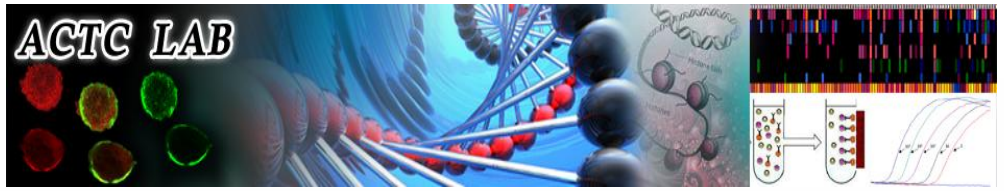
HELLENIC REPUBLIC
National and Kapodistrian University of Athens
EST. 1837



Detection rate for *ESR1* mutations is higher in circulating-tumor-cell-derived genomic DNA than in paired plasma cell-free DNA samples as revealed by ddPCR

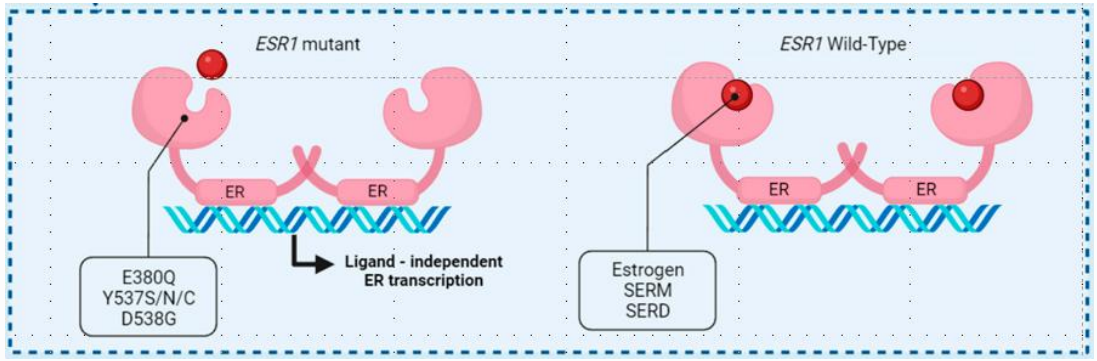
Aliki Ntzifa, PhD

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

11 June 2025

RESISTANCE TO ENDOCRINE THERAPY-*ESR1* GENE MUTATIONS

- More than 70% of breast cancers express *ESR1*
- Aromatase inhibitors or selective ER modulators/ degraders (SERM/SERD) are conventional treatments for ER$^+$ breast cancer.
- Resistance to these endocrine therapies is a clinical challenge → 30%–40% of endocrine-resistant metastatic breast cancer (MBC) is enriched in *ESR1* somatic missense mutations
- Ligand-binding domain (LBD) *ESR1* mutations correlate with poor outcomes in patients with advanced disease.
- *ESR1* hotspot mutations drive constitutive ER activity and decrease sensitivity toward ER antagonists



Betz M, et al. Cancers (Basel). 2023 Oct 27;15(21):5169

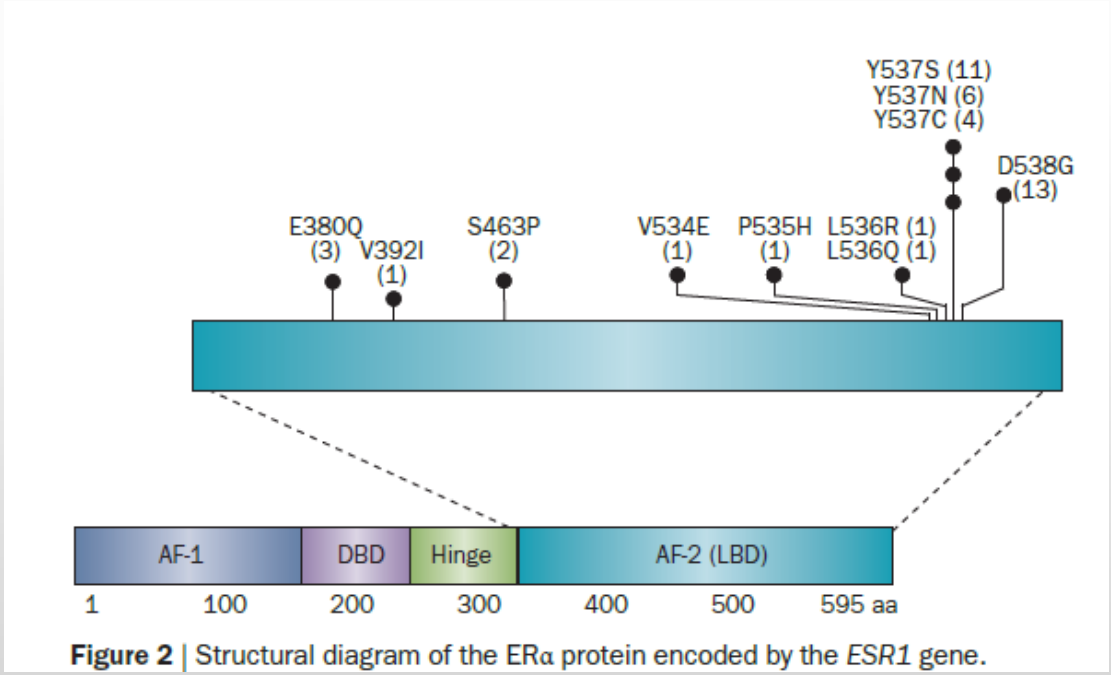
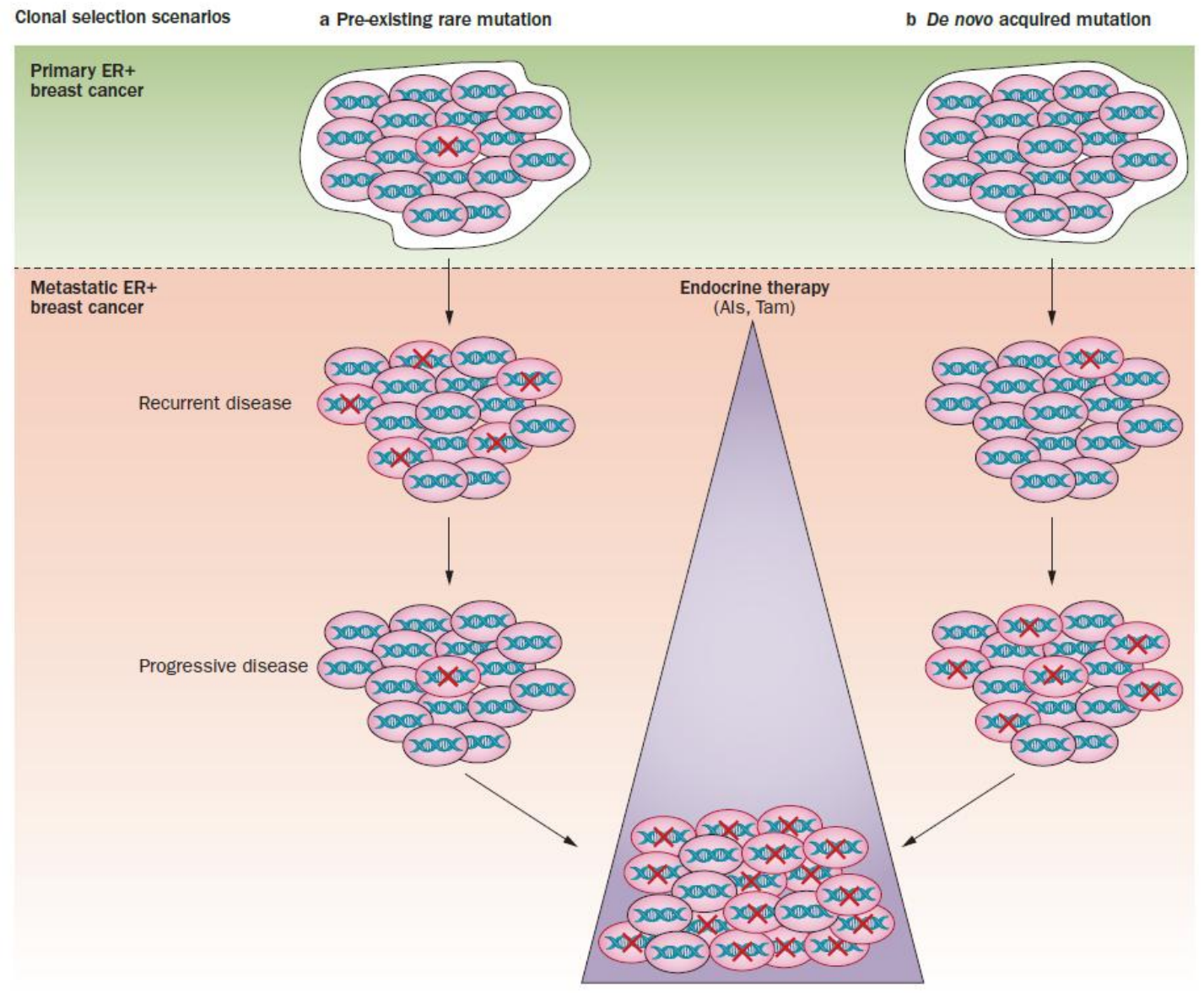


Figure 2 | Structural diagram of the ERα protein encoded by the *ESR1* gene.

