COR2ED

THE HEART OF MEDICAL EDUCATION

CLINICAL TOPIC

THE EVOLVING ROLE OF LIQUID BIOPSY IN HR+/HER2- METASTATIC BREAST CANCER

Prof. Frédérique Penault-Llorca
Molecular Pathologist, University of Clermont-Ferrand, France
Dr Aditya Bardia
Medical Oncologist, David Geffen School of Medicine at UCLA, United States

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HR, hormone receptor

DEVELOPED BY PRECISION ONCOLOGY CONNECT

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THIS PROGRAMME HAS BEEN DEVELOPED BY A GROUP OF EXPERTS

Prof. Frédérique Penault-Llorca
University of Clermont-Ferrand, France

Dr Aditya Bardia
David Geffen School of Medicine at UCLA, United States

CLINICAL TAKEAWAYS

- **Liquid biopsies** offer a **less invasive alternative to traditional tissue biopsies** in patients with advanced breast cancer, to examine molecular features
- The role of **liquid biopsy** should be seen as a **complementary testing method to tissue-based assays** and the information derived should be reviewed in combination with tissue results
- **Liquid biopsy can detect mutations** such as *ESR1* and *PIK3CA* that are often **associated with resistance to endocrine therapies** and may be therapeutic targets
- **Multigene NGS testing is recommended by the ESMO and NCCN guidelines** to ensure a broad spectrum of genetic alterations can be detected

NGS, next-generation sequencing

EDUCATIONAL OBJECTIVES

1. Know the **testing landscape** in HR+/HER2- metastatic breast cancer, **when** and **how** to test using **liquid and tumour biopsies**

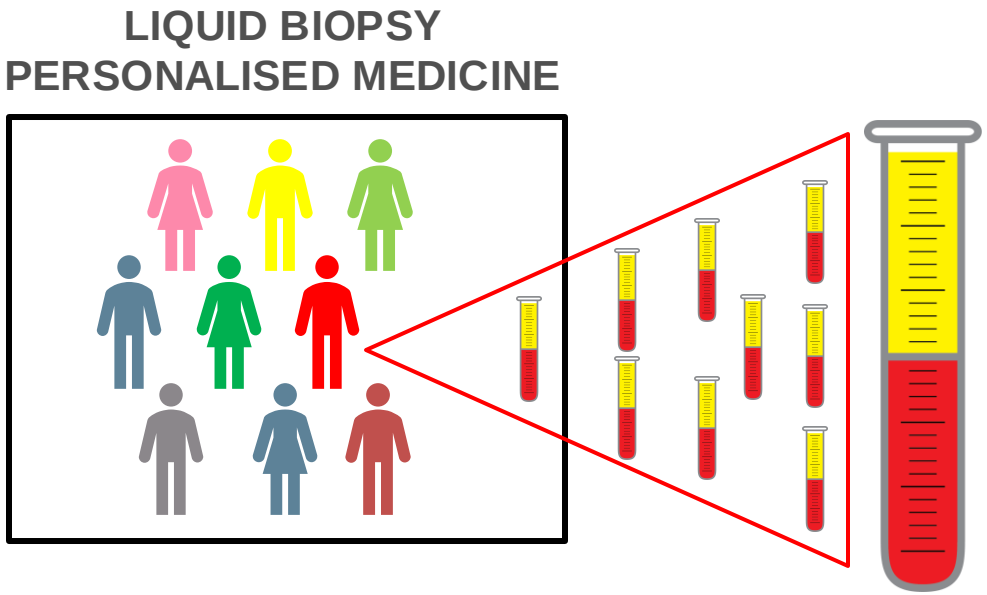
2. Be able to **include all relevant biomarkers** in test requests across the breast cancer patient journey

3. Understand the **implications of biomarker testing results** on the management of HR+/HER2- metastatic breast cancer patients

HR, hormone receptor

WHAT IS A LIQUID BIOPSY?

- A liquid biopsy can be derived from the blood and other body fluids, e.g., saliva, ascites fluid, urine, cerebrospinal and pleural fluid



Enrichment and detection from plasma/serum of:

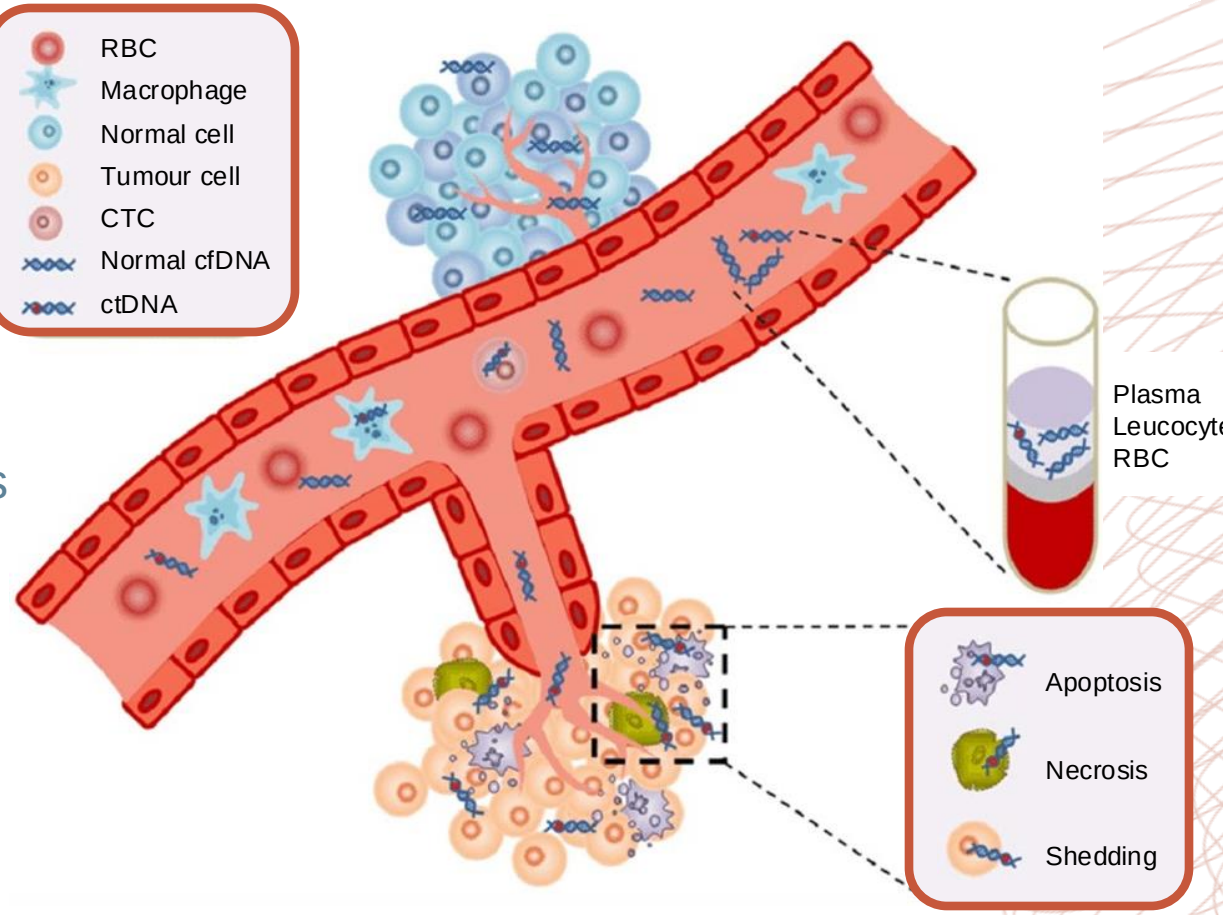
- Circulating tumour cells
- Circulating tumour nucleic acids (cell-free DNA, microRNA, mRNA, lncRNA)
- Circulating tumour microvesicles/exosomes

lncRNA, long non-coding RNA; mRNA, messenger RNA

Raimondi L, et al. Oncotarget. 2017;8:100831-51; Nikanjam M, et al. J Hematol Oncol. 2022;15:131

