Carcinoma mammario metastatico: quali informazioni deve fornire oggi l'anatomopatologo al clinico? Quali sottogruppi sono oggi identificabili?

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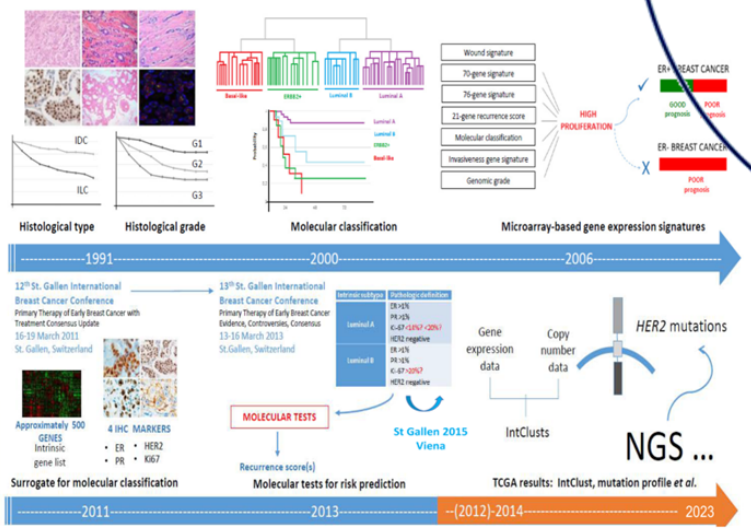
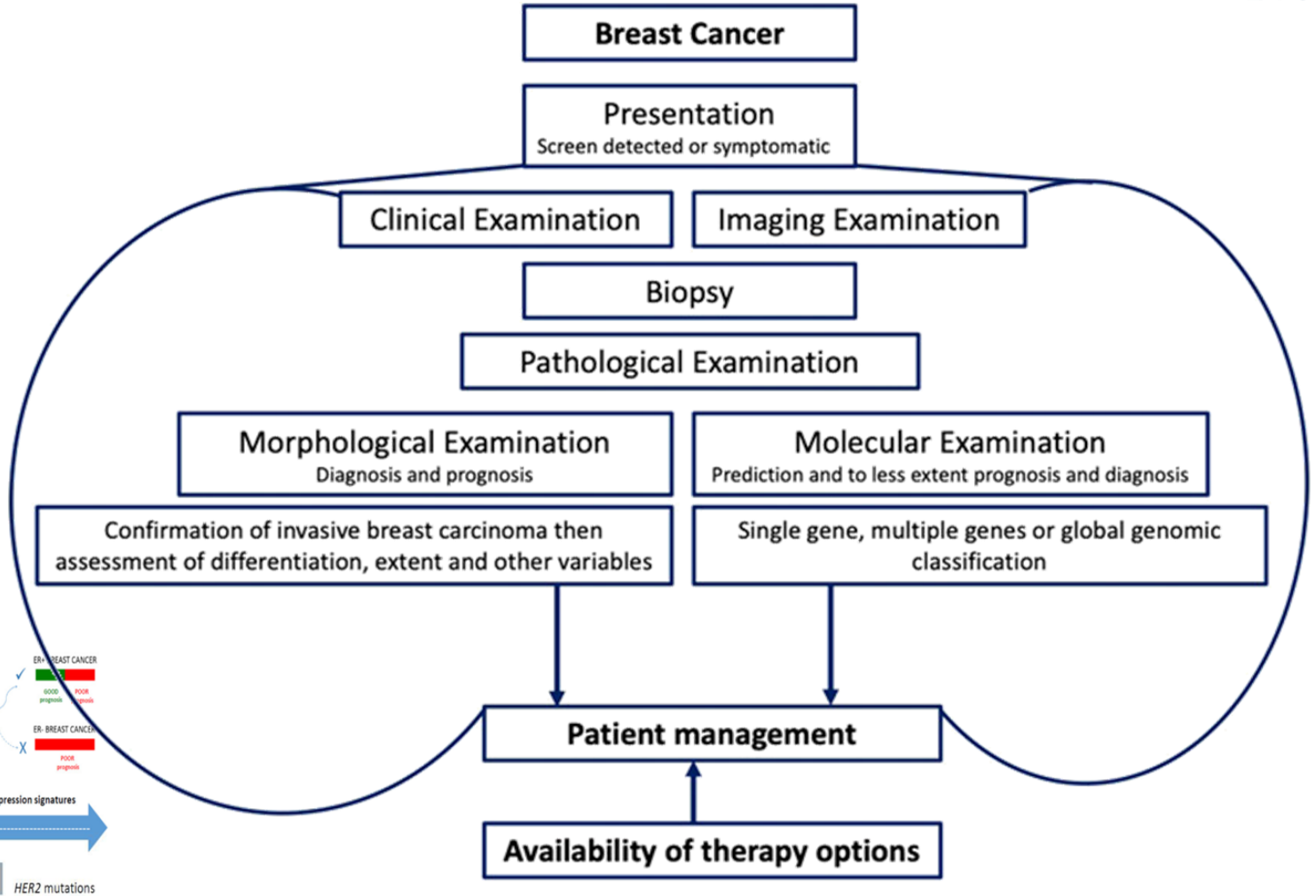
DISCLOSURES

Commercial Interest	Relationships
MSD, Novartis, AstraZeneca, Diaceutics, Adicet Bio, Sysmex, Roche, Menarini, Gilead, Veracyte Inc, Sakura.	Consulting/advisory role
MSD, Novartis, AstraZeneca, Daiichi Sankyo, GSK, Gilead, Roche, Leica Biosystems, Sysmex, Diatech, Lilly, Pfizer.	Speaker bureau
Novartis, Reply, Gilead, AstraZeneca, GSK, Pfizer.	Research grants
Roche, Novartis.	Travel grants

mBC BIOMARKERS IN 2024

- The pathology report
- HRs (ER/PgR), Ki67
- HER2 spectrum
- PD-L1 (CPS/IC)
- PIK3CA (& pathway)
- ESR1
- gBRCA






