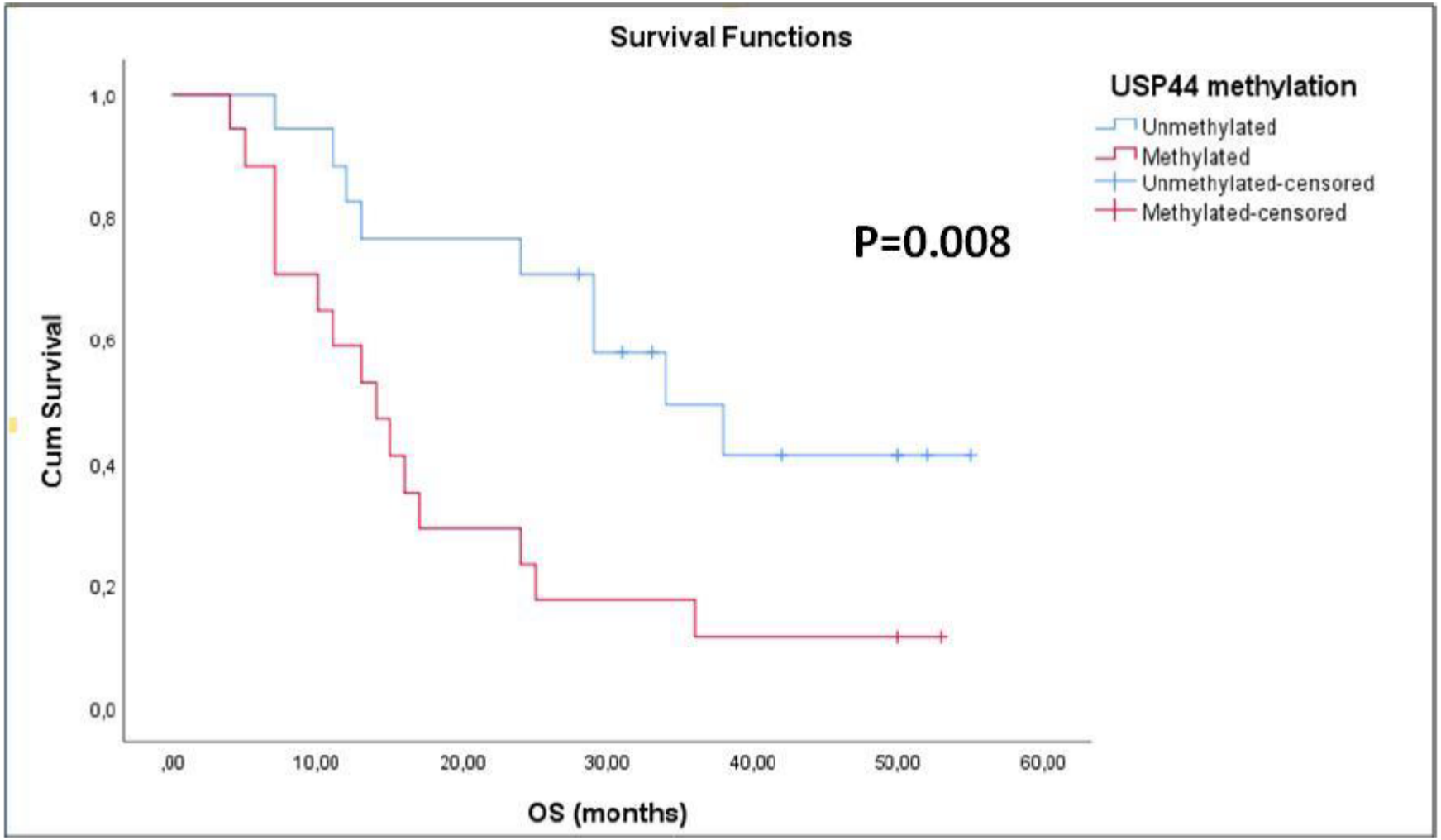
Londra T[1], Mastoraki S[1], Bournakis E[2], Zavridou M[1], Thanos A[3],Rampias T[4], Lianidou E[1].