WHAT IS CELL-FREE DNA (cfDNA) AND CIRCULATING TUMOUR DNA (ctDNA)

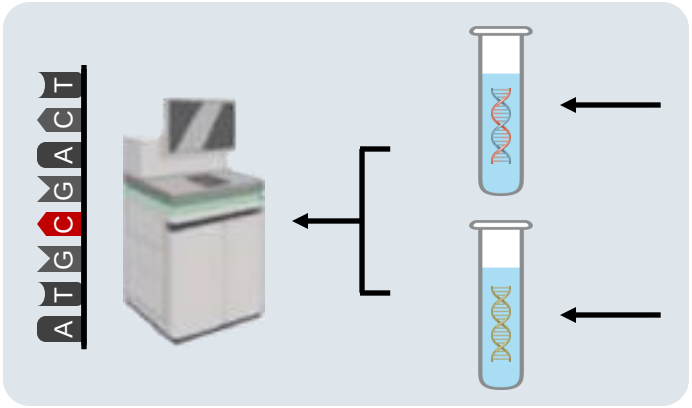
- cfDNA
  - dsDNA fragments associated with histones in circulation that have been released by cells
- Circulating tumour DNA (ctDNA)
  - cfDNA derived from cancer cells
  - Characterised by somatic, cancer-specific alterations, cancer-specific methylation patterns
- Most cfDNA is released by normal leukocytes
- Higher levels of ctDNA associated with:
  - Certain cancer subtypes
  - Higher burden of disease
  - Liver metastases



cfDNA, cell-free DNA, CTC, circulating tumour cell; ctDNA, circulating tumour DNA, dsDNA, double-stranded DNA; RBC, red blood cell

Hahn AW, et al. Kidney Cancer 3, 2019;7-13; Qi T, et al. Int J. Mol Sci. 2023;24:1503; Sanchez-Herrero E, et al. Front. Oncol. 2022;12:943253; Parsons HA. ASCO 2024

Tissue biopsy



GOLD STANDARD

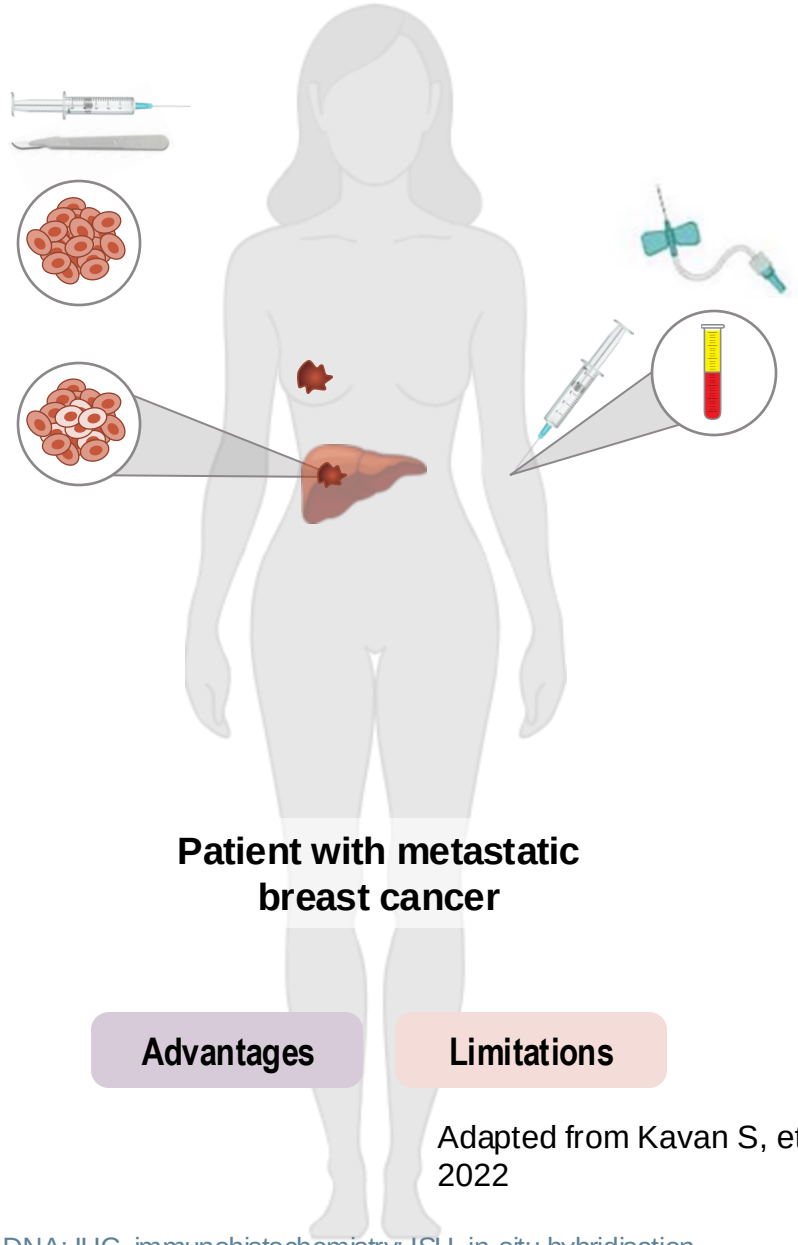
Provides important histopathological information