Jeselsohn R, et al. Nat Rev Clin Oncol. 2015

ESR1 MUTATIONS IN ctDNA

CLONAL EVOLUTION OF *ESR1* MUTATIONS



Jeselsohn R, et al. Nat Rev Clin Oncol. 2015

cancers

MDPI

Article

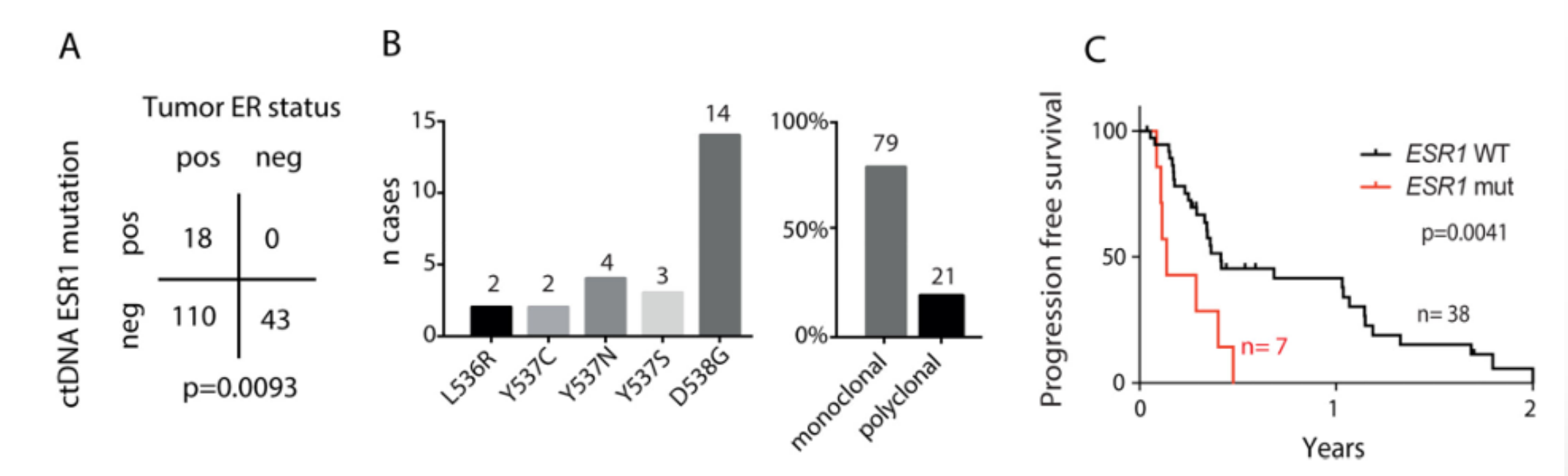
Detection of *ESR1* Mutations in Tissue and Liquid Biopsy with Novel Next-Generation Sequencing and Digital Droplet PCR Assays: Insights from Multi-Center Real Life Data of Almost 6000 Patients

Srushti Borkar [1,2,†], Fenja Markus [3,4,†], Agnes Oetting [3], Stefanie Schmidt [3], Christine Vössing [3,4], David Horst [5,6], Markus Möbs [5,6], Elena I. Braicu [7], Frank Griesinger [1,2,4], Katja Horling [3], Katharina Tiemann [3], Lukas C. Heukamp [2,3,4,*], Eva-Maria Willing [3] and Claudia Vollbrecht [5,6,*]

*"We evaluated NGS results of a pan-cancer cohort of almost **6000 patients** from two major German institutes of pathology, to show that **the occurrence of ESR1 mutations is extremely rare (<1%) in ET-naive patients.** This suggests that ESR1 mutations **arise almost exclusively under the pressure of ET.**"*

Borkar S, et al. Cancers (Basel). 2025

CLINICAL UTILITY OF ctDNA *ESR1* MUTATION TESTING



- *ESR1* mutations were detected in the plasma of 10.5% (18/171, 95% CI 6%-16%) patients with advanced breast cancer, exclusively in patients with ER positive breast cancer
- All patients with *ESR1* mutations detected had prior AI exposure
- Patients with *ESR1* mutations detected in plasma had a substantially shorter PFS on subsequent AI-based therapy

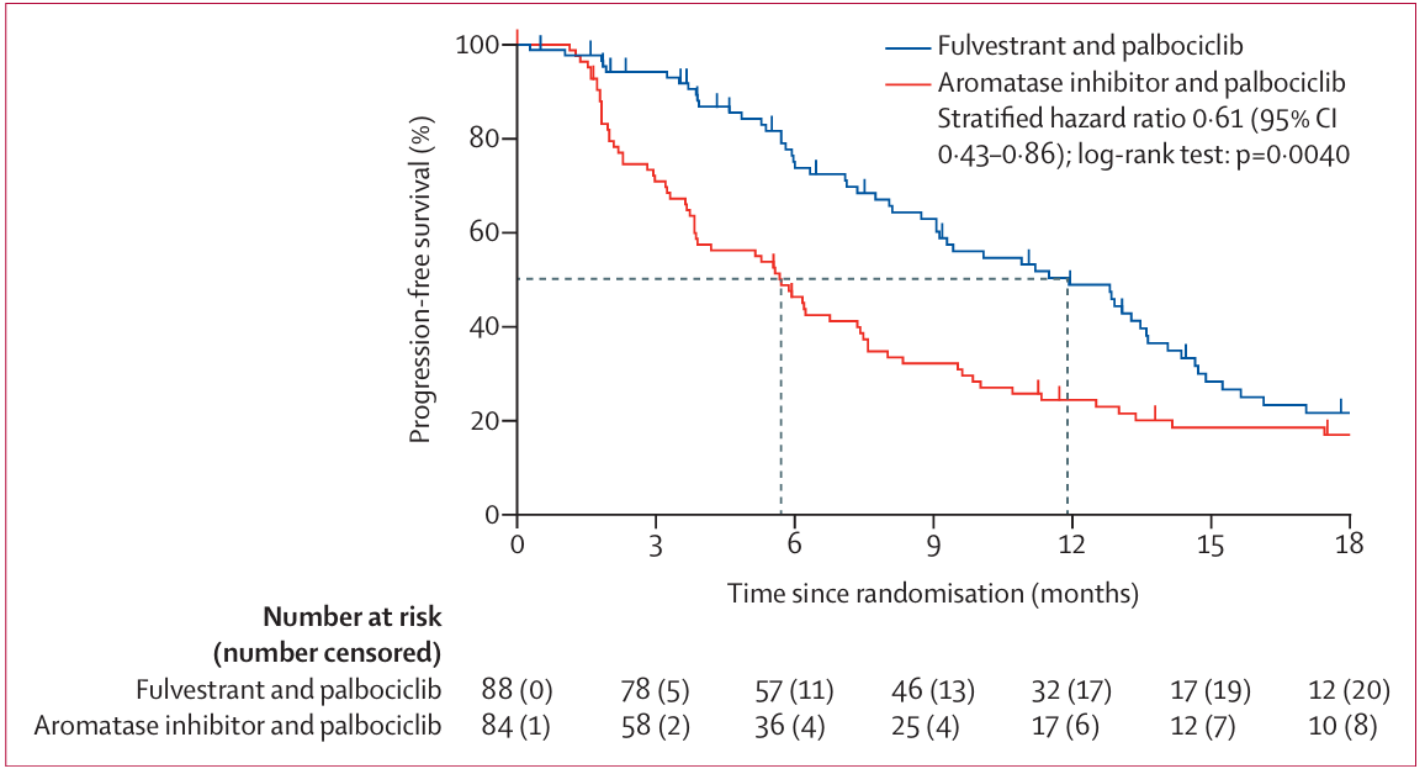
Schiavon G, et al. Sci Transl Med. 2015

ctDNA *ESR1* MUTATION MONITORING FOR THERAPY DECISIONS

PADA-1 trial

- **molecular stratification** of patients with MBC under 1st line aromatase inhibitor (AI) and palbociclib based on *ESR1* mutations detected through liquid biopsy

- to detect these mutations early, potentially **before clinical progression** is evident, which could allow **for earlier intervention** with alternative therapies: **early switch** from AI therapy **to the SERD fulvestrant, with palbociclib** being continued



Bidard FC, et al. Lancet Oncol. 2022

CLINICAL UTILITY OF *ESR1* MUTATION TESTING

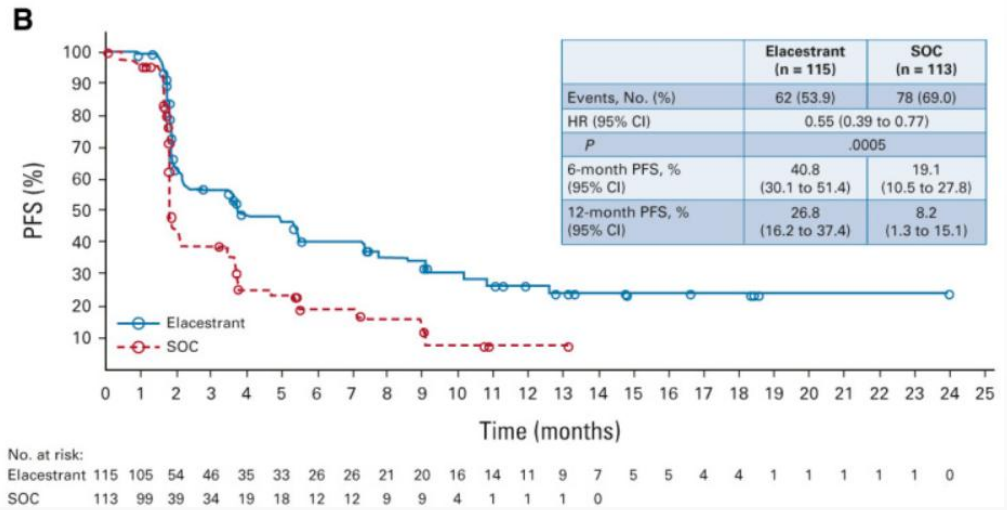
Table 1. Frequency of *ESR1*m after AI (mono or combination therapy).