Histopathology

An update on the pathological classification of breast cancer

Targeting ‘low’ and ‘ultralow’ HER2-expressing tumours in mBC

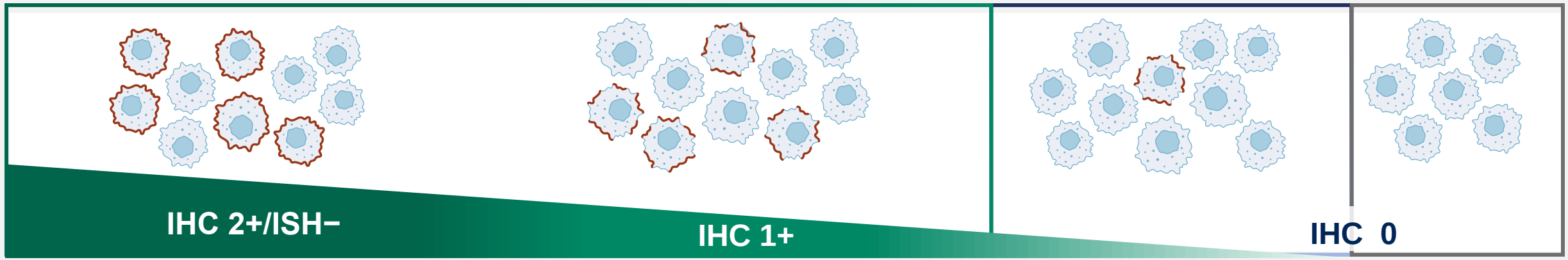
HER2 IHC categories within HR+, HER2– mBC (per ASCO/CAP[1])

DESTINY-Breast06 patient population: ~85% of HR+, HER2− mBC

HER2-low
~60–65%[2,3]

HER2-ultralow
~20–25%[2–4]

Figure adapted from Venetis K, et al. Front Mol Biosci. 2022;9:834651



IHC 2+/ISH−	IHC 1+	IHC 0	
Weak-to-moderate complete membrane staining in >10% tumour cells	Faint, incomplete membrane staining in >10% tumour cells	**Faint, incomplete membrane staining in ≤10% tumour cells**	Absent / no observable membrane staining

ASCO/CAP=American Society of Clinical Oncology / College of American Pathologists; HER2=human epidermal growth factor receptor 2; HR+=hormone receptor-positive; HER2=human epidermal growth factor receptor 2; IHC=immunohistochemistry; ISH=in situ hybridization; mBC=metastatic breast cancer; T-DXd=trastuzumab deruxtecan.

1. Wolff AC, et al. *J Clin Oncol.* 2023;41:3867–3872; 2. Denkert C, et al. *Lancet Oncol.* 2021;22:1151–1161; 3. Chen Z, et al. *Breast Cancer Res Treat.* 2023;202:313–323; 4. Mehta S, et al. *J Clin Oncol.* 2024;42(Suppl.16):Abstract e13156.

Phase 3 DESTINY-Breast06

ESMO 2024

HER2-Low or -Ultralow Status Determination

Nicola

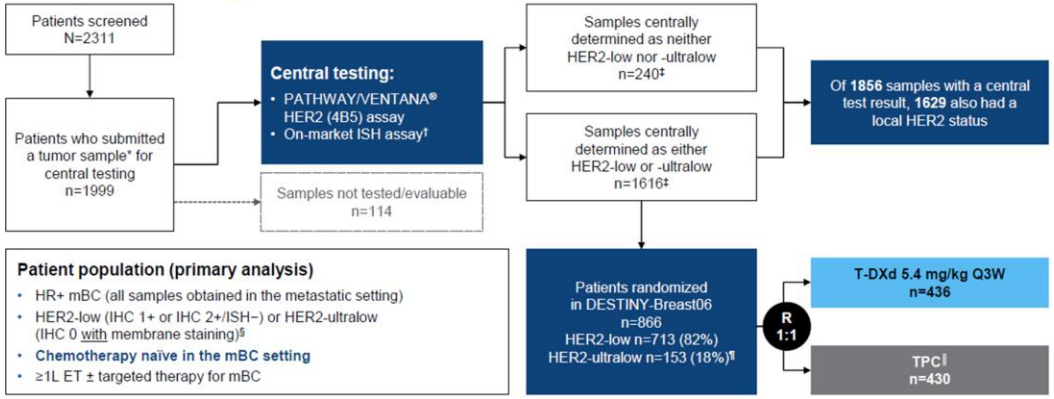
Central HER2 IHC score prevalence consistent across key variables in the population locally scored as HER2-negative

Variable, n (%)	IHC 0 absent membrane staining*	HER2-ultralow (IHC 0 with membrane staining)†	IHC 1+	IHC 2+/ISH−	IHC 2+/ISH+	IHC 3+	Total
Overall	**225 (12)**	**402 (22)**	**829 (45)**	**385 (21)**	**11 (<1)**	**4 (<1)**	**1856**
Sample type							
Biopsy	202 (12)	344 (21)	729 (45)	338 (21)	8 (<1)	4 (<1)	1625
Resection	23 (10)	58 (25)	100 (43)	47 (20)	3 (1)	0	231
Sample age							
<3 months	83 (11)	133 (18)	362 (48)	168 (22)	3 (<1)	1 (<1)	750
3 to ≤6 months	14 (10)	31 (22)	59 (42)	35 (25)	3 (2)	0	142
>6 months to ≤12 months	23 (10)	44 (20)	100 (45)	50 (23)	1 (<1)	2 (<1)	220
>1 to ≤3 years	62 (13)	126 (26)	208 (42)	95 (18)	2 (<1)	1 (<1)	494
>3 years	43 (17)	68 (27)	100 (40)	37 (15)	2 (1)	0	250

No difference in prevalence observed between

- **Primary vs metastatic sample site**‡
- **Region** (America, Europe, Asia [excluding China], China)

Study design



Concordance between central and local results

Results from central scoring

- Of samples scored as HER2-low locally, **94%** met DESTINY-Breast06 inclusion criteria (were either **HER2-low** or **HER2-ultralow** by central testing)
- **Overall percent agreement** was **77.8%** for HER2-low*
- Of samples scored as IHC 0 locally, central testing found
  - 35% were IHC 0 absent membrane staining
  - **40%** were **HER2-ultralow**
  - **24%** were **HER2-low**

  → **64% with membrane staining**

Central vs local HER2 scores in patients screened for DESTINY-Breast06†

HER2 status by central testing, n		HER2 status by local result, n: IHC 0†	HER2-low	IHC 2+/ISH+	IHC 3+	Total
IHC 0†	Absent membrane staining‡	123	65	0	1	189
	With membrane staining (HER2-ultralow)§	140	196	2	1	339
HER2-low		85	999	6	0	1090
IHC 2+/ISH+		1	7	0	0	8
IHC 3+		0	3	0	0	3
Total		349	1270	8	2	1629

Note: The sample used for central testing may not have been the same as that used for the local test result