Captures neither tumour heterogeneity nor dynamic changes

Invasive procedure

Allows IHC, ISH, CGP, targeted molecular testing

Versus

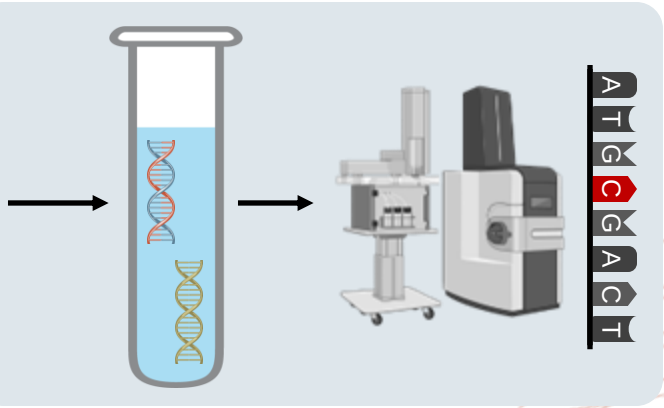


Patient with metastatic breast cancer

Advantages | **Limitations**

Adapted from Kavan S, et al. 2022

Liquid biopsy



Rapid and minimally invasive

Captures tumour heterogeneity

Allows serial, dynamic testing

Requires sensitive assays, and high ctDNA shedding

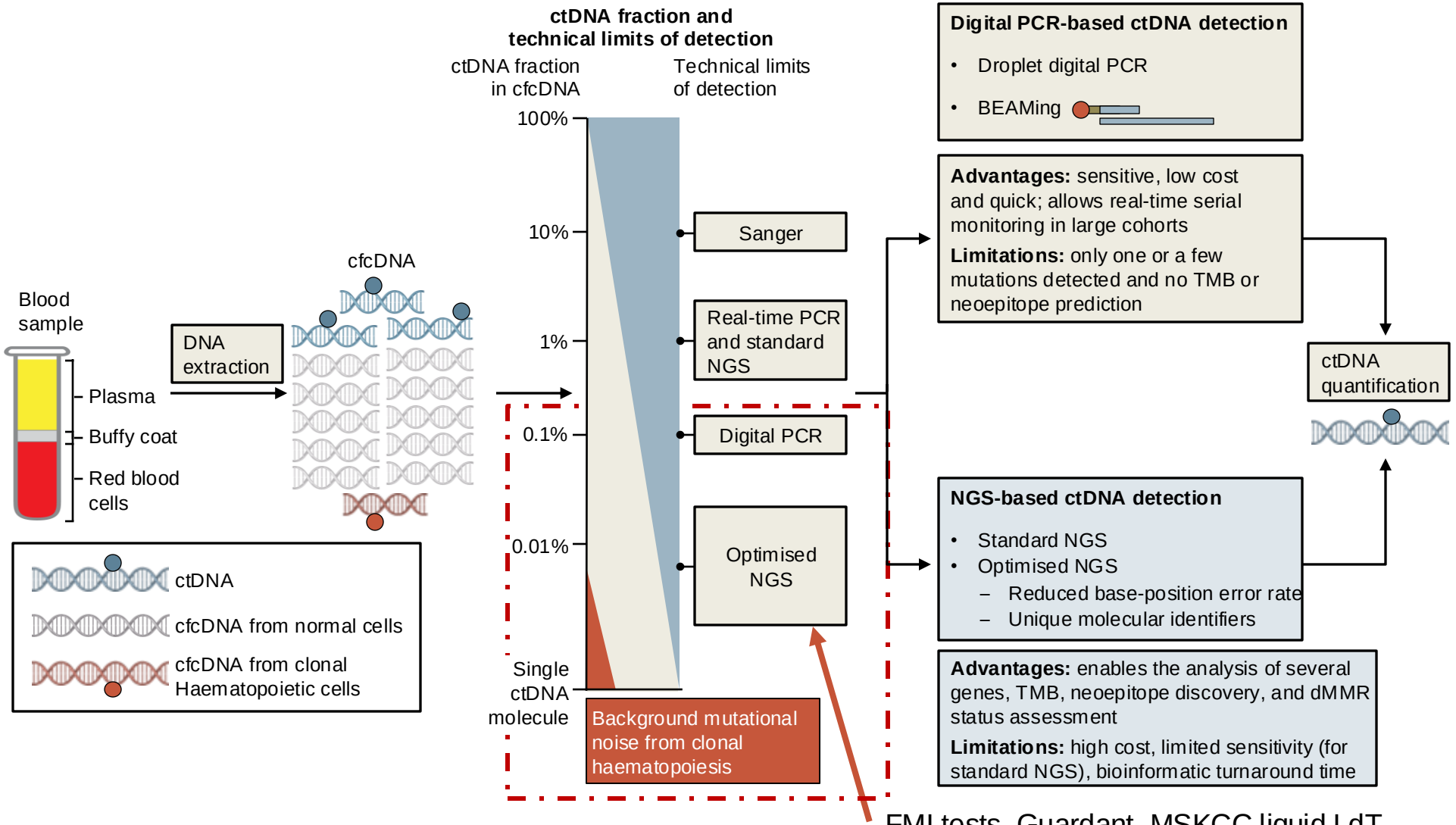
Cannot replace histopathological assessment

Allows targeted molecular testing, CGP (with some limitations)

CGP, comprehensive genomic profiling; ctDNA, circulating tumour DNA; IHC, immunohistochemistry; ISH, in-situ hybridisation

Kavan S, et al. Cancer and Metastasis Rev. 2022;41:433-446; Penault-Llorca F. ESMO Advanced Course on Breast Cancer: Filling the Gaps Following Progression on CDK4/6 in Hormone Receptor Positive HER2 Negative Advanced Breast Cancer 2024: Doha. Session 3 – What to do after CDK4/6 inhibition in advanced breast cancer

TECHNOLOGIES FOR cfDNA HAVE TO BE SENSITIVE



BEAMing, beads, emulsion, amplification, magnetics; cf(c)DNA, cell-free (circulating) DNA; ctDNA, circulating tumour DNA; dMMR, deficient DNA mismatch repair; FMI, Foundation Medicine; LdT, laboratory developed test; MSKCC, Memorial Sloane Kettering Cancer Center; NGS, next-generation sequencing; PCR, polymerase chain reaction; TMB, tumour mutational burden

Cabel L, et al. Nat Rev Clin Oncol. 2018;15:639-650; Penault-Llorca F. ESMO Advanced Course on Breast Cancer: Filling the Gaps Following Progression on CDK4/6 in Hormone Receptor Positive HER2 Negative Advanced Breast Cancer 2024: Doha. Session 3 – What to do after CDK4/6 inhibition in advanced breast cancer

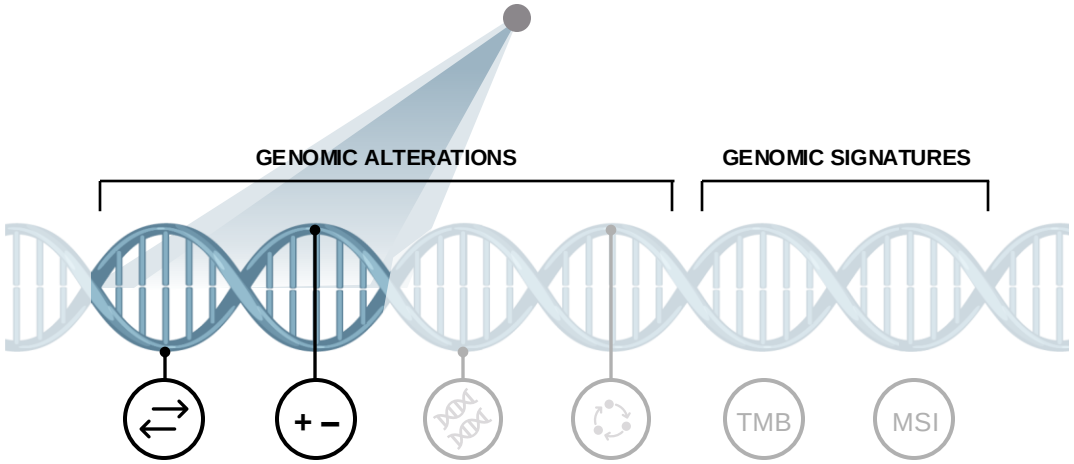
HOW TO TEST – WHICH TECHNIQUE?