Trial	Tumor characteristics	Timing of test	Test (sample)	*ESR1*m frequency	N, *ESR1*m/total	Ref.
MONARCH 3	Endocrine-therapy-naive HR+/HER2- ABC treated with 1L AI monotherapy (control arm)	End of 1L AI treatment	NGS (plasma)	31%	NR	[20]
EMERALD	One or two previous lines of endocrine therapy, at least one in combination with CDK4/6i	Start of 2L or 3L treatment	NGS (plasma)	48%	228/477	[12]
GuardantINFORM database	At least one previous AI therapy	Post-AI therapy	NGS (plasma)	31%	2044/6541	[21]
SoFEA/EFECT	HR+ mBC that had progressed on previous AI monotherapy	Start of 2L treatment	ddPCR (plasma)	30%	151/383	[18]
BOLERO-2	HR+ ABC that had progressed on previous AI monotherapy	Start of 2L or 3L treatment	ddPCR (plasma)	29%	156/541	[19]
PEARL	AI-resistant HR+/HER2- mBC	Start of 2L or 3L treatment	ddPCR (plasma)	29%	164/557	[22]
PALOMA-3†	HR+/HER2- mBC that had relapsed or progressed on previous AI or tamoxifen monotherapy	Start of 2L or 3L treatment	ddPCR (plasma)	26%	114/445	[23]

Turner N et al. Future Oncol. 2023

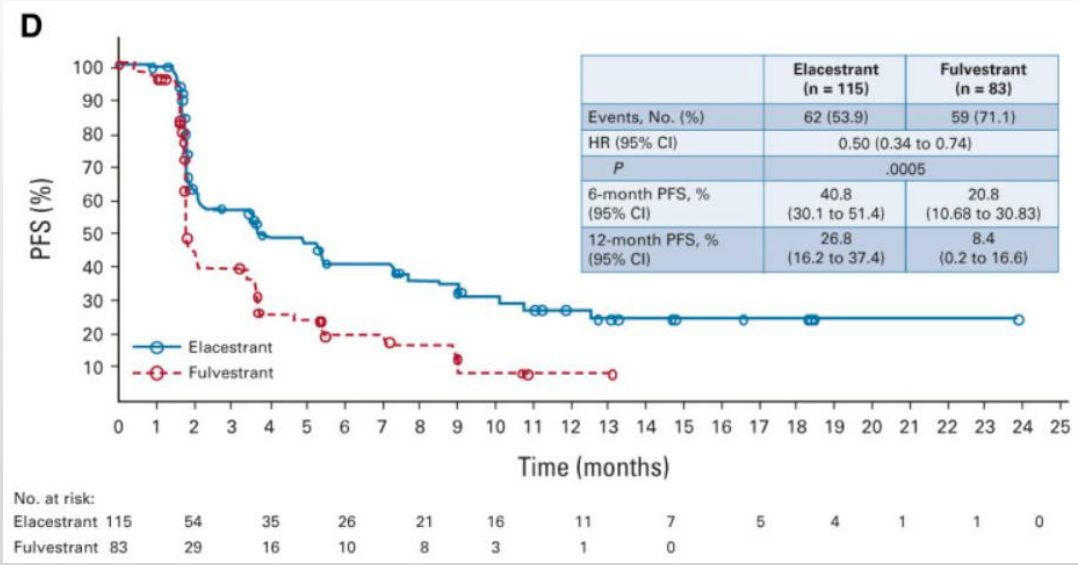
CLINICAL UTILITY OF ctDNA *ESR1* MUTATION TESTING

EMERALD trial



	Elacestrant (n = 115)	SOC (n = 113)
Events, No. (%)	62 (53.9)	78 (69.0)
HR (95% CI)	0.55 (0.39 to 0.77)	
P	.0005	
6-month PFS, % (95% CI)	40.8 (30.1 to 51.4)	19.1 (10.5 to 27.8)
12-month PFS, % (95% CI)	26.8 (16.2 to 37.4)	8.2 (1.3 to 15.1)

- Randomizing patients with pre-treated MBC to receive the novel oral SERD elacestrant or the standard of care ET.
- Patients harboring ***ESR1* mutations and treated with elacestrant had a longer progression-free survival (PFS),** with a PFS of 3.8 months compared with the 1.9 months of the standard-of-care (SOC) arm
- The efficacy of elacestrant was maintained when compared with the fulvestrant subgroup



	Elacestrant (n = 115)	Fulvestrant (n = 83)
Events, No. (%)	62 (53.9)	59 (71.1)
HR (95% CI)	0.50 (0.34 to 0.74)	
P	.0005	
6-month PFS, % (95% CI)	40.8 (30.1 to 51.4)	20.8 (10.68 to 30.83)
12-month PFS, % (95% CI)	26.8 (16.2 to 37.4)	8.4 (0.2 to 16.6)

Bidard FC, et al. J Clin Oncol. 2022

FDA APPROVED LIQUID BIOPSY TESTS

Table 1. Liquid biopsy tests cleared by the FDA (up to March 2023).

Test	Company	Cancer type	Biomarkers	Intended use	Technology	Year	Matrix
CTC enumeration CellSearch®	Menarini Silicon Biosystems	Metastatic Breast Metastatic Colorectal Metastatic Prostate	CTC detection ($CK^+/DAPI^+/CD45^-$)	Prognostic significance	Ep-CAM based CTC enrichment & CTC detection with IF	2004 2007 2008	Whole blood
cobas® EGFR Mutation Test v2	Roche	Metastatic NSCLC	*EGFR* exon 19 deletions or exon 21 L858R	CDx for erlotinib (TARCEVA, Genentech) and osimertinib (TAGRISSO, AstraZeneca)	Real-time PCR	2016	Plasma
Epi proColon®	Epigenomics AG	Colorectal cancer	*Septin9* gene DNA methylation	Screening test	Bisulfite conversion & Real-time PCR	2016	Plasma
cobas® EGFR Mutation Test v2	Roche	Metastatic NSCLC	*EGFR* exon 19 deletions or exon 21 L858R	CDx for gefitinib (IRESSA, AstraZeneca)	Real-time PCR	2018	Plasma
therascreen® *PIK3CA* RGQ PCR Kit	Qiagen	Advanced or Metastatic Breast Cancer	*PIK3CA* mutations	CDx for alpelisib (PIQRAY, Novartis)	Real-time PCR	2019	Plasma
Guardant360® CDx assay	Guardant Health	Metastatic NSCLC	*EGFR* exon 19 deletions, L858R and T790M	CDx for osimertinib (TAGRISSO, AstraZeneca Pharmaceuticals LP)	NGS	2020	Plasma
Guardant360® CDx assay	Guardant Health	All Solid Cancers	SNVs. Indels, amplifications and fusions in 55 genes	Comprehensive genomic profiling (CGP)	NGS	2020	Plasma
FoundationOne® Liquid CDx	FoundationOne	Metastatic Castration Resistance Prostate Cancer (mCRPC)	*BRCA1* and/ or *BRCA2* mutations	CDx for rucaparib (RUBRACA, Clovis Oncology, Inc.)	NGS	2020	Plasma
FoundationOne® Liquid CDx	FoundationOne	Advanced or Metastatic Breast Cancer	*PIK3CA* mutations	CDx for alpelisib (PIQRAY, Novartis)	NGS	2020	Plasma
FoundationOne® Liquid CDx	FoundationOne	Metastatic NSCLC	*ALK* rearrangements	CDx for alectinib (ALECENSA, Genetech USA, Inc.)	NGS	2020	Plasma
FoundationOne® Liquid CDx	FoundationOne	Advanced Ovarian cancer	*BRCA1* and/ or *BRCA2* mutations	CDx for rucaparib (RUBRACA, Clovis Oncology, Inc.)	NGS	2020	Plasma
cobas® EGFR Mutation Test v2	Roche	Metastatic NSCLC	*EGFR* exon 19 deletions or exon 21 L858R	CDx for expanded EGFR TKIs: osimertinib (TAGRISSO, AstraZeneca), erlotinib (TARCEVA, Genentech), gefitinib (IRESSA, AstraZeneca), afatinib (GILOTRIF, Boehringer Ingelheim), and dacomitinib (VIZIMPRO, Pfizer)	Real-time PCR	2020	Plasma
FoundationOne® Liquid CDx	FoundationOne	Metastatic Castration Resistance Prostate Cancer (mCRPC)	*BRCA1*, *BRCA2* and *ATM* mutations	CDx for olaparib (LYNPARZA, AstraZeneca Pharmaceuticals LP)	NGS	2020	Plasma
Guardant360® CDx assay	Guardant Health	Locally Advanced or Metastatic NSCLC	*EGFR* Exon 20 Insertion Mutations	CDx for amivantamab-vmjw (RYBREVANT, Janssen)	NGS	2021	Plasma
Guardant360® CDx assay	Guardant Health	Locally Advanced or Metastatic NSCLC	*KRAS* G12C mutation	CDx for sotorasib (LUMAKRAS, Amgen Inc.)	NGS	2021	Plasma
FoundationOne® Liquid CDx	FoundationOne	Metastatic NSCLC	*MET* exon 14 skipping	CDx for capmatinib (TABRECTA, Novartis)	NGS	2021	Plasma
FoundationOne® Liquid CDx	FoundationOne	Metastatic NSCLC	*EGFR* exon 19 deletions or exon 21 L858R substitutions	CDx for erlotinib (TARCEVA, Genentech), osimertinib (TAGRISSO), and gefitinib (IRESSA)	NGS	2022	Plasma
therascreen®KRAS RGQ PCR kit	Qiagen	NSCLC	*KRAS* G12C mutation	CDx for adagrasib (KRAZATI, Mirati Therapeutics)	Real-time PCR	2022	Plasma
CTC isolation / enrichment	ANGLE	Metastatic Breast Cancer	*Different biomarkers*	CTC isolation	Size-based enrichment microfluidics	2022	Whole blood
FoundationOne® Liquid CDx	FoundationOne	Metastatic NSCLC	*ROS1* mutations or *NTRK* fusions	CDx for entrectinib (ROZLYTREK, Roche)	NGS	2023	Plasma
Guardant360® CDx assay	Guardant Health	Advanced or Metastatic Breast Cancer	*ESR1* mutations	CDx for elacestrant (ORSERDU, Menarini)	NGS	2023	Plasma