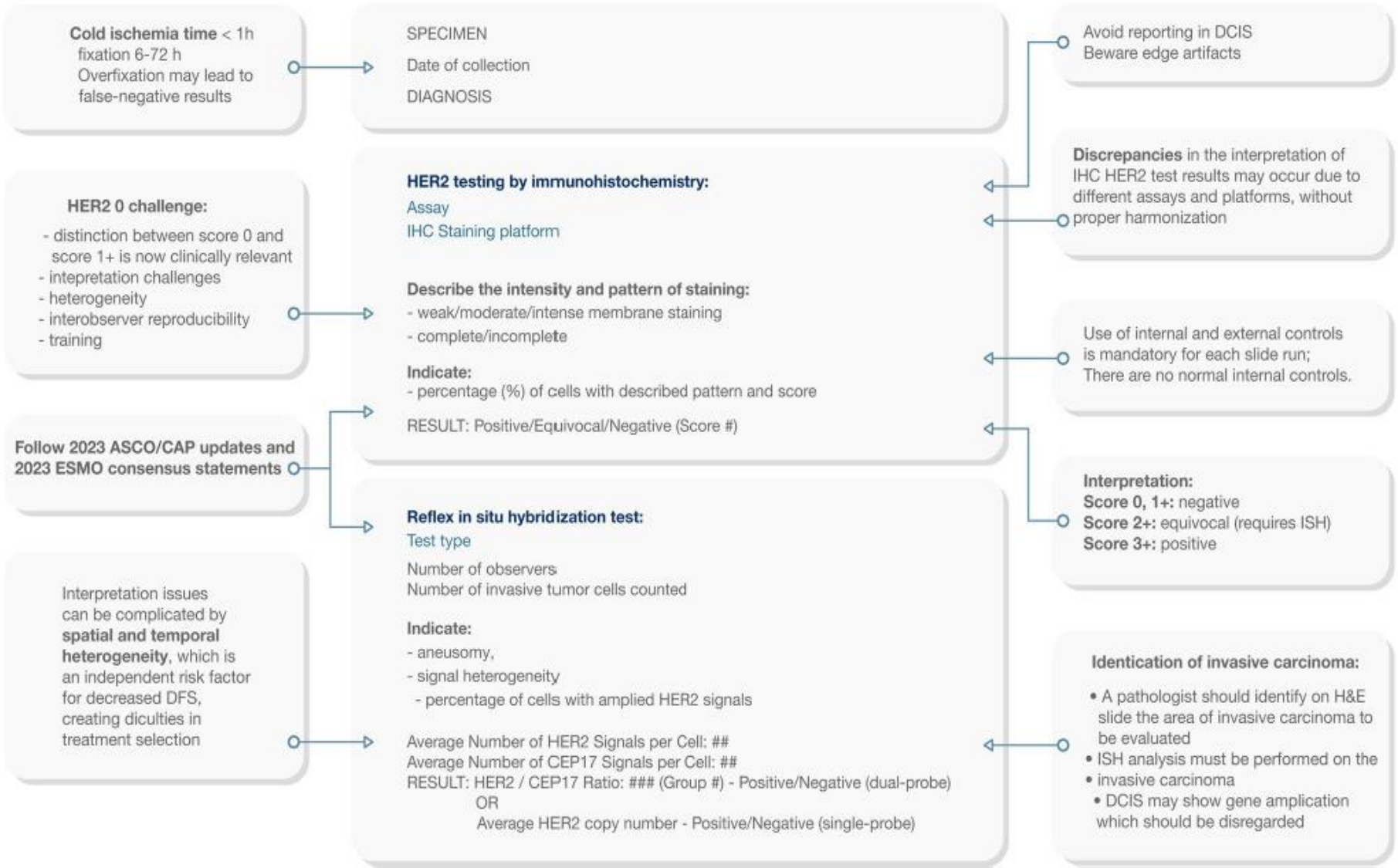
Viale G, et al. ESMO 2024. LBA21.

HER2-Low or -Ultralow Status Determination

Conclusions and future directions

- Patients with HR+, HER2-low or HER2-ultralow mBC derived clinically meaningful benefit from T-DXd vs TPC
- Patients likely to benefit from T-DXd could be identified regardless of sample type or location
- Overall percent agreement for HER2-low between local and central results was **78%**
  - Almost all (**94%**) of patients with a local HER2-low score were centrally scored as either HER2-low or HER2-ultralow and hence were eligible to participate in DESTINY-Breast06
- A majority (**64%**) of patients with a local HER2 IHC 0 score were centrally scored as HER2-low (**24%**) or HER2-ultralow (**40%**)
  - It may be advisable for patients with HR+ mBC scored as HER2 IHC 0 to be reassessed to determine if they may be eligible for treatment with T-DXd
- Increased awareness of low HER2 expression levels is desirable

SYNOPTIC REPORT FOR HER2 TEST TO ADDRESS THE EVOLVING CLINICAL RATIONALE



Challenges to be addressed:

- Different antibodies and detection systems
- Different platforms
- Different scoring systems (ASCO/CAP vs Ventana)
- Spatial and temporal heterogeneity

Standardized pathology report for HER2 testing in compliance with 2023 ASCO/CAP updates and 2023 ESMO consensus statements on HER2-low breast cancer

Mariia Ivanova[1] · Francesca Maria Porta[1] · Marianna D'Ercole[1] · Carlo Pescia[1] · Elham Sajjadi[1,2] · Giulia Cursano[1] · Elisa De Camilli[1] · Oriana Pala[1] · Giovanni Mazzarol[1] · Konstantinos Venetis[1] · Elena Guerini-Rocco[1,2] · Giuseppe Curigliano[2,3] · Giuseppe Viale[1] · Nicola Fusco[1,2]