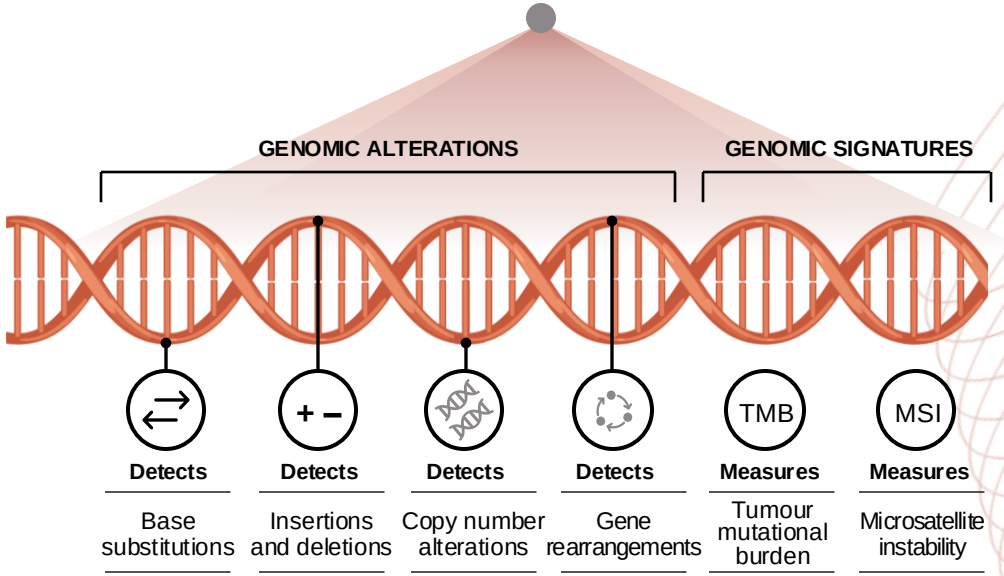
PCR and dPCR

- Detects a **predefined** set of specific genetic alterations (mutations-deletions>fusions>CNV)
- Can only detect predefined mutations (hot spot mutations**)**
- In general, rare mutations are not investigated

NGS

- Allows the detection of alterations in several genes in a single test, without preconceived ideas
- Able to detect the **four main classes of genetic alterations**
- It can also be used to characterise new alterations



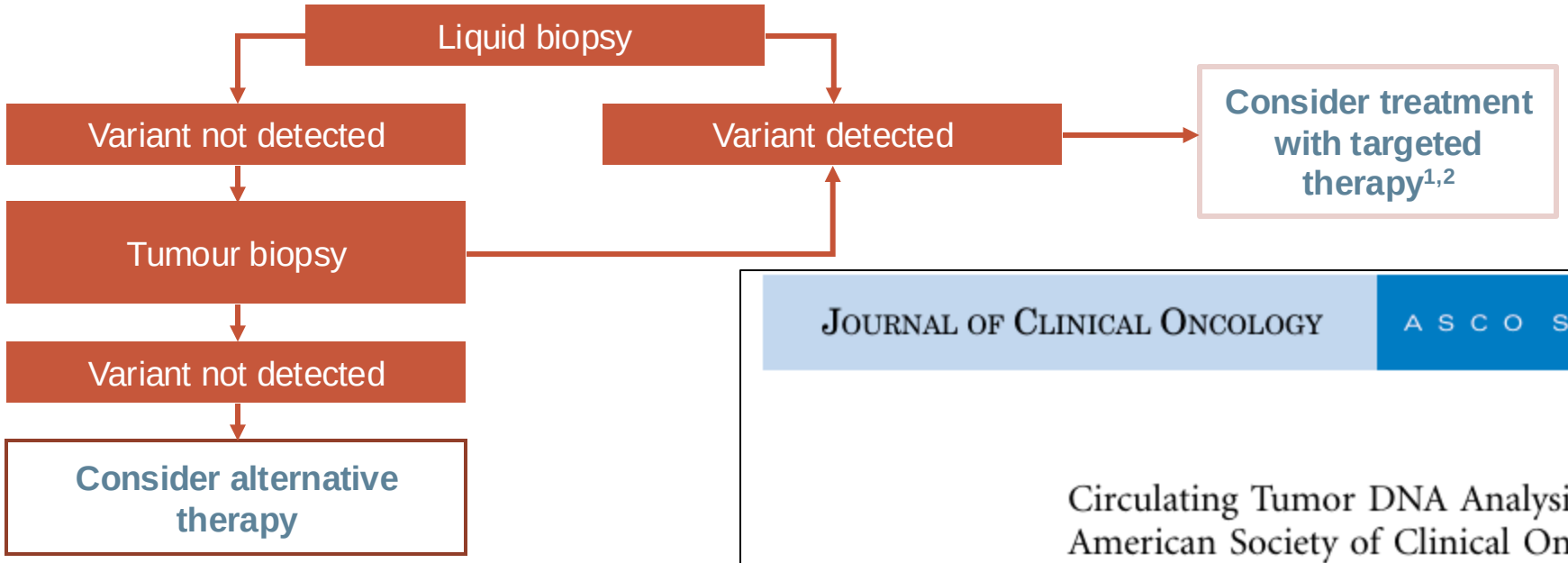


CNV, copy number variation; dPCR, digital PCR; MSI-H, microsatellite instability-high; NGS, next-generation sequencing; PCR, polymerase chain reaction; TMB, tumour mutational burden

Penault-Llorca F. ESMO Advanced Course on Breast Cancer: Filling the Gaps Following Progression on CDK4/6 in Hormone Receptor Positive HER2 Negative Advanced Breast Cancer 2024: Doha. Session 3 – What to do after CDK4/6 inhibition in advanced breast cancer

MUTATION TESTING IN HR+/HER2− mBC: LIQUID BIOPSY[1,2]

- Samples should be taken at disease progression for tumour genotyping[1]
- Consider tumour biopsy testing to confirm negative results[1]




JOURNAL OF CLINICAL ONCOLOGY | ASCO SPECIAL ARTICLE

Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review

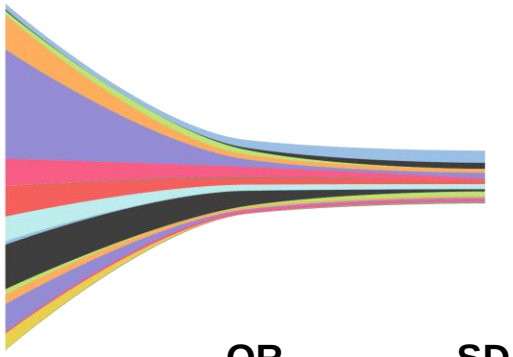
Jason D. Merker, Geoffrey R. Oxnard, Carolyn Compton, Maximilian Diehn, Patricia Hurley, Alexander J. Lazar, Neal Lindeman, Christina M. Lockwood, Alex J. Rai, Richard L. Schilsky, Apostolia M. Tsimberidou, Patricia Vasalos, Brooke L. Billman, Thomas K. Oliver, Suanna S. Bruinooge, Daniel F. Hayes, and Nicholas C. Turner

HR, hormone receptor; mBC, metastatic breast cancer

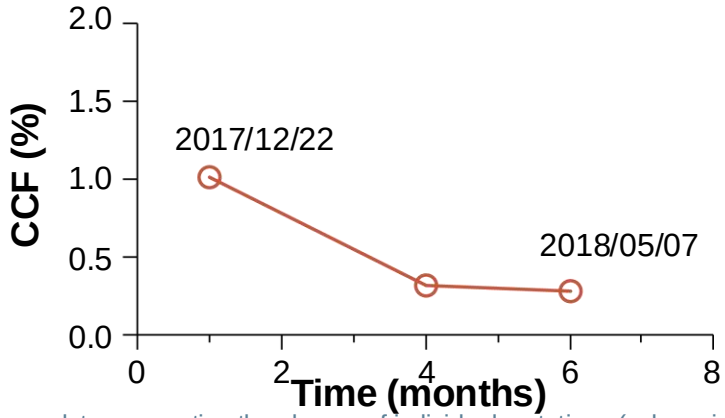
1. Merker JD, et al. J Clin Oncol. 2018;36:1631-41; 2. NCCN Guidelines Version 6.2024 for Invasive Breast Cancer. Available at: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf (accessed January 2025); Penault-Llorca F. ESMO Advanced Course on Breast Cancer: Filling the Gaps Following Progression on CDK4/6 in Hormone Receptor Positive HER2 Negative Advanced Breast Cancer 2024: Doha. Session 3 – What to do after CDK4/6 inhibition in advanced breast cancer

LIQUID BIOPSY: WHEN TO USE IT?