Ntzifa A. & Lianidou E, Crit Rev Clin Lab Sci, 2023

ctDNA *ESR1* MUTATION MONITORING FOR THERAPY DECISIONS

SERENA-6 trial

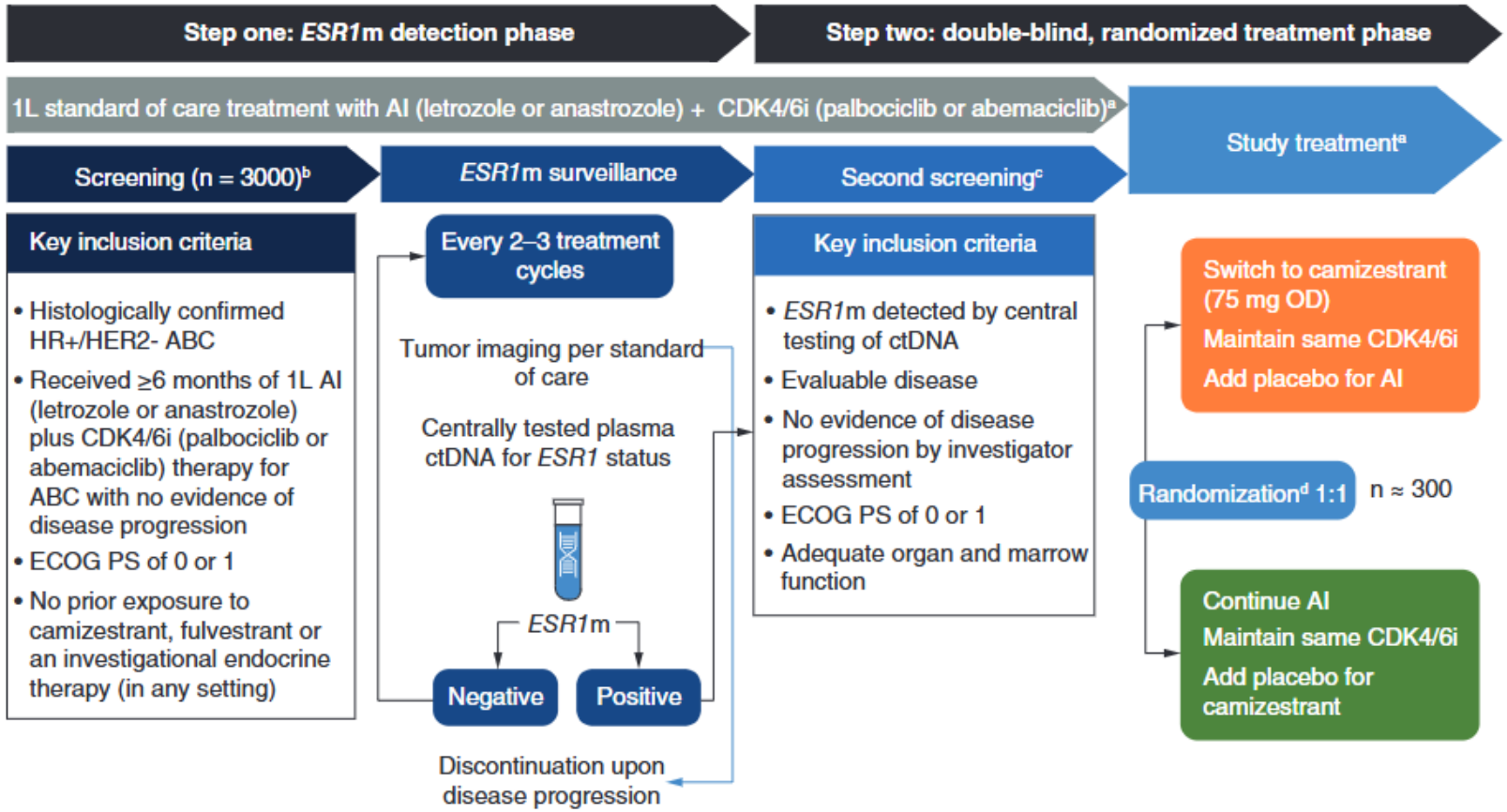


Figure 2. SERENA-6 trial schema.

*"The results from SERENA-6 showed that switching from an aromatase inhibitor to **camizestrant in combination with any of the three CDK4/6** inhibitors after the emergence of ESR1 mutations **delays the progression of disease** and extends the benefit of 1st-line treatment, representing an important step forward for the treatment management of patients."*

Turner N et al. Future Oncol. 2023

ctDNA *ESR1* MUTATION MONITORING FOR THERAPY DECISIONS

The NEW ENGLAND JOURNAL of MEDICINE

CURRENT ISSUE SPECIALTIES TOPICS

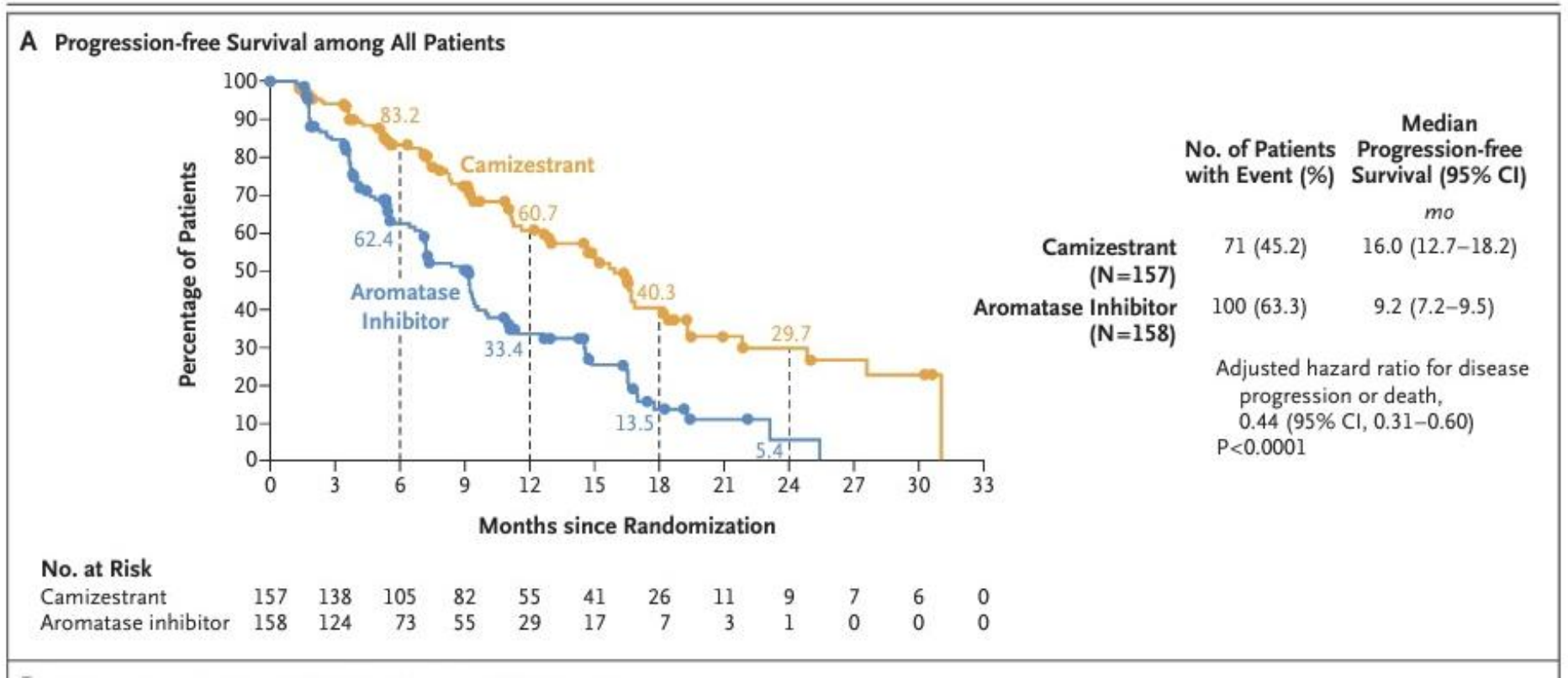
ORIGINAL ARTICLE

First-Line Camizestrant for Emerging ESR1-Mutated Advanced Breast Cancer

Authors: François-Clément Bidard, M.D., Ph.D., Erica L. Mayer, M.D., M.P.H., Yeon Hee Park, M.D., Ph.D., Wolfgang Janni, M.D., Ph.D., Cynthia Ma, M.D., Ph.D., Massimo Cristofanilli, M.D., Giampaolo Bianchini, M.D., +18, for the SERENA-6 Study Group* Author Info & Affiliations

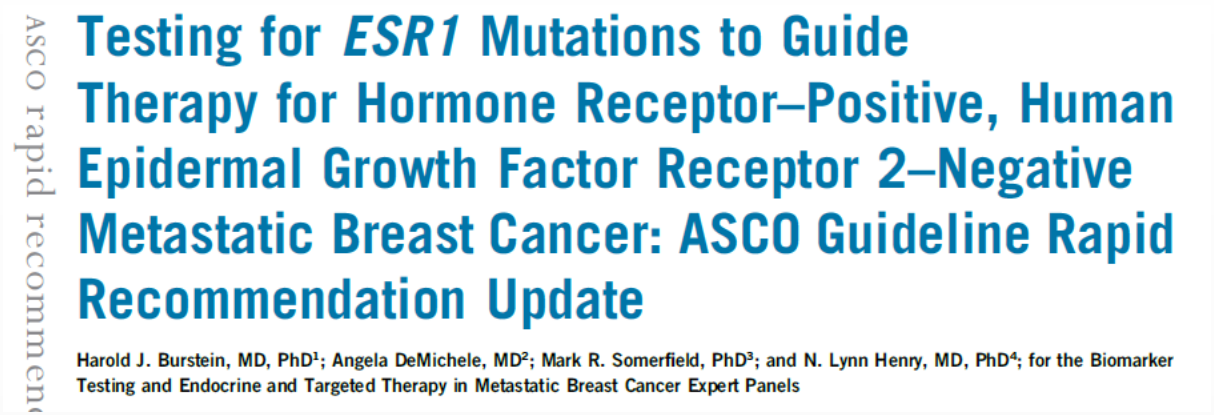
Published June 1, 2025 | DOI: 10.1056/NEJMoa2502929 |

- the median progression-free survival was **16.0 months** (95% confidence interval [CI], 12.7 to 18.2) **in the camizestrant group and 9.2 months** (95% CI, 7.2 to 9.5) **in the aromatase-inhibitor group**