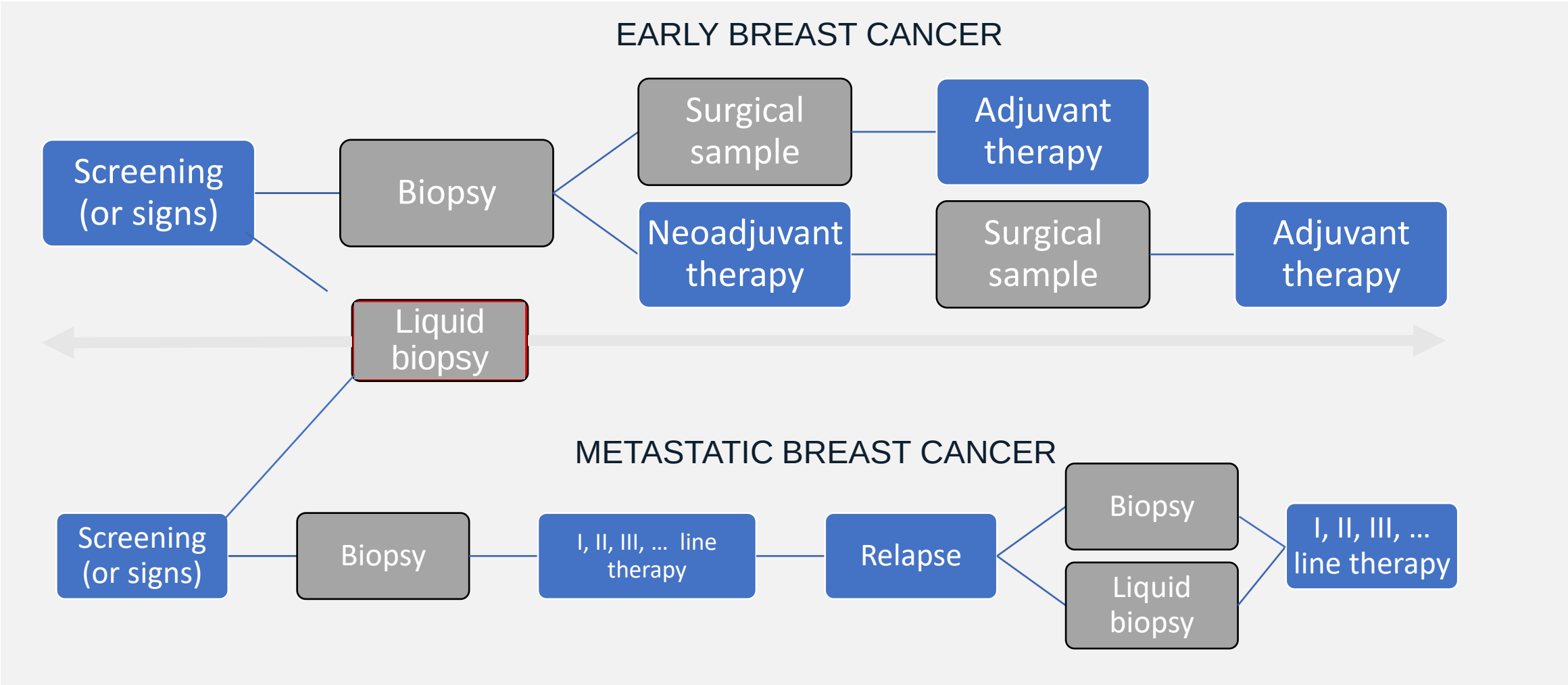
Received: 20 July 2023 / Revised: 3 September 2023 / Accepted: 13 September 2023 / Published online: 28 September 2023

Virchows Archiv (2024) 484:3–14

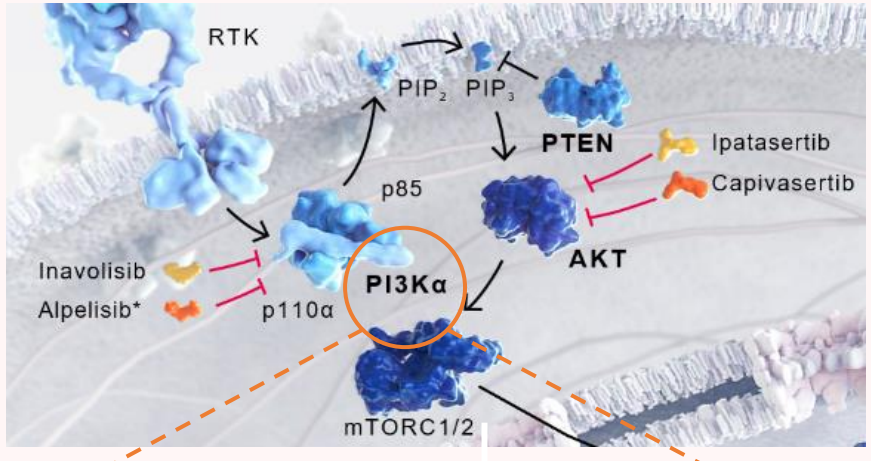
ESTABLISHED MOLECULAR BIOMARKERS IN MBC TODAY

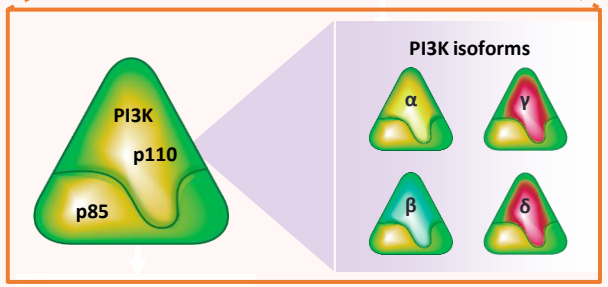
- **PIK3CA**
- **ESR1**
- **gBRCA**
- (HER2 mut)

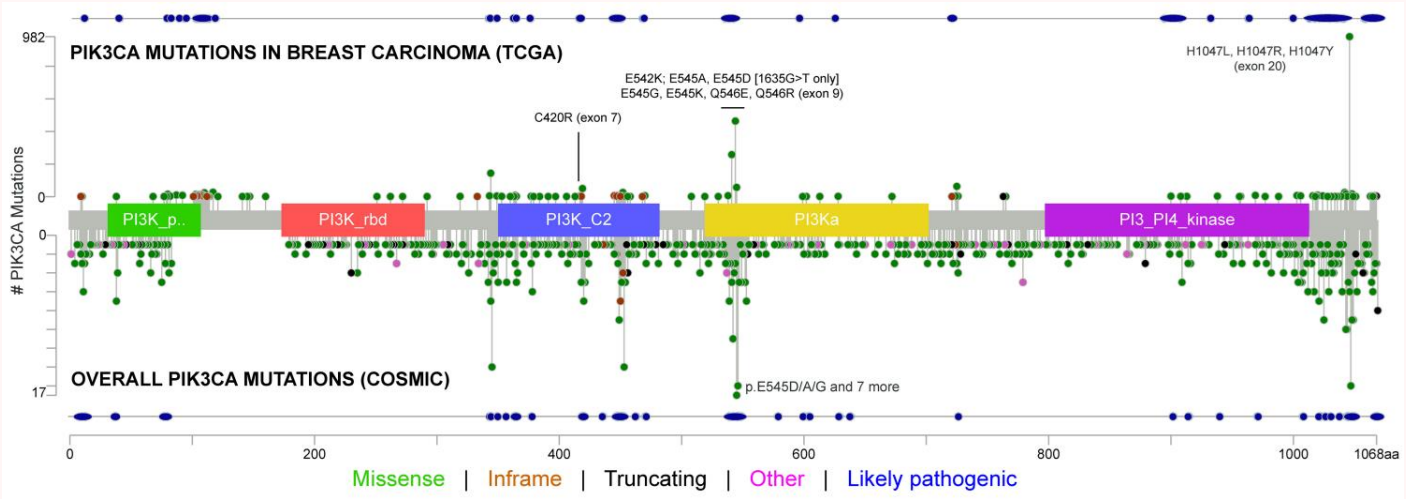
Diagnostic testing can inform treatment decisions in breast cancer