USP44 promoter is methylated at a high percentage in plasma cfDNA of metastatic prostate cancer patients but not in healthy donors

USP44 promoter methylation in plasma cell free DNA provides significant prognostic information in metastatic prostate cancer.

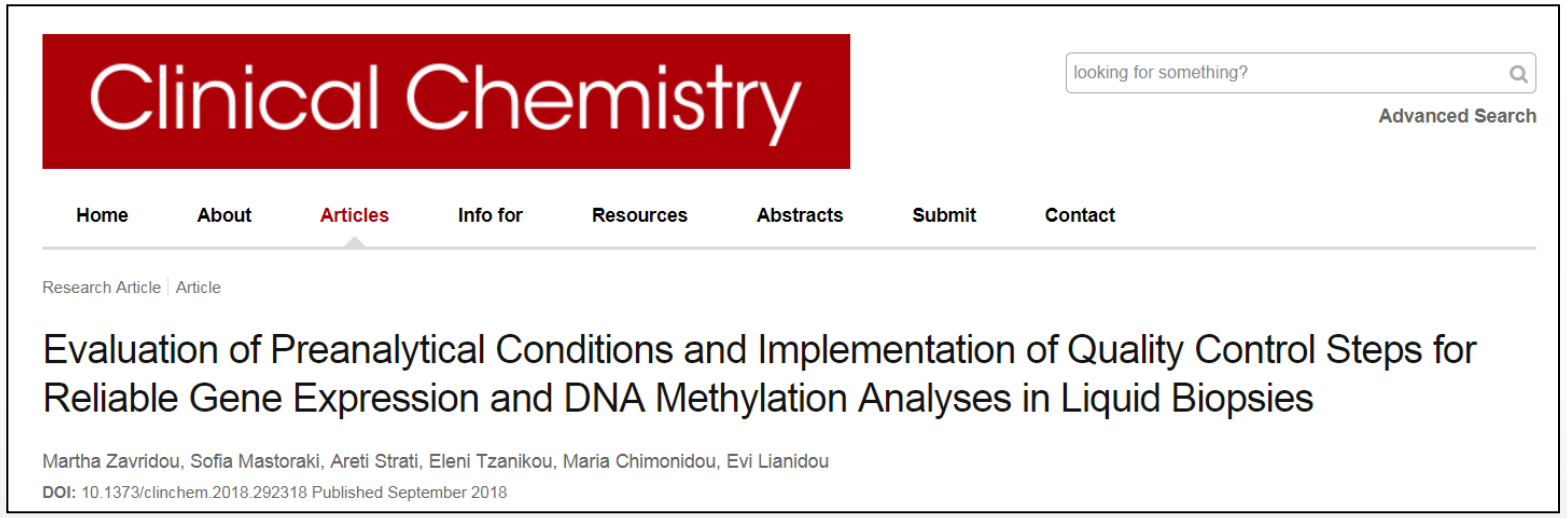
USP44 promoter methylation is significantly associated with overall survival (OS) (P=0.008).

Kaplan–Meier estimates of patients with metastatic prostate cancer in relation to USP44 methylation in ctDNA (n=39)