Bidard FC, N Engl J Med. 2025

GUIDELINES

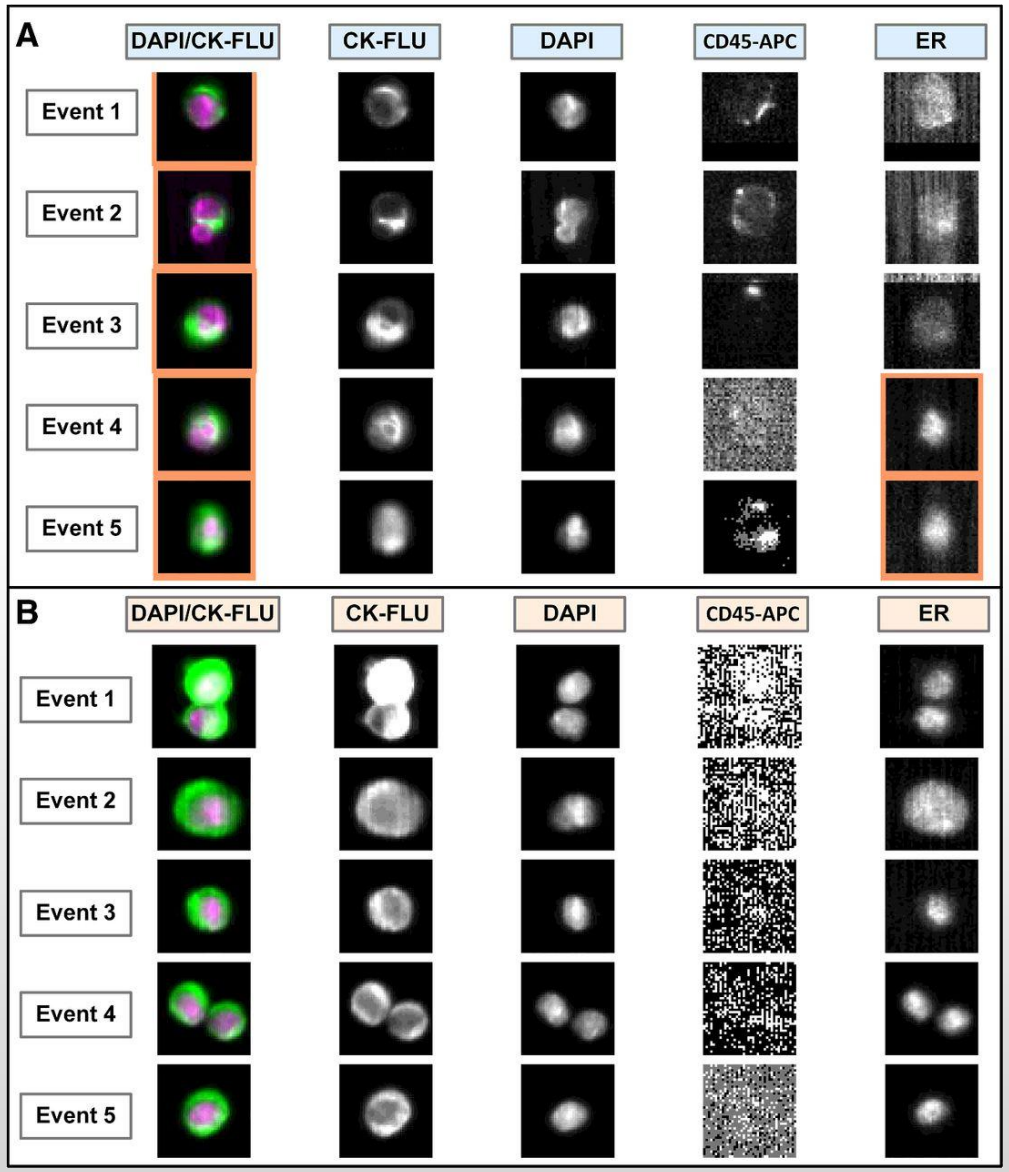


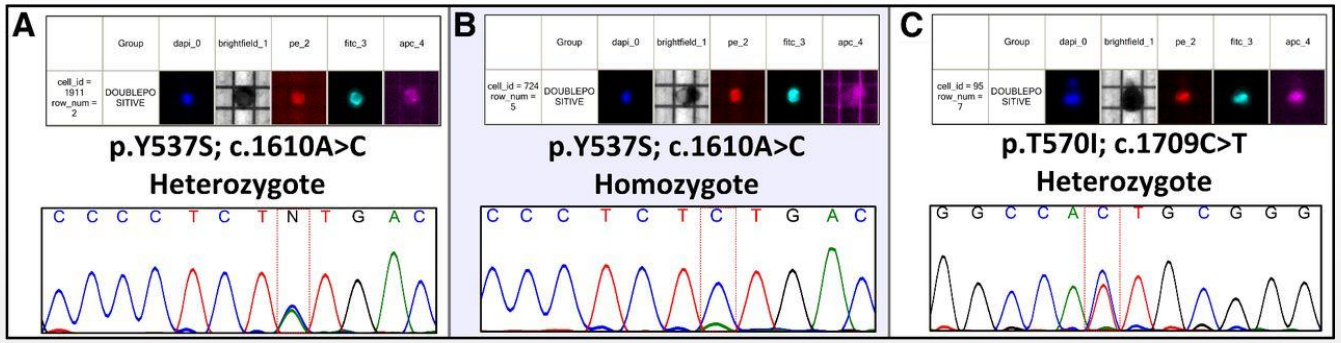
"To aid in treatment selection, the Expert Panel recommends routine testing for emergence of ESR1 mutations at recurrence or progression on ET (given with or without CDK4/6 inhibitor) in patients with ER-positive, HER2-negative MBC. Testing with a ***Clinical Laboratory Improvement Amendments–certified assay*** *should be performed on blood or tissue obtained* ***at the time of progression****, as ESR1 mutations develop in response to selection pressure during treatment and are typically undetectable in the primary tumor.* ***Blood-based ctDNA is preferred owing to greater sensitivity"***

Burstein Hjet al. J Clin Oncol. 2023

ESR1 MUTATIONS IN CTCs

ESR1 MUTATIONS IN CTCs





Paolillo C, et al. Clin Cancer Res. 2017

Eur J Cancer. 2021 Jan;143:147-157. doi: 10.1016/j.ejca.2020.11.005. Epub 2020 Dec 8.

Understanding the organ tropism of metastatic breast cancer through the combination of liquid biopsy tools

Lorenzo Gerratana [1], Andrew A Davis [2], Maurizio Polano [3], Qiang Zhang [4], Ami N Shah [4], Chenyu Lin [4], Debora Basile [5], Giuseppe Toffoli [3], Firas Wehbe [6], Fabio Puglisi [5], Amir Behdad [7], Leonidas C Platanias [4], William J Gradishar [4], Massimo Cristofanilli [8]

Cancer Res. 2022 Apr 1;82(7):1321-1339. doi: 10.1158/0008-5472.CAN-21-2576.

Hotspot ESR1 Mutations Are Multimodal and Contextual Modulators of Breast Cancer Metastasis

Zheqi Li [1 2], Yang Wu [2 3], Megan E Yates [2 4 5], Nilgun Tasdemir [1 2], Amir Bahreini [2 6], Jian Chen [2], Kevin M Levine [2 7], Nolan M Priedigkeit [1 2], Azadeh Nasrazadani [2], Simak Ali [8], Laki Buluwela [8], Spencer Arnesen [9 10], Jason Gertz [9 10], Jennifer K Richer [11], Benjamin Troness [12], Dorraya El-Ashry [12], Qiang Zhang [13], Lorenzo Gerratana [13 14], Youbin Zhang [13], Massimo Cristofanilli [13], Maritza A Montanez [15], Prithu Sundd [15], Callen T Wallace [16], Simon C Watkins [16], Caterina Fumagalli [17], Elena Guerini-Rocco [17 18], Li Zhu [19], George C Tseng [19], Nikhil Wagle [20], Jason S Carroll [21], Paul Jank [22], Carsten Denkert [22], Maria M Karsten [23], Jens-Uwe Blohmer [23], Ben H Park [24], Peter C Lucas [7], Jennifer M Atkinson [1 2], Adrian V Lee [1 2 4 6], Steffi Oesterreich [1 2 4 6 7]

Breast Cancer Res Treat. 2021 July ; 188(1): 43–52. doi:10.1007/s10549-021-06270-z.

Evaluation of endocrine resistance using *ESR1* genotyping of circulating tumor cells and plasma DNA

Tilak K. Sundaresan[1,9,12], Taronish D. Dubash[1,12], Zongli Zheng[1,4,11,12], Aditya Bardia[1,2,12], Ben S. Wittner[1,12], Nicola Aceto[1,10,12], Erin J. Silva[1,12], Douglas B. Fox[1,12], Matthew Liebers[1,12], Ravi Kapur[3], John Iafrate[1,4,12], Mehmet Toner[1,5,6,7,12], Shyamala Maheswaran[1,7,12], Daniel A. Haber[1,2,8,12]

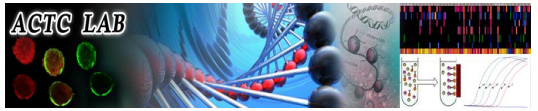
Molecular Oncology

FEBS PRESS

Serial monitoring of genomic alterations in circulating tumor cells of ER-positive/HER2-negative advanced breast cancer: feasibility of precision oncology biomarker detection

Andi K. Cani[1,2], Emily M. Dolce[1,2], Elizabeth P. Darga[1,2], Kevin Hu[3,4,5], Chia-Jen Liu[3,4], Jackie Pierce[6], Kieran Bradbury[6], Elaine Kilgour[6], Kimberly Aung[1,2], Gaia Schiavon[7], Danielle Carroll[7], T. Hedley Carr[7], Teresa Klinowska[8], Justin Lindemann[7], Gayle Marshall[7], Vicky Rowlands[7], Elizabeth A. Harrington[7], J. Carl Barrett[9], Nitharsan Sathiyayogan[7], Christopher Morrow[7], Valeria Sero[10], Anne C. Armstrong[11], Richard Baird[12], Erika Hamilton[13], Seock-Ah Im[14], Komal Jhaveri[15], Manish R. Patel[16], Caroline Dive[6], Scott A. Tomlins[3,4], Aaron M. Udager[2,3,4], Daniel F. Hayes[1,2] and Costanza Paoletti[1,2]

ACTC LAB: *ESR1* MUTATIONS IN CTCs & ctDNA



cancers

MDPI

Article

ESR1 NAPA Assay: Development and Analytical Validation of a Highly Sensitive and Specific Blood-Based Assay for the Detection of *ESR1* Mutations in Liquid Biopsies