PI3K pathway increased relevance in HR+ Breast Cancer. Molecular information to guide treatment & improve patient outcomes







frontiers in Oncology
published: 25 March 2021
doi: 10.3389/fonc.2021.644737

***PIK3CA* Mutations as a Molecular Target for Hormone Receptor-Positive, HER2-Negative Metastatic Breast Cancer**

Nicola Fusco[1,2†], Umberto Malapelle[3†], Matteo Fassan[4,5], Caterina Marchiò[6,7], Simonetta Buglioni[8], Simonetta Zupo[9], Carmen Criscitiello[2,10], Paolo Vigneri[11,12], Angelo Paolo Dei Tos[4,5], Eugenio Maiorano[13] and Giuseppe Viale[1,2]*

- **~40% of HR+/HER2– aBC patients have a mutation in the *PIK3CA* gene** and can have endocrine resistance and/or shorter mPFS
- **Hotspot regions in *PIK3CA*: ex 7, 9, 20** but also outside hot spots
- *PIK3CA* mutations can be detected in tissue (FFPE) or plasma samples
- *PIK3CA* mutations are **considered to be truncal**; samples from both primary and metastatic tumours can be used for testing[1–4]

1. Chang DY, *et al. Ther Clin Risk Manag* 2021; 2. O'Leary B, *et al. Cancer Discov* 2018; 3. Mroz EA and Rocco JW. *Cancer* 2017; 4. Miron A, et al. *Cancer Res* 2010.

Leveraging past experiences to identify the ‘holy grail’ of PI3K inhibitors

Pan-PI3K inhibitor	Pan-PI3K/ mTOR inhibitor	β-sparing inhibitor and ‘mut degradation facilitator’	PI3Kα- and PI3Kδ-specific	α-selective inhibitor	α-selective inhibitor and ‘mut degradation facilitator’	Mutant-selective PI3Kα-specific inhibitors
Pictilisib[1] Buparlisib[2] *Discontinued*[3,4]	Gedatolisib *Under investigation*[5]	Taselisib *Discontinued*[6]	Copanlisib *Under investigation*[7,8]	Alpelisib *First Global approval 2019*[9,10]*	Inavolisib *FDA approval expected Q4 2024*[11,12]	RLY-2608[13,14] STX-478[15] *Under investigation* LOXO-783[16] *Discontinued*

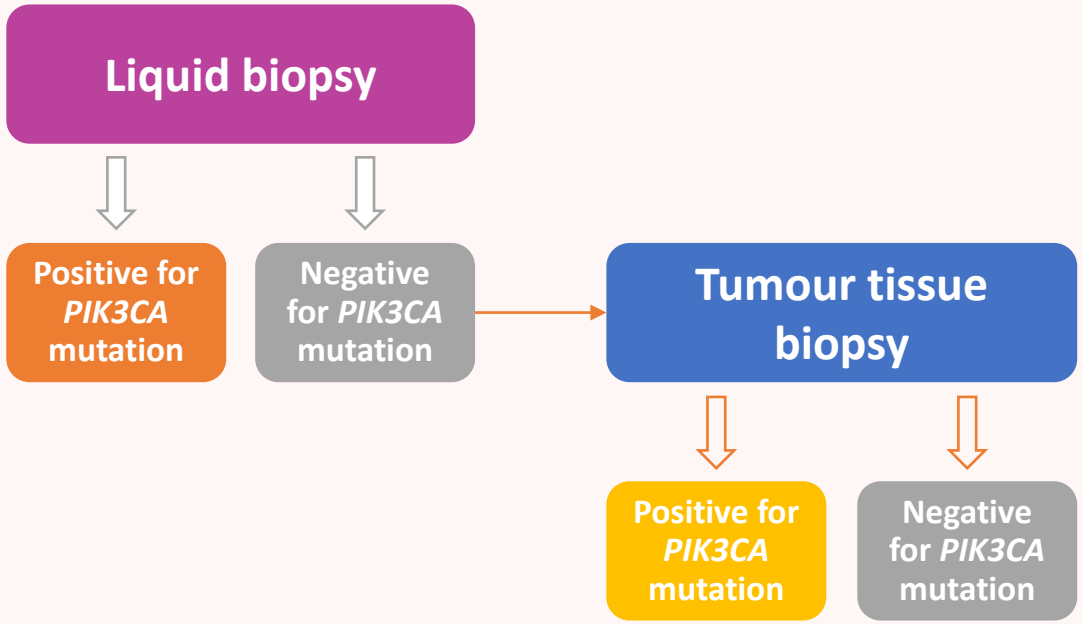
- **Achieving** an acceptable therapeutic index has been a challenge due to on-target toxicities
- **Specificity** toward p110α is paramount to enable a greater therapeutic index in a biomarker-defined population

Alpelisib is also approved in the EU.[17]

1. Krop IE, *et al. Lancet Oncol* 2016; 2. Di Leo A, *et al. Lancet Oncol* 2018; 3. https://ascopost.com/News/40583 (accessed March 2024);
4. https://www.cancernetwork.com/view/buparlisib-effective-toxic-advanced-breast-cancer-patients (accessed March 2024); 5. https://clinicaltrials.gov/ct2/show/NCT05501886 (accessed May 2023); 6. Jhaveri K, *et al. Clin Cancer Res* 2021; 7. https://clinicaltrials.gov/ct2/show/NCT03939897 (accessed March 2024);
8. https://www.hcp.aliqopa-us.com/mechanism-of-action (accessed March 2024); 9. PIQRAY PI 2024;
10. https://www.novartis.com/news/media-releases/fda-approves-novartis-piqray-first-and-only-treatment-specifically-patients-pik3ca-mutation-hrher2-advanced-breast-cancer (accessed March 2024); 11. Dey A, *et al*. SABCS 2019 (Poster P3-11-23); 12. Roche. Data on file; 13. https://clinicaltrials.gov/ct2/show/NCT05216432 (accessed March 2024);
14. Varkaris A, *et al.* AACR 2023 (Oral CT017); 15. https://clinicaltrials.gov/study/NCT05768139 (accessed March 2024); 16. https://clinicaltrials.gov/study/NCT05307705 (accessed March 2024); 17. PIQRAY SmPC 2024.