Londra et al, CANCERS, 2021

PRE-ANALYTICAL CONSIDERATIONS



Clinical Chemistry

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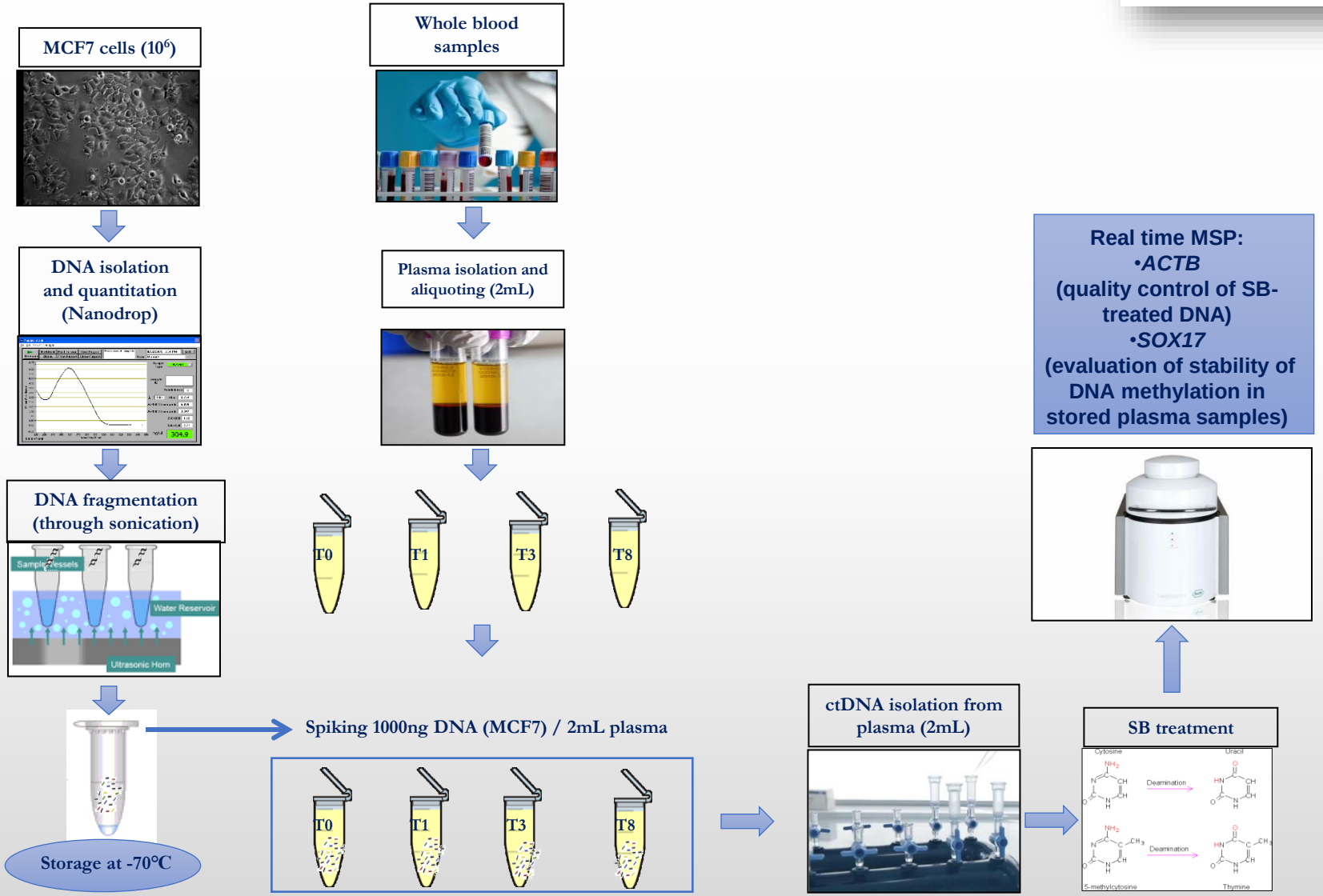
Evaluation of Preanalytical Conditions and Implementation of Quality Control Steps for Reliable Gene Expression and DNA Methylation Analyses in Liquid Biopsies

Martha Zavridou, Sofia Mastoraki, Areti Strati, Eleni Tzanikou, Maria Chimonidou, Evi Lianidou