A. Change of individual mutations in a patient with BC[1, a]



B. Overall change (sum) of all mutations on the panels[1]



Risk of false negative in ctDNA if disease is responding to therapy (no shedding)[2,4]

Better to test with liquid biopsy when the tumour is progressing (higher probability of shedding, higher tumour burden) ➔ either before 1L, or at progression before 2L)[2,3,4]

Don't anticipate testing when the patient is stable under therapy as the risk of false negative is very high[2,4]

[a] Stream plot representing the change of individual mutations (colours indicate different genes and specific mutations on the panels)

1L, first-line; 2L, second-line; BC, breast cancer; CCF, ctDNA content fraction; ctDNA, circulating tumour DNA; OR, objective response; SD, stable disease

1. Li J, et al. J Transl Med. 2020;18:293; 2. Kuligina ES, et al. Cancer Genet. 2021;256-257:165-178; 3. Pascual J, et al. Annals of Oncology 2022; 33: 750-768; 4. Penault-Llorca F. ESMO Advanced Course on Breast Cancer: Filling the Gaps Following Progression on CDK4/6 in Hormone Receptor Positive HER2 Negative Advanced Breast Cancer 2024: Doha. Session 3 – What to do after CDK4/6 inhibition in advanced breast cancer

BIOMARKERS FOR TARGETED THERAPY

FREQUENCY OF LEVEL IA BIOMARKERS IN HR+ BREAST CANCER[1]

PIK3CAm
30-40%

AKT1m
5%

PTEN
7%

gBRCAm
5%

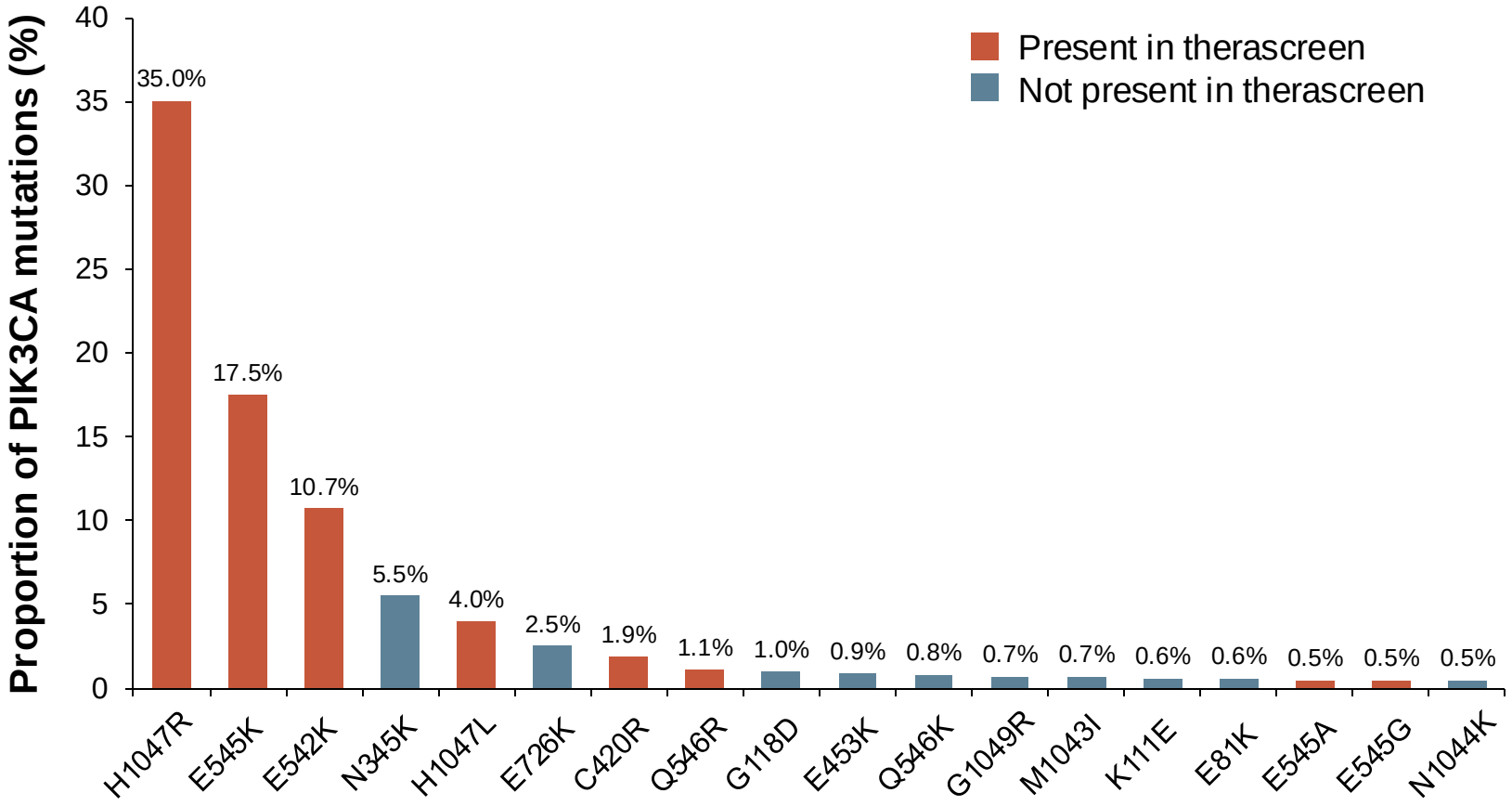
ESR1m
~30-40%

g, germline; HR+, hormone receptor positive; m, mutated

1. Mosele M, et al. Annals of Oncology 2024; 35: 588-606; 2. Penault-Llorca F. ESMO Advanced Course on Breast Cancer: Filling the Gaps Following Progression on CDK4/6 in Hormone Receptor Positive HER2 Negative Advanced Breast Cancer 2024: Doha. Session 3 – What to do after CDK4/6 inhibition in advanced breast cancer

IMPORTANCE OF USING 'BROAD' NGS-TYPE TESTS INSTEAD OF PCR

Proportion of the 18 most-frequent *PIK3CA* mutations in *PIK3CA*mut BC from a combined dataset