Dimitra Stergiopoulou [1], Athina Markou [1,*], Eleni Tzanikou [1], Ioannis Ladas [2], G. Mike Makrigiorgos [2], Vassilis Georgoulias [3,4] and Evi Lianidou [1,*]

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Development and validation of a multi-marker liquid bead array assay for the simultaneous detection of *PIK3CA* and *ESR1* hotspot mutations in single circulating tumor cells (CTCs)

Dimitra Stergiopoulou [a], Vassilis Georgoulias [b], Athina Markou [a], Evi Lianidou [a,*]

[a] *Analysis of Circulating Tumor Cells Lab, Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, 15771 Athens, Greece*
[b] *First Department of Medical Oncology, METROPOLITAN General Hospital, 264, Mesogion Av, Cholargos, Athens, Greece*

The Journal of Liquid Biopsy 5 (2024) 100154

Contents lists available at ScienceDirect

The Journal of Liquid Biopsy

journal homepage: www.sciencedirect.com/journal/the-journal-of-liquid-biopsy

Multiplex detection of ten *ESR1* mutations and *AKT1* E17K in breast cancer using digital PCR

Stavroula Smilkou [a], Aliki Ntzifa [a], Dimitra Stergiopoulou [a], Vasilis Georgoulias [b], Evi Lianidou [a,*]

[a] *Analysis of Circulating Tumor Cells, Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, 15771, Athens, Greece*
[b] *First Department of Medical Oncology, Metropolitan General Hospital, Athens, 15562, Greece*

DIRECT COMPARISON OF CTCs & ctDNA FOR THE DETECTION OF *ESR1* MUTATIONS

Molecular Oncology

FEBS PRESS

Detection rate for *ESR1* mutations is higher in circulating-tumor-cell-derived genomic DNA than in paired plasma cell-free DNA samples as revealed by ddPCR

Stavroula Smilkou[1], Aliki Ntzifa[1], Victoria Tserpeli[1], Ioanna Balgkouranidou[2], Alkistis Papatheodoridi[3], Evangelia Razis[4], Helena Linardou[5], Christos Papadimitriou[6], Amanda Psyrri[7], Flora Zagouri[3], Stylianos Kakolyris[2] and Evi Lianidou[1]

- In the present study, we investigated whether the analysis of *ESR1* mutations in CTCs and ctDNA provide complementary information on resistance to ET using a highly sensitive 6-plex ddPCR assay.

MATERIALS & METHODS

Metastatic breast cancer patients

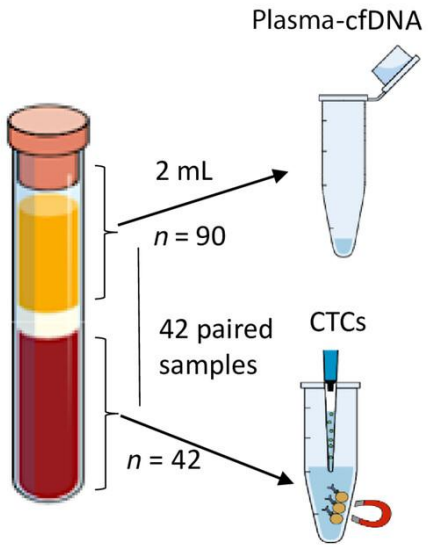
- 90 patients
- HR+/ HER2-
- Treated with endocrine therapy (ET)

Control group

- 10 healthy donors.
- Peripheral blood was processed exactly in the same way as clinical samples

**Clinical samples were collected in the context of the Operational Program 'Competitiveness, Entrepreneurship and Innovation', under the call RESEARCH-CREATE-INNOVATE (project code: T1RCI-02935)*

MATERIALS & METHODS

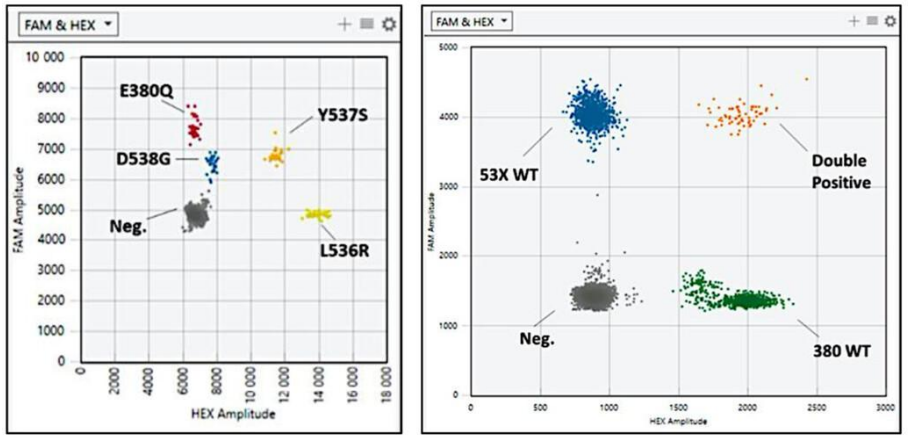


cfDNA extraction with QIAamp DSP cNA Kit (Qiagen, Hilden, Germany)

CTC enrichment with EpCAM+ immunomagnetic beads

gDNA extraction from CTCs with TRIzol™ LS

Detection of *ESR1* mutations using 6plex ddPCR, Bio-Rad, USA

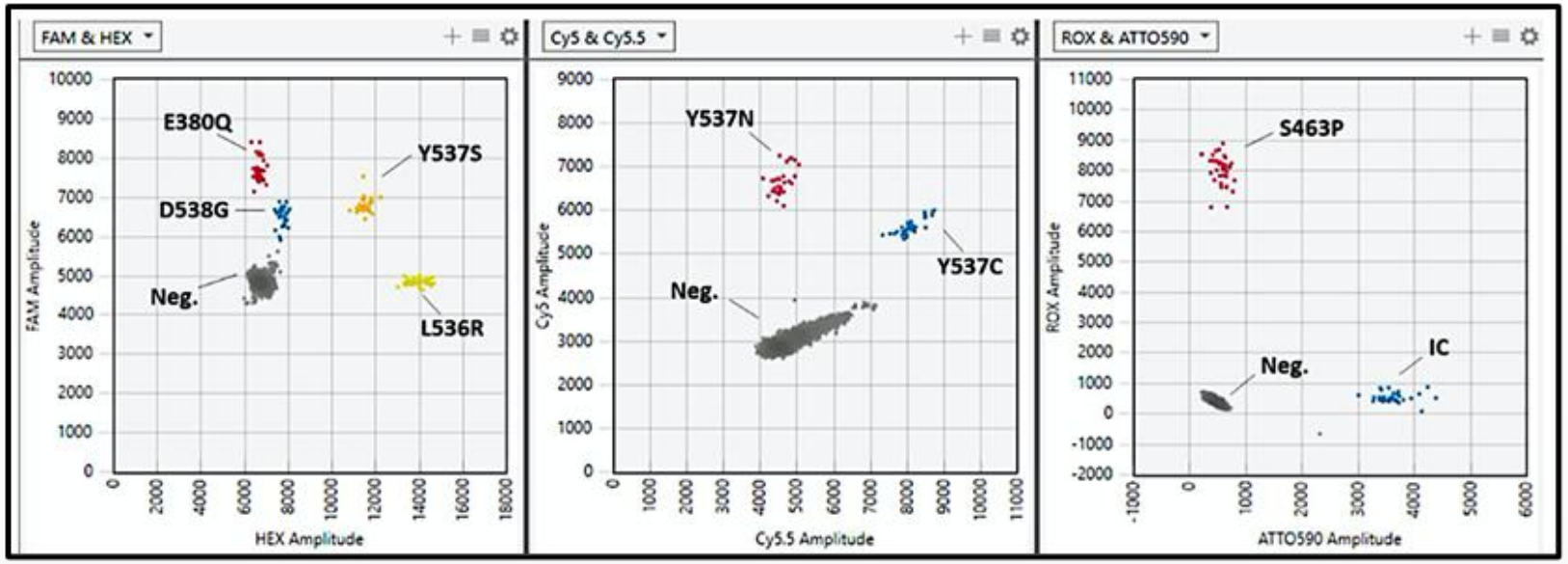


- **mBC patients under ET**
- **Time point: before any switch of treatment**

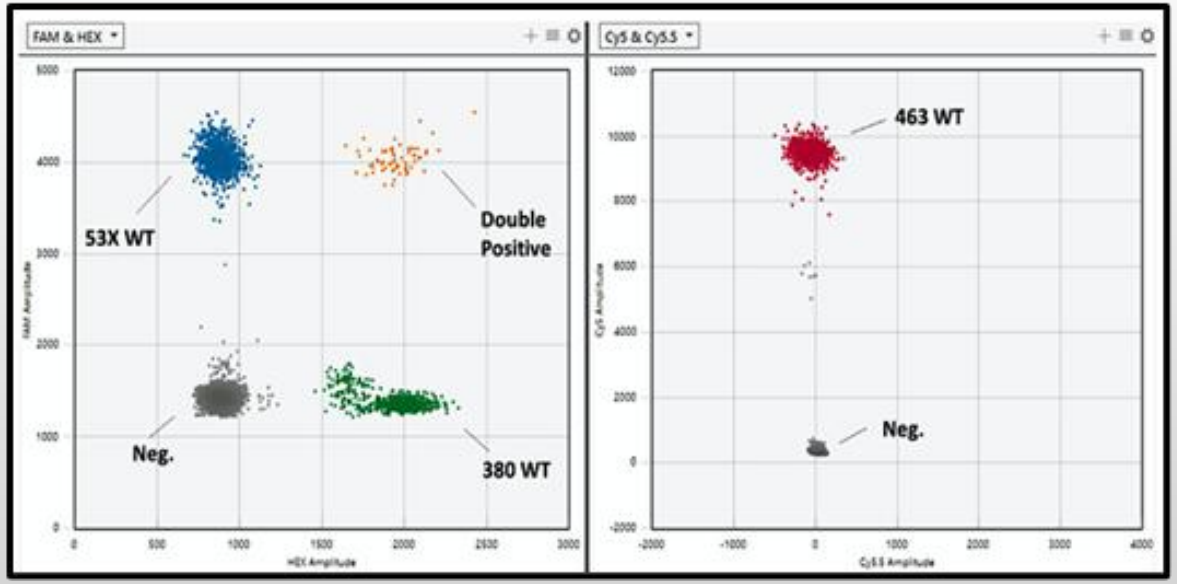
Smilkou S, et al. Mol Oncol. 2025

MATERIALS & METHODS

A



B



ddplex *ESR1* assay mutations

- exon 5 (E380Q)
- exon 7 (S463P)
- exon 8 (L536R, Y537C, Y537S, Y537N, D538G)