DOI: 10.1373/clinchem.2018.292318 Published September 2018

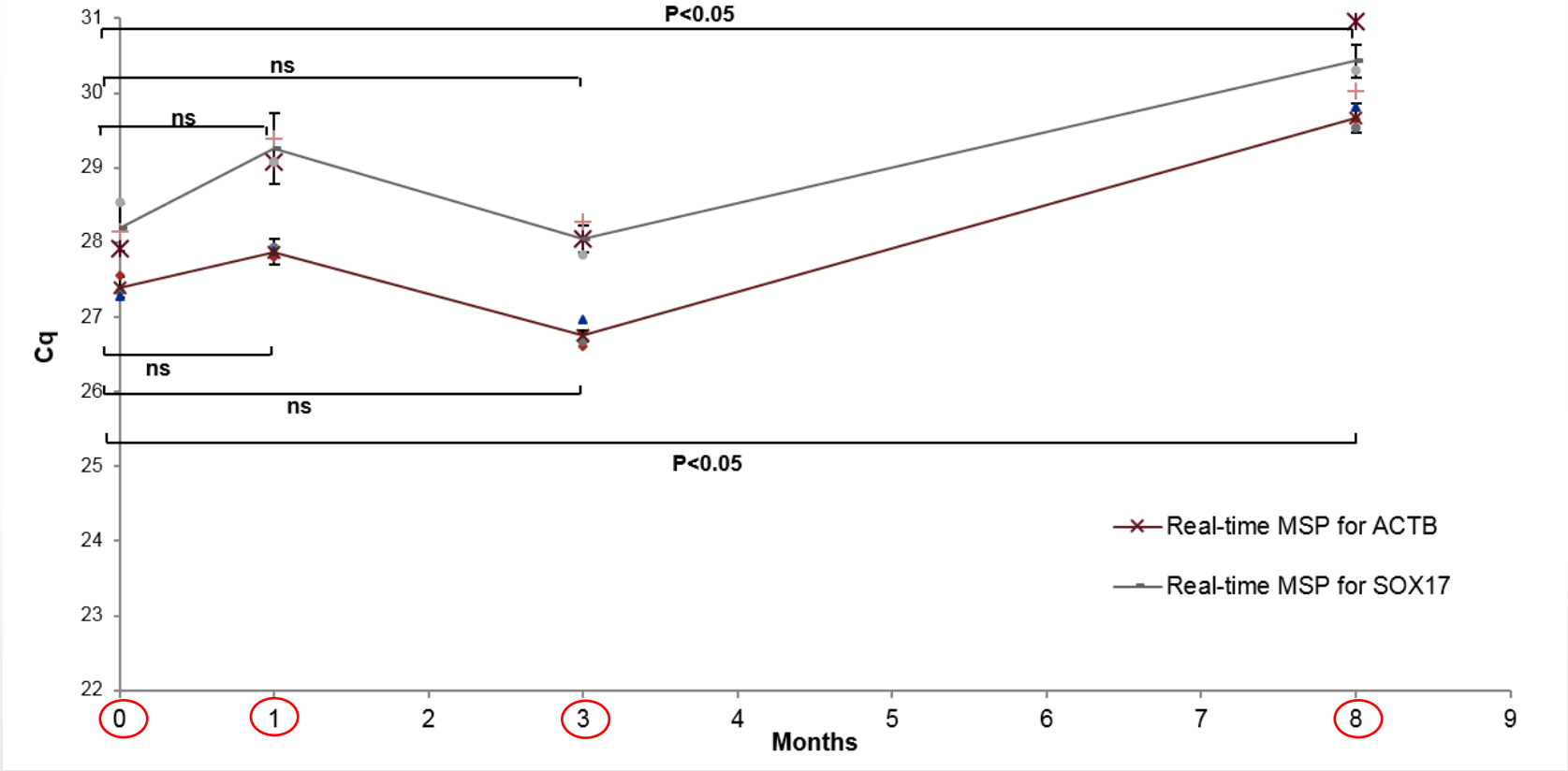
CANCER ID

Stability of DNA methylation in plasma



Martha Zavridou et al. (Clin Chem, 2018)