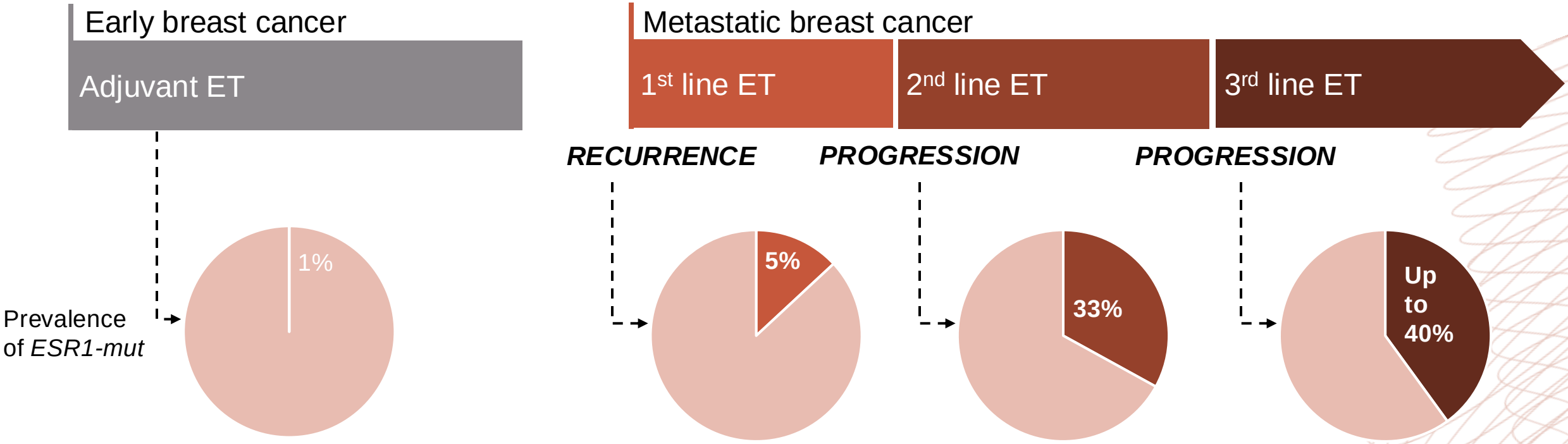


The currently validated therascreen companion diagnostic PCR test, which covers 11 hotspot mutations, might not capture up to 20% of patients with PIK3CA mutations

BC, breast cancer; NGS, next-generation sequencing; PCR, polymerase chain reaction

Martínez-Sáez O, et al. Breast Cancer Res. 2020;22:45; Penault-Llorca F. ESMO Advanced Course on Breast Cancer: Filling the Gaps Following Progression on CDK4/6 in Hormone Receptor Positive HER2 Negative Advanced Breast Cancer 2024: Doha. Session 3 – What to do after CDK4/6 inhibition in advanced breast cancer

LONGER EXPOSURE TO ET IN 1L INCREASES THE CHANCE OF DEVELOPING *ESR1* MUTATION DURING TREATMENT



ESR1-mut occur almost exclusively after aromatase inhibitors in the metastatic setting[5]
Testing for _ESR1_-mut should occur at each progression on ET if not detected[11-13]

1L, first line; ET, endocrine therapy; ESR1, estrogen receptor 1

Modified from: 1. Jeselsohn R, et al. Clin. Cancer Res. 2014;20:1757-67; 2. Jeselsohn R, et al. Cancer Cell. 2018;33:173-86;
3. Allouchery V, et al. Breast Cancer Res. 2018;20:40; 4. Schiavon G, et al. Sci Transl Med. 2015;7(313);313ra182;
5. Brett JO, et al. Breast Cancer Res. 2021;23(1):85; 6. Toy W, et al. Nat Genet. 2013;45(12):1439-45; 7. Bidard FC et al. J Clin Oncol 2022;40:3246-56;
8. Jhaveri et al, Annals of Oncology. 2023;34 (suppl_2): S334-90; 10.1016/annonc/annonc1299; 9. Lin et al, Annals of Oncology. 2023;34 (suppl_2): S334-90;
10.1016/annonc/annonc1299; 10. Bhave et al, SABCS 2023_PO2-1605; 11. Lee N, et al Int J Mol Sci. 2020;21(22):8807;
12. Gennari A, et al. Ann Oncol. 2021;32(12):1475-95; 13. Burstein HJ, et al J Clin Oncol. 2023;41(18):3423-5

ESR1: TESTING RECOMMENDATIONS

Test at progression of first-line therapy[1]

Breast cancer[1,2]	*PIK3CA* mutations	IA	*ESR1* mutations should preferentially be tested in ctDNA[2]
	ERBB2 amplification	IA	
	BRCA1/2 mutations	IA	
	ESR1 mutations	IB	IA[3]
	MSI-H	IC	
	NTRK 1/2/3 fusions	IC	

REFERENCE METHOD in PADA-1 trial: droplet digital PCR[4]

REFERENCE METHOD in EMERALD trial: *Guardant360® CDx* (Guardant Health)[5]

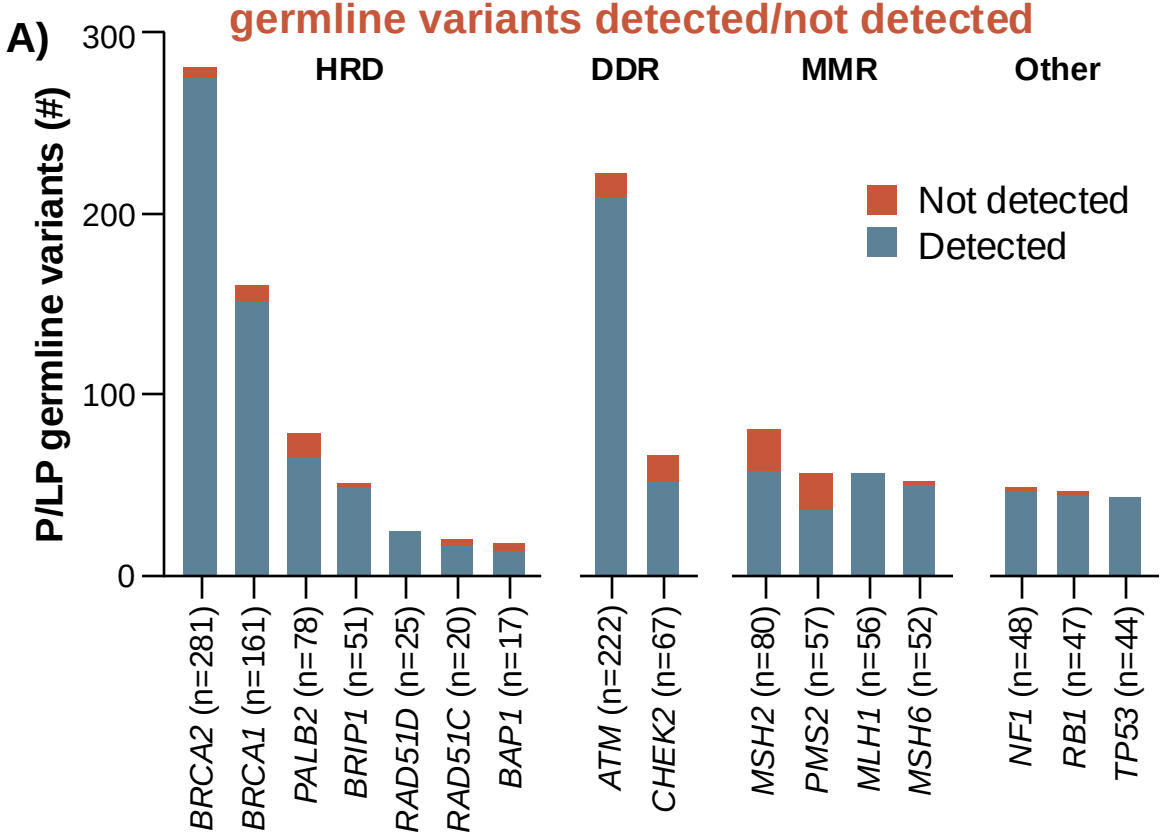
CDx, companion diagnostic; ctDNA, circulating tumour DNA; MSI-H, microsatellite instability-high; NGS, next-generation sequencing; PCR, polymerase chain reaction