ddplex WT assay

wild-type loci in exons 5, 7, 8 of the *ESR1* gene

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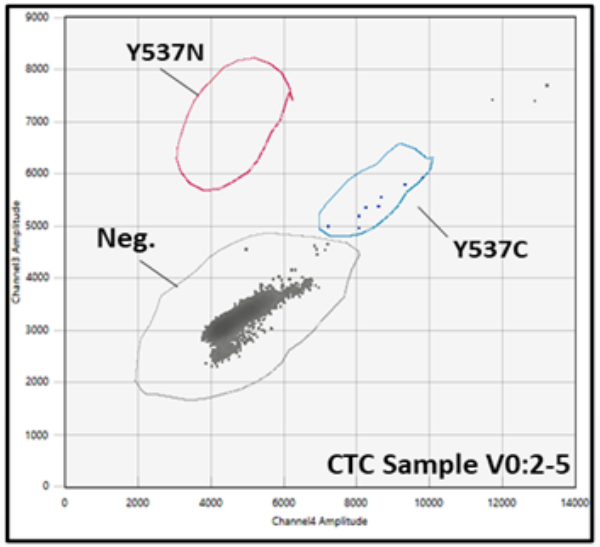
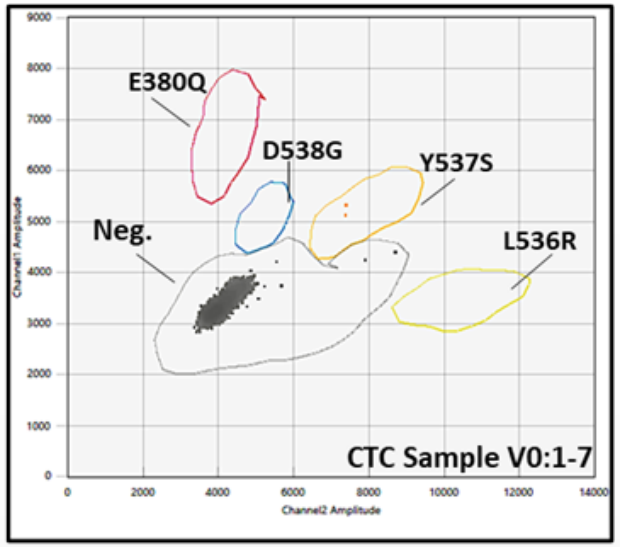
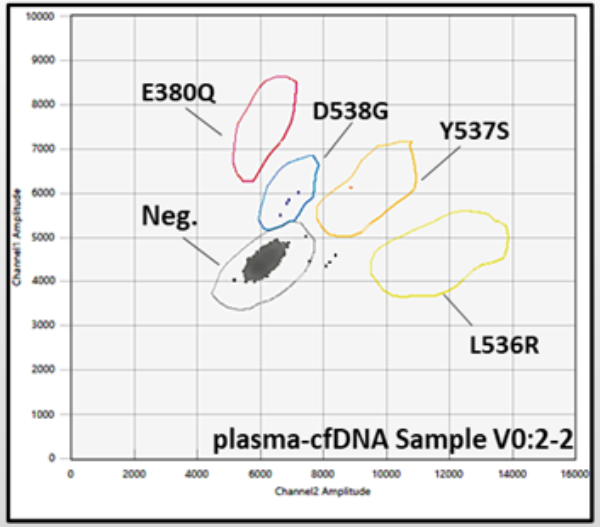
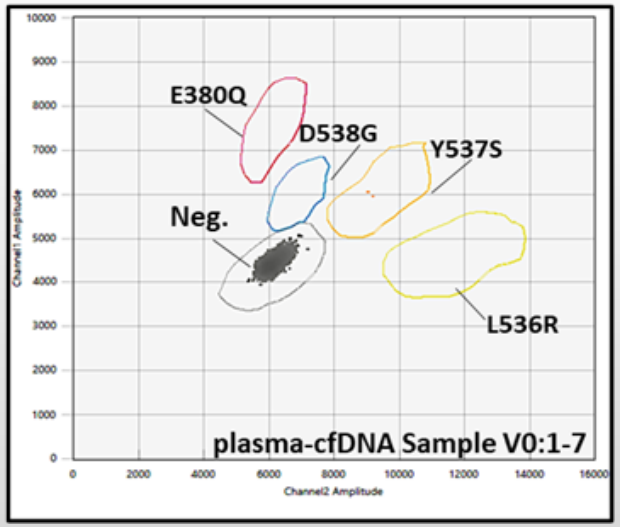
RESULTS: 2D ddPCR plots for *ESR1* mutations in clinical samples









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ctDNA

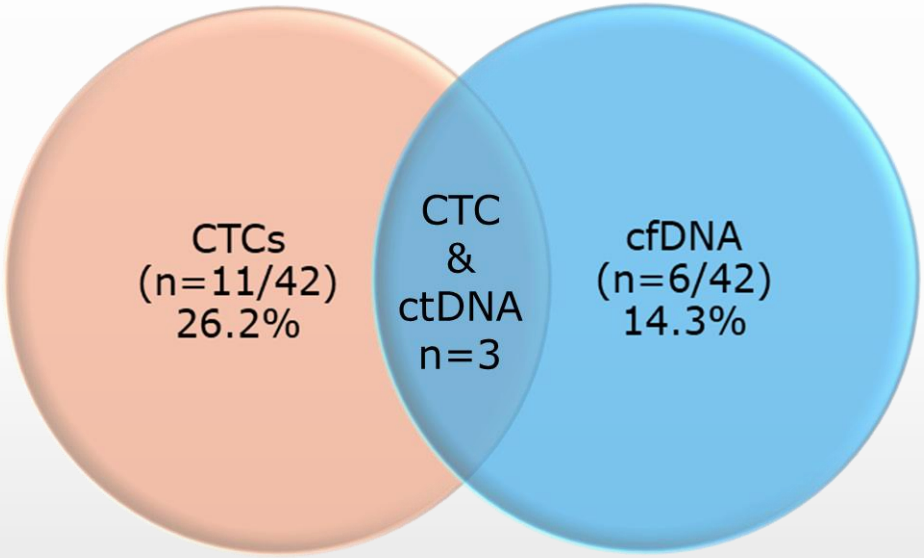
8/90 (8.9%) positive for *ESR1* mutations
6/8: Y537S [%MAF: 0.15-9.51%]
2/8: D538G [%MAF: 0.20-0.64%]

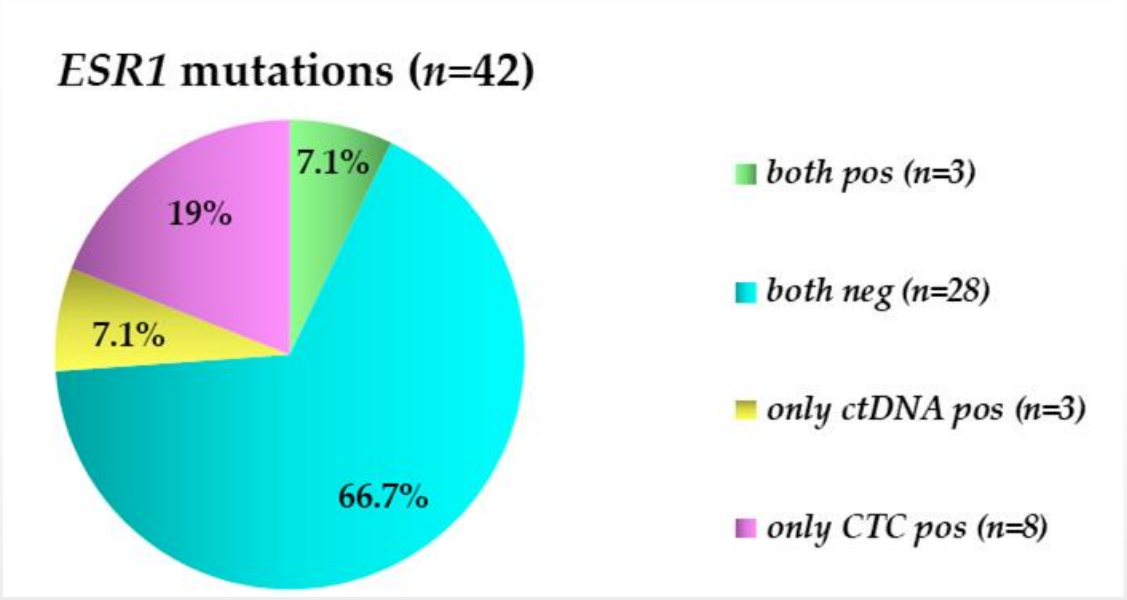
CTCs

11/42 (26.2%) positive for *ESR1* mutations
6/11: Y537S [%MAF: 0.1-4.4%]
5/11: Y537C [%MAF: 0.03-6.7%]
Concurrent mutations: Y537S, D538G, L536R in one sample

***ESR1* mutations were not detected in any of HDs' CTC or cfDNA samples**

RESULTS: Direct comparison of cfDNA & paired CTC samples





Concordance: 31/42 (73.81%), p < 0.152, chi-squared test

RESULTS: Direct comparison of cfDNA & paired CTC samples

Patiend ID	plasma-cfDNA	CTC-derived gDNA
V0:1-1	WT	WT
V0:1-2	WT	Y537S
V0:1-3	WT	WT
V0:1-4	WT	WT
V0:1-5	WT	WT
V0:1-6	WT	Y537C
V0:1-7	Y537S	Y537S
V0:1-8	Y537S	Y537C
V0:1-9	WT	WT
V0:2-1	WT	WT
V1:2-1	WT	WT
V0:2-2	D538G	WT
V0:2-3	WT	Y537S
V0:2-4	WT	WT
V0:2-5	WT	Y537C
V0:2-6	WT	D538G, Y537S, L536R
V0:3-1	WT	WT
V1:3-1	WT	WT
V0:3-2	WT	WT
V1:3-2	WT	WT
V0:3-3	WT	WT