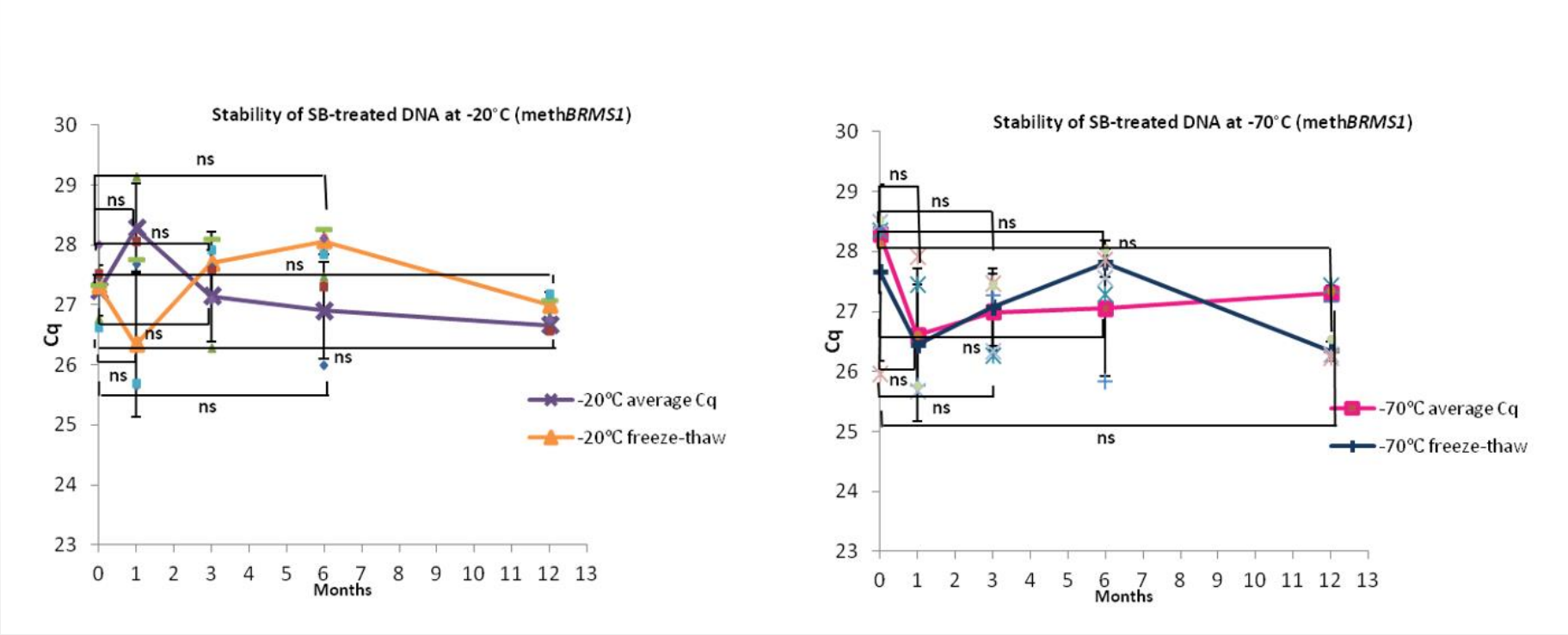
Stability of DNA methylation in plasma



Real-time MSP for *ACTB* and *SOX17* during plasma storage at -70°C for up to eight months; Cq (n=3) and SD values of SB-treated DNA

Martha Zavridou et al. (Clin Chem, 2018)

Stability of SB-treated DNA during storage at -20ºC and -70ºC



Real-time MSP for *BRMS1;* Cq (n=3) and SD values of SB-treated DNA.

Martha Zavridou et al. (Clin Chem, 2018)


ANY
QUESTIONS?