1. Burstein H, et al. J Clin Oncol 2024; 42: 1450-1453; 2. Pascual J, et al. Ann Oncol. 2022;33:750-768; 3. Mosele MF, et al. Ann Oncol. 2024;35:588-606; 4. Callens C, et al. Anal Chem. 2022;94:6297-6303; 5. Bidard F-C, et al. J Clin Oncol. 2022:3246-3256

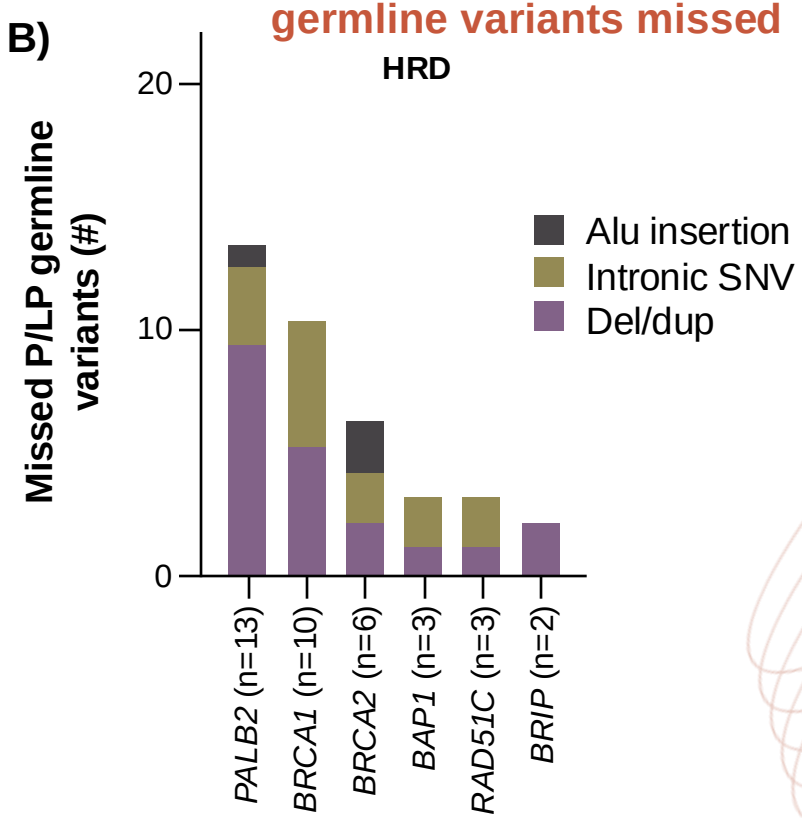
Penault-Llorca F. ESMO Advanced Course on Breast Cancer: Filling the Gaps Following Progression on CDK4/6 in Hormone Receptor Positive HER2 Negative Advanced Breast Cancer 2024: Doha. Session 3 – What to do after CDK4/6 inhibition in advanced breast cancer

IMPORTANT MESSAGE: TUMOUR NGS DOES NOT REPLACE GERMLINE TESTING

Tumour-only sequencing: germline variants detected/not detected



Tumour-only sequencing: germline variants missed



Breast cancer variants not detected affecting **HRD** or **DDR genes**: **13.2%** (95% CI: 8.9-19.1)

More frequently missed: *BRCA 1/2, PALB2* **intronic** variants, insertions, large rearrangements ➔ **false negative**

CI, confidence interval; DDR, DNA damage response; del, deletion; dup, duplication; HRD, homologous recombination deficiency; MMR, mismatch repair; NGS, next-generation sequencing; P/LP, pathogenic/likely pathogenic; SNV, single-nucleotide variant

Terraf P, et al. Ann Oncol. 2022;33:426-433; Penault-Llorca F. ESMO Advanced Course on Breast Cancer: Filling the Gaps Following Progression on CDK4/6 in Hormone Receptor Positive HER2 Negative Advanced Breast Cancer 2024: Doha. Session 3 – What to do after CDK4/6 inhibition in advanced breast cancer

NCCN TARGETED THERAPY RECOMMENDATIONS

TARGETED THERAPIES AND ASSOCIATED BIOMARKER TESTING FOR RECURRENT UNRESECTABLE (LOCAL OR REGIONAL) OR STAGE IV (M1) DISEASE

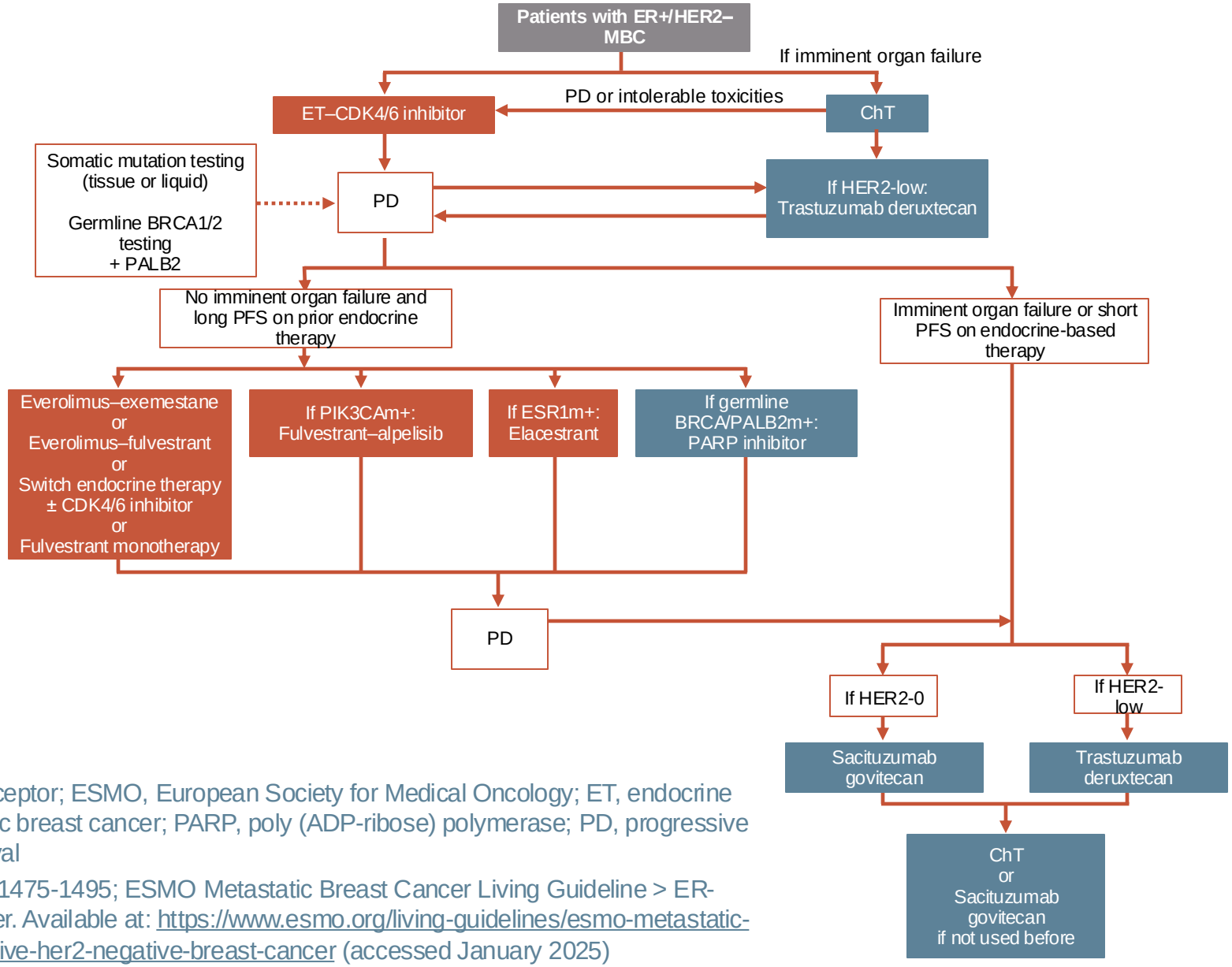
Biomarkers associated with FDA-approved therapies					
Breast cancer subtype	**Biomarker**	**Detection**	**FDA-approved agents**	**NCCN category of evidence**	**NCCN category of preference**
HR-positive, HER2-negative	*PIK3CA* activating mutation	NGS, PCR (tumour tissue or blood)	Inavolisib + palbociclib + fulvestrant	Category 1	Useful in certain circumstances first-line therapy
HR-positive/ HER2-negative	*PIK3CA* activating mutation	NGS, PCR (tumour tissue or blood)	Alpelisib + fulvestrant	Category 1	Preferred second- or subsequent-line therapy
HR-positive/ HER2-negative	*PIK3CA* or *AKT1* activating mutations or *PTEN* alterations	NGS, PCR (tumour tissue or blood)	Capivasertib + fulvestrant	Category 1	Preferred second- or subsequent-line therapy in select patients
HR-positive/ HER2-negative	*ESR1* mutation	NGS, PCR (blood preferred)	Elacestrant	Category 2A	Other recommended regimen