WT
Y537S
Y537C
D538G
L536R

Patiend ID	plasma-cfDNA	CTC-derived gDNA
V0:3-5	WT	WT
V0:4-1	WT	WT
V0:4-2	WT	WT
V0:4-3	WT	WT
V0:4-4	WT	WT
V0:5-1	WT	WT
V0:5-2	WT	Y537C
V0:6-1	WT	WT
V1:6-1	WT	WT
V0:6-10	Y537S	Y537C
V1:6-10	WT	WT
V0:7-1	WT	Y537S
V0:7-2	WT	WT
V0:7-3	WT	WT
V0:7-4	WT	WT
V0:7-5	Y537S	WT
V0:7-6	WT	Y537S
V0:7-7	WT	WT
V0:7-8	WT	WT
V0:7-9	D538G	WT
V0:7-10	WT	WT

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RESULTS: Direct comparison of cfDNA & paired CTC samples

Patiend ID	plasma-cfDNA	CTC-derived gDNA
V0:1-1	WT	WT
V0:1-2	WT	Y537S
V0:1-3	WT	WT
V0:1-4	WT	WT
V0:1-5	WT	WT
V0:1-6	WT	Y537C
V0:1-7	Y537S	Y537S
V0:1-8	Y537S	Y537C
V0:1-9	WT	WT
V0:2-1	WT	WT
V1:2-1	WT	WT
V0:2-2	D538G	WT
V0:2-3	WT	Y537S
V0:2-4	WT	WT
V0:2-5	WT	Y537C
V0:2-6	WT	D538G + Y537S + L536R
V0:3-1	WT	WT
V1:3-1	WT	WT
V0:3-2	WT	WT
V1:3-2	WT	WT
V0:3-3	WT	WT

WT
Y537S
Y537C
D538G
L536R

Patiend ID	plasma-cfDNA	CTC-derived gDNA
V0:3-5	WT	WT
V0:4-1	WT	WT
V0:4-2	WT	WT
V0:4-3	WT	WT
V0:4-4	WT	WT
V0:5-1	WT	WT
V0:5-2	WT	Y537C
V0:6-1	WT	WT
V1:6-1	WT	WT
V0:6-10	Y537S	Y537C
V1:6-10	WT	WT
V0:7-1	WT	Y537S
V0:7-2	WT	WT
V0:7-3	WT	WT
V0:7-4	WT	WT
V0:7-5	Y537S	WT
V0:7-6	WT	Y537S
V0:7-7	WT	WT
V0:7-8	WT	WT
V0:7-9	D538G	WT
V0:7-10	WT	WT

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RESULTS: Direct comparison of cfDNA & paired CTC samples

Patiend ID	plasma-cfDNA	CTC-derived gDNA
V0:1-1	WT	WT
V0:1-2	WT	Y537S
V0:1-3	WT	WT
V0:1-4	WT	WT
V0:1-5	WT	WT
V0:1-6	WT	Y537C
V0:1-7	Y537S	Y537S
V0:1-8	Y537S	Y537C
V0:1-9	WT	WT
V0:2-1	WT	WT
V1:2-1	WT	WT
V0:2-2	D538G	WT
V0:2-3	WT	Y537S
V0:2-4	WT	WT
V0:2-5	WT	Y537C
V0:2-6	WT	D538G + Y537S + L536R
V0:3-1	WT	WT
V1:3-1	WT	WT
V0:3-2	WT	WT
V1:3-2	WT	WT
V0:3-3	WT	WT

WT
Y537S
Y537C
D538G
L536R

Patiend ID	plasma-cfDNA	CTC-derived gDNA
V0:3-5	WT	WT
V0:4-1	WT	WT
V0:4-2	WT	WT
V0:4-3	WT	WT
V0:4-4	WT	WT
V0:5-1	WT	WT
V0:5-2	WT	Y537C
V0:6-1	WT	WT
V1:6-1	WT	WT
V0:6-10	Y537S	Y537C
V1:6-10	WT	WT
V0:7-1	WT	Y537S
V0:7-2	WT	WT
V0:7-3	WT	WT
V0:7-4	WT	WT
V0:7-5	Y537S	WT
V0:7-6	WT	Y537S
V0:7-7	WT	WT
V0:7-8	WT	WT
V0:7-9	D538G	WT
V0:7-10	WT	WT

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RESULTS: Direct comparison of cfDNA & paired CTC samples

Patiend ID	plasma-cfDNA	CTC-derived gDNA
V0:1-1	WT	WT
V0:1-2	WT	Y537S
V0:1-3	WT	WT
V0:1-4	WT	WT
V0:1-5	WT	WT
V0:1-6	WT	Y537C
V0:1-7	Y537S	Y537S
V0:1-8	Y537S	Y537C
V0:1-9	WT	WT
V0:2-1	WT	WT
V1:2-1	WT	WT
V0:2-2	D538G	WT
V0:2-3	WT	Y537S
V0:2-4	WT	WT
V0:2-5	WT	Y537C
V0:2-6	WT	D538G + Y537S + L536R
V0:3-1	WT	WT
V1:3-1	WT	WT
V0:3-2	WT	WT
V1:3-2	WT	WT
V0:3-3	WT	WT

WT
Y537S
Y537C
D538G
L536R

Patiend ID	plasma-cfDNA	CTC-derived gDNA
V0:3-5	WT	WT
V0:4-1	WT	WT
V0:4-2	WT	WT
V0:4-3	WT	WT
V0:4-4	WT	WT
V0:5-1	WT	WT
V0:5-2	WT	Y537C
V0:6-1	WT	WT
V1:6-1	WT	WT
V0:6-10	Y537S	Y537C
V1:6-10	WT	WT
V0:7-1	WT	Y537S
V0:7-2	WT	WT
V0:7-3	WT	WT
V0:7-4	WT	WT
V0:7-5	Y537S	WT
V0:7-6	WT	Y537S
V0:7-7	WT	WT
V0:7-8	WT	WT
V0:7-9	D538G	WT
V0:7-10	WT	WT

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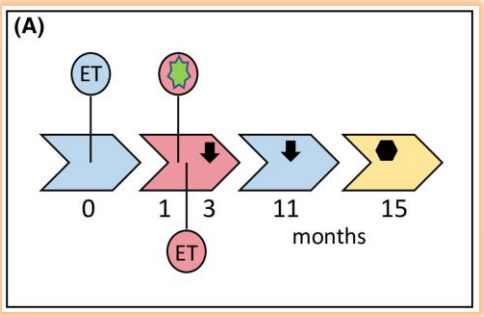
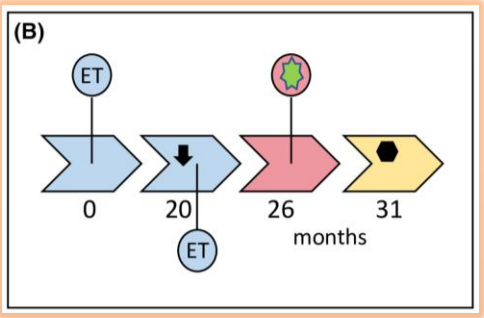
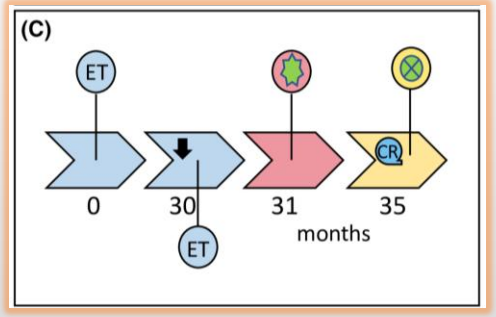
RESULTS: Case Studies



- ET for 15 months until the date of blood sampling
- then switched to chemotherapy plus immunotherapy
- 2 months later had PD. At this time point, ***ESR1* p.D538G was detected in plasma-cfDNA**.
- the patient kept receiving chemotherapy plus immunotherapy, and after 4 months from new PD and resistance to therapy, the patient passed away.



- ET as a first-line treatment, 20 months later progressed
- After 1 month from relapse, the patient received again ET
- ESR1 mutations were not detected in plasma-cfDNA of this patient
- 6 months after PD and before the 2nd-line ET, ***ESR1* p.Y537S was detected in CTC-derived gDNA**.



- ET as first-line treatment and progressed 30 months after diagnosis
- One month after the relapse, ***ESR1* p.Y537S and *ESR1* p.Y537C were detected in plasma-cfDNA and in CTC-derived gDNA, respectively**.
- continued on ET plus immunotherapy and 4 months later **none of these mutations were detected either in plasma-cfDNA or in CTC-derived gDNA**.
- The patient had complete response to treatment.

PD
ESR1 mutation
Death
ET Endocrine therapy
CR Complete response
Non-mutated *ESR1*

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CONCLUSIONS

- **Liquid biopsy as a minimally invasive approach plays important role in the clinical setting of ER+/HER2- metastatic breast cancer patients**
- **Detection of *ESR1* mutations in plasma cfDNA upon resistance to ET has proven to be crucial for the treatment management of ER+/HER2- metastatic breast cancer patients, as this was shown in clinical trials**
- **6-plex ddPCR offers high sensitivity and accuracy and in combination with the multiplexing capacity offers a potential for the detection of *ESR1* mutations both in CTCs and cfDNA**
- **Targeted approach of ddPCR for the detection of *ESR1* mutations is essential for the longitudinal monitoring of metastatic BrCa patients during treatment to guide early treatment interventions**

CONCLUSIONS

- **Our results clearly indicate that a higher detection rate was revealed in CTC-derived genomic DNA than in paired plasma-cfDNA samples**
- **CTC-derived gDNA analysis should be further evaluated as an important and complementary tool to ctDNA for identifying patients with *ESR1* mutations and for guiding individualized therapy**
- **Future perspectives: to integrate CTC analysis in larger clinical studies to further confirm the importance of detecting *ESR1* mutations both in CTCs and plasma for guiding personalized treatment**