Preferred sample type will depend on different criteria.
Sample quality and DNA quantity, Turn Around Time required, and cost and availability.
Multidisciplinary teams should consider these criteria and define protocols

Clinical guidelines recommend liquid or tumour tissue biopsy; if liquid biopsy is negative, tumour tissue testing is recommended



FDA approves inavolisib with palbociclib and fulvestrant for endocrine-resistant, PIK3CA-mutated, HR-positive, HER2-negative, advanced breast cancer

On October 10, 2024, the Food and Drug Administration approved inavolisib (Itovebi, Genentech, Inc.) with palbociclib and fulvestrant for adults with endocrine-resistant, PIK3CA-mutated, hormone receptor (HR)-positive, human epidermal growth-factor receptor 2 (HER2)-negative, locally advanced or metastatic breast cancer, as detected by an FDA-approved test, following recurrence on or after completing adjuvant endocrine therapy.

FDA also approved the FoundationOne Liquid CDx assay as a companion diagnostic device to identify patients with breast cancer for treatment with inavolisib with palbociclib and fulvestrant.

FDA U.S. FOOD & DRUG ADMINISTRATION

1. Henry LN, *et al. J Clin Oncol* 2022;
2. NCCN Breast Cancer Guidelines; Version 1, 2024;
3. Gennari A, *et al. Ann Oncol* 2021.

Clinically relevant *PIK3CA* alterations may be detected using different techniques (e.g. real-time PCR and next-generation sequencing)[1–4]

Immunohistochemistry (IHC)

- ***Single biomarker analysis (protein expression)***

Fluorescence *in situ* hybridisation (FISH)

- ***Single biomarker analysis***
- ***Copy number alterations***

Polymerase chain reaction (PCR)

Single biomarker and/or multi-gene hotspot NGS panel tests

NGS

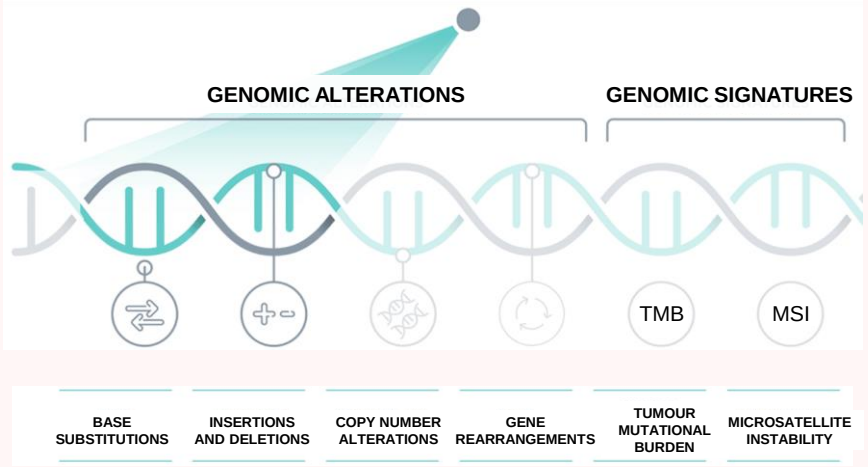
Hybrid capture-based NGS/CGP

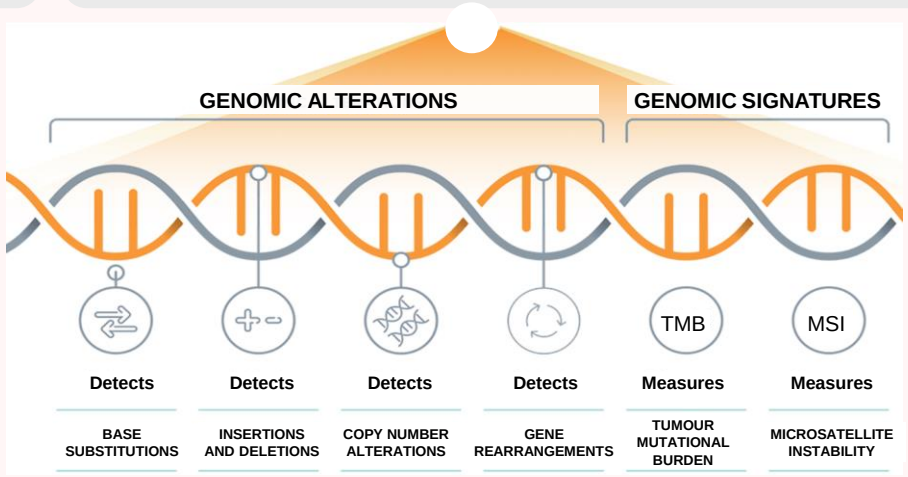
Conventional methods for identifying targetable alterations in cancer, including IHC and FISH, identify single protein or nucleic acid biomarker

Not suitable for *PIK3CA* mut identification

- Detects a predefined set of specific gene alterations that are associated with an effective targeted therapy
- Can **miss** indels, copy number alterations, rearrangements