If liquid biopsy is negative, tumour tissue testing is recommended

FDA, Food and Drug Administration; HR, hormone receptor; M, metastatic stage; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; PCR, polymerase chain reaction

NCCN Guidelines Version 6.2024 for Invasive Breast Cancer. Available at: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf (accessed January 2025)

ESMO TARGETED THERAPY RECOMMENDATIONS



ChT, chemotherapy; ER, estrogen receptor; ESMO, European Society for Medical Oncology; ET, endocrine therapy; m, mutation; MBC, metastatic breast cancer; PARP, poly (ADP-ribose) polymerase; PD, progressive disease; PFS, progression-free survival

Gennari A, et al. Ann Oncol. 2021;32:1475-1495; ESMO Metastatic Breast Cancer Living Guideline > ER-positive HER2-negative Breast Cancer. Available at: https://www.esmo.org/living-guidelines/esmo-metastatic-breast-cancer-living-guideline/er-positive-her2-negative-breast-cancer (accessed January 2025)

TARGETED THERAPY FOR LESS FREQUENT ALTERATIONS

IF DRUGS ARE AVAILABLE

HER2m[1,2]

If lobular carcinoma

(up to 25%)

MSI-H[3]

~1%

TMB-H[4]

~10%

NTRK fusions[5]

<1%

HR, hormone receptor; MSI-H, microsatellite instability-high; TMB-H, tumour mutational burden-high

1. Ma J, et al. Transl Oncol. 2022;21:101444; 2. Kennedy LC, et al. J Clin Oncol. 2024;42(16 suppl):TPS630 (presented at 2024 ASCO Annual Meeting I); 3. Vidula N, et al. NPJ Breast Cancer. 2022;8:117; 4. Barroso-Sousa R, et al. Cancers (Basel). 2023;15:3997; 5. Theik NWY, et al. Int J Mol Sci. 2024;25:2366

Mosele M, et al. Annals of Oncology 2024; 35: 588-606; Penault-Llorca F. ESMO Advanced Course on Breast Cancer: Filling the Gaps Following Progression on CDK4/6 in Hormone Receptor Positive HER2 Negative Advanced Breast Cancer 2024: Doha. Session 3 – What to do after CDK4/6 inhibition in advanced breast cancer; NCCN Guidelines Version 6.2024 for Invasive Breast Cancer. Available at: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf (accessed January 2025)

NCCN TARGETED THERAPY RECOMMENDATIONS

Biomarkers associated with FDA-approved therapies					
Breast cancer subtype	**Biomarker**	**Detection**	**FDA-approved agents**	**NCCN category of evidence**	**NCCN category of preference**
Any	Germline *BRCA1* or *BRCA2* mutation	Germline sequencing	Olaparib Talazoparib	Category 1	Preferred
Any	*NTRK* fusion	FISH, NGS, PCR (tumour tissue or blood)	Larotrectinib Entrectinib Repotrectinib	Category 2A	Useful in certain circumstances
Any	MSI-H/dMMR	IHC, NGS, PCR, (tumour tissue or blood)	Pembrolizumab	Category 2A	
			Dostarlimab-gxly		
Any	TMB-H (≥10 mut/Mb)	NGS (tumour tissue)	Pembrolizumab	Category 2A	
Any	RET-fusion	NGS (tumour tissue or blood	Selpercatinib	Category 2A	

FDA, Food and Drug Administration; FISH, fluorescence in-situ hybridisation; IHC, immunohistochemistry; Mb, megabase; MSI-H, microsatellite instability-high; mut, mutation; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; PCR, polymerase chain reaction; TMB-H, tumour mutational burden-high

NCCN Guidelines Version 6.2024 for Invasive Breast Cancer. Available at: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf (accessed January 2025)

SUMMARY

- Liquid biopsy has completely changed the diagnostic pathway of our patients
  - It provides **quick results** and is **useful for serial testing of alterations** and the dynamic evaluation of the **appearance or disappearance of some alterations**
- Tissue biopsy remains important - plasma and tissue generally provide **complementary** information
- Biomarkers with clinical utility in HR+ HER2- metastatic breast cancer are **somatic *PIK3CA/AKT1/PTEN, ESR1, BRCA* alterations** and **germline *BRCA/PALB2***
- Multigene NGS testing is **recommended** by the ESMO and NCCN guidelines. Tumour NGS does not replace germline testing
- **Timing of testing** depends on the alteration
- Use of plasma to detect **resistance** mechanisms to prior therapy should occur when the disease is **progressing** (e.g. *ESR1m*)
- A negative liquid biopsy does not mean there are no relevant alterations – confirm with tissue testing

ESMO, European Society for Medical Oncology; HR, hormone receptor; NGS, next-generation sequencing; NCCN, National Comprehensive Cancer Network

NCCN Guidelines Version 6.2024 for Invasive Breast Cancer. Available at: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf (accessed January 2025); Turner B, et al. Human Pathology Reports 2021: doi.org/10.1016/j.hpr.2021.300574; Mosele M, et al. Annals of Oncology 2024; 35: 588-606;

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