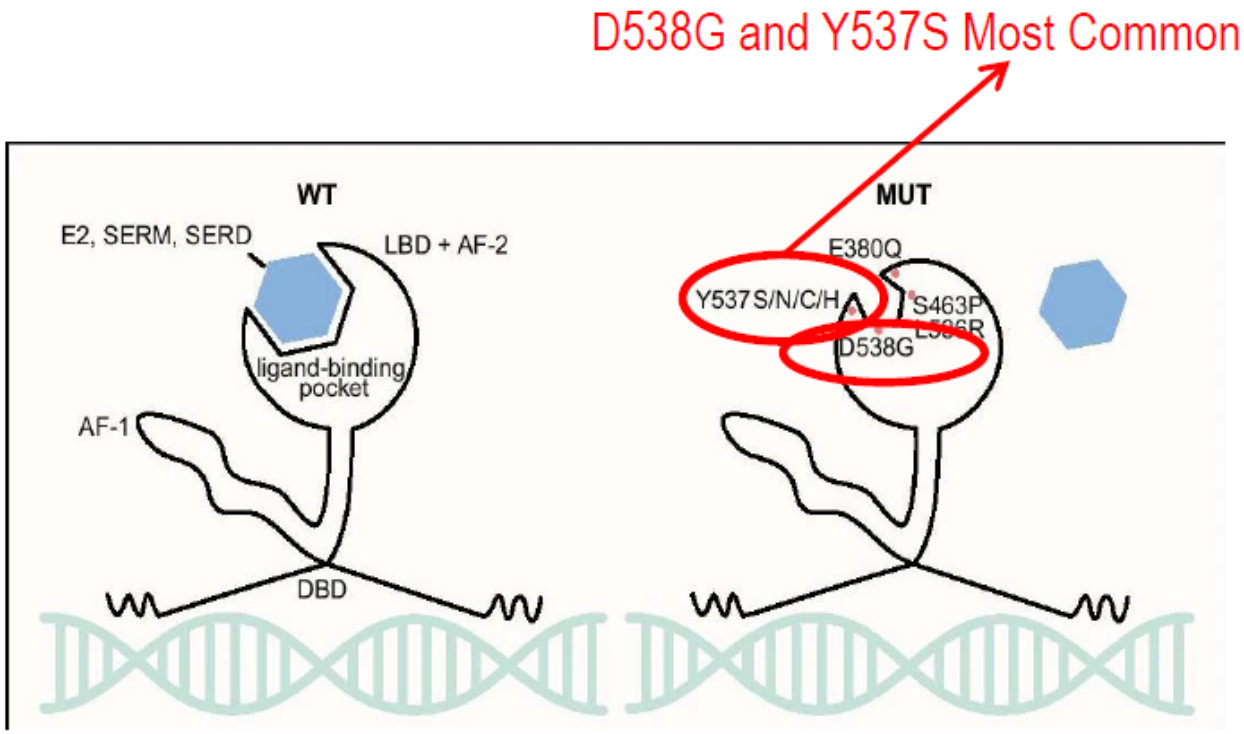
- Can target the whole coding sequence of a gene and is able to detect all four main classes of gene alterations
- Also allows for the characterisation of both **known and novel variants** for discovery-related applications





1. Dong L, *et al. Current Genomics* 2015; 2. Gray PN, *et al. Cancers* 2015;
3. Frampton GM, *et al. Nat Biotechnol* 2013; 4. Roche. Data on file.

ESR1 mutations



AI, aromatase inhibitor; ESR1, estrogen receptor 1; mut, mutation; SERD, selective estrogen receptor downregulator; SERM, selective estrogen receptor modulator; *wt*, wild-type. D538G and Y537S, Estrogen Receptor Alpha Somatic Mutations Brett et al. Breast Cancer Res. 2021;23(1):85.; Jeselsohn et al. Nat Rev Clin Oncol. 2015;12:573-583.

- *ESR1* mutations (*ESR1m*) commonly occur in breast cancers exposed to aromatase inhibitors.
- Most mutations affect the estrogen receptor (ER) ligand-binding domain (amino acids 282-595).
- Few studies have reported the frequency of ESR1 mutations in the AMEA region.

HOW TO TEST ESR1?

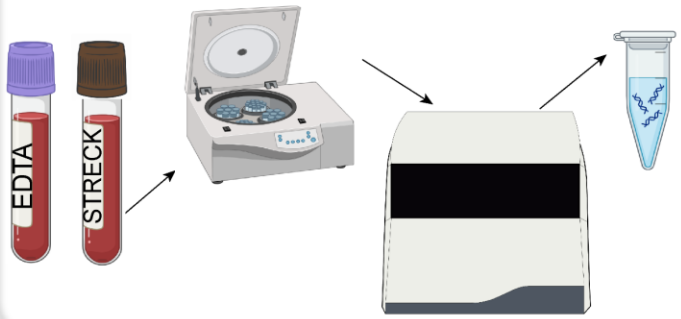
Pre-analytical Phase

Timing of blood sampling — disease progression

Collection tubes
- EDTA (need to be processed within 1 hour upon blood collection)
- Preservative tubes (up to 7 days at 6°C to 37°C)

Centrifugation → Two-step 2000 x g at 4° centrifugation 10' each

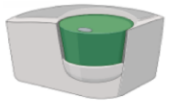
cfDNA extraction → Automated extraction methods are preferred over manual ones



Analytical Phase

PCR-based approaches

- **LOD**: ddPCR up to 0.0001%; RT-PCR between 1% and 5%
- **TAT**: 3-5 days
- **Reportable range**: mainly hotspot codons 380, 536, 537, 538



NGS-based approaches

- **LOD**: between 0.1% to 1.1%
- **TAT**: around 7 days
- **Reportable range**: multiple *ESR1* exons to full gene coverage

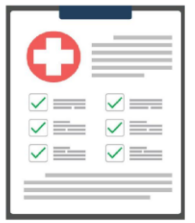
In case of *ESR1* wt consider retesting of patients at subsequent progression(s)

Post-analytical Phase

- Result → WT
- Result → Mutated → specific mutation

clinical annotation + ESCAT Score
- oncogenic
- likely oncogenic
- VUS
- likely neutral

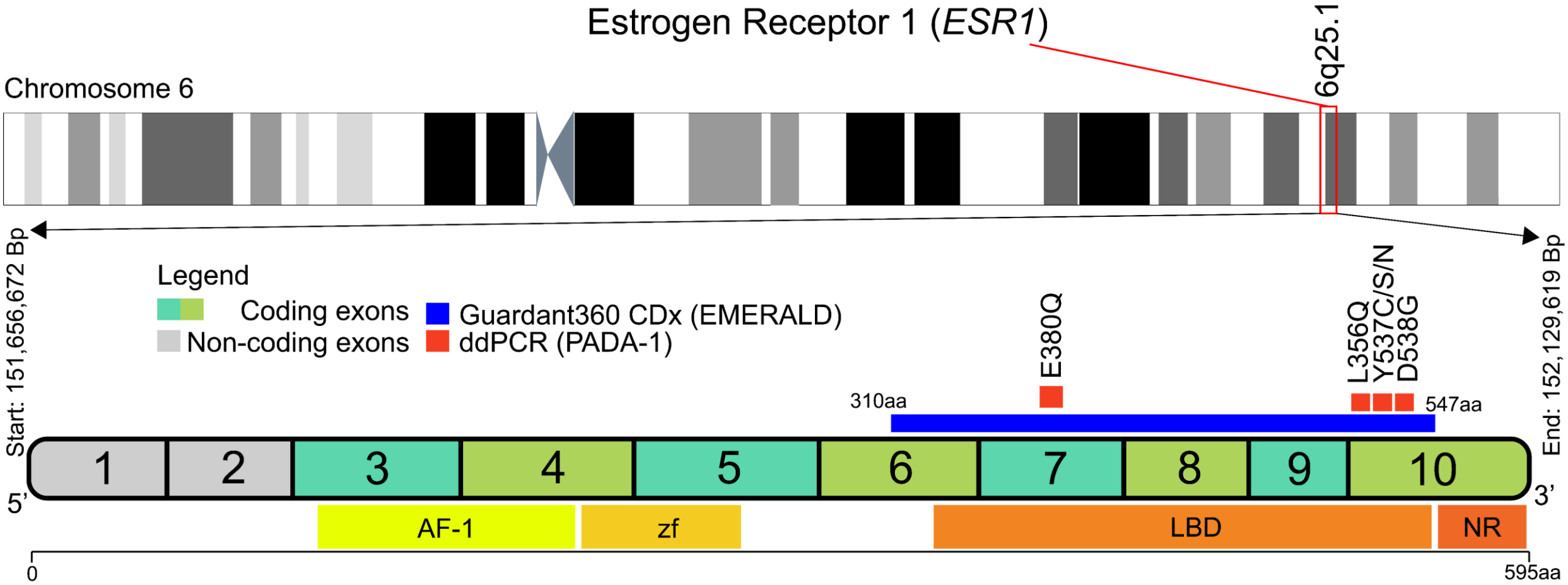
- Contextualization of mutations detected below the reportable cut-off values
- Rigorous SOPs, describing the diagnostic workflow from the blood collection to *ESR1* report
- Collaboration between pathologists and molecular biologists
- Clinical report



Guerini-Rocco et al. 2024

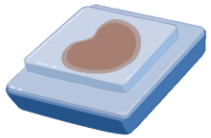
ESR1 TESTING ON LIQUID BIOPSY

- ESR1 mutations occurrence in LBD lead to ET resistance (mainly to AI)
- Rationale: to select patients with HR+/HER2- MBC for treatment with the SERD Elacestrant



Venetis et al. Cancer Treat Rev 2023

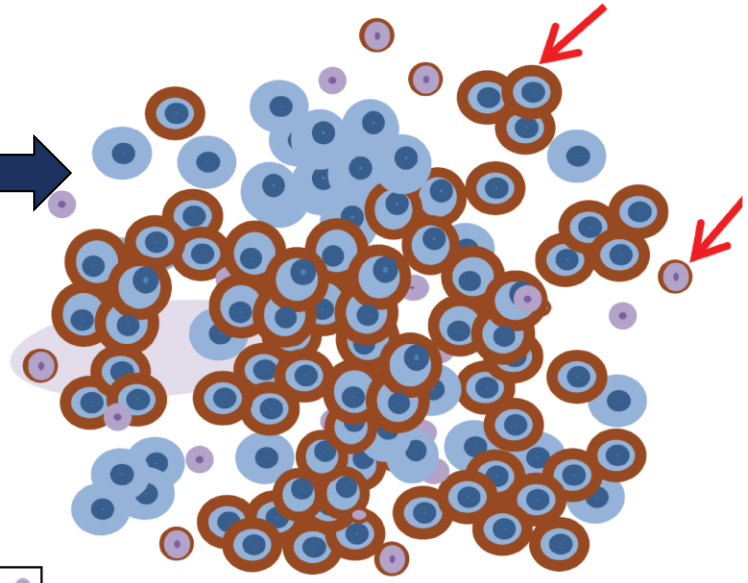
GENOMIC TESTING: WHAT, WHEN, HOW AND WHERE TO TEST? TIPS FOR ONCOLOGISTS

Sample type		DNA quantity	DNA quality		Biomarkers	RT-PCR	dPCR	Target NGS	CGP
Tissue (FFPE) 	Metastatic site 	→ / ↓	↑ If recent sample	→ / ↓ if old sample (more than 5 years or de-calcified bone metastasis)	ESR1	🔴	🟡	🟡	🟢
					PIK3CA	🟢	🟢	🟢	🟢
					PIK3CA pathway	🔴	🔴	🟢	🟢
	Primary tumor 	↑	↑ If recent sample	→ / ↓ if old sample (more than 5 years or de-calcified bone metastasis)	ESR1	🔴	🔴	🔴	🔴
					PIK3CA	🟢	🟢	🟢	🟢
					PIK3CA pathway	🔴	🔴	🟢	🟢
Liquid Biopsy 	ctDNA 	↓	↑		ESR1	🔴	🟡	🟢	🟢
					PIK3CA	🟢	🟡	🟢	🟡
					PIK3CA pathway	🔴	🔴	🟡	🟡

De Angelis et al. 2024, in preparation

PD-L1 TEST IN mTNBC

PEMBROLIZUMAB



Unstained mononuclear cell
PD-L1pos mononuclear cell
Unstained tumor cell
PD-L1 pos Tumor cell

CPS

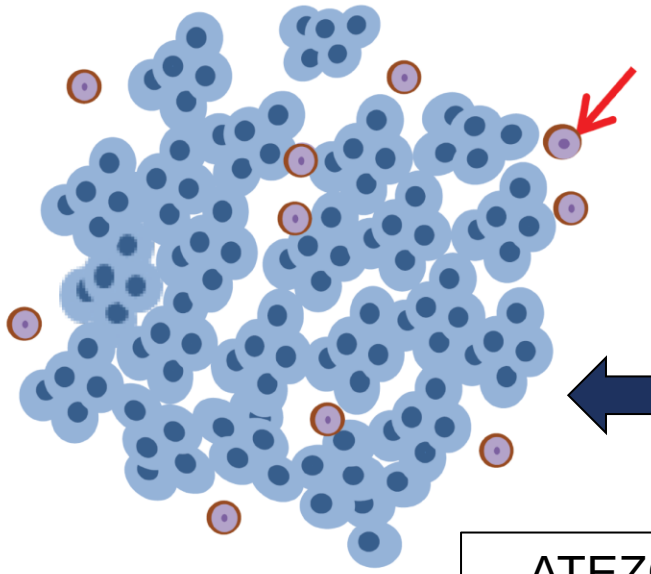
$$CPS = \frac{\text{PD- L1 staining} \left[\begin{array}{l}\text{Tumor cells}\\ \text{Lymphocytes}\\ \text{Macrophages}\end{array}\right]}{\text{viable tumor cells}} 100$$

Validated assays for CPS:
- Ventana SP263
- Dako 22-C3

TNBCs are scored and divided into:

CPS < 10 IC < 1

CPS ≥ 10 IC ≥ 1



ATEZOLIZUMAB

IC

$$IC = \frac{\text{PD- L1 staining} \left[\begin{array}{l}\text{Plasma cells}\\ \text{Lymphocytes}\\ \text{Macrophages}\end{array}\right]}{\text{tumor area}} 100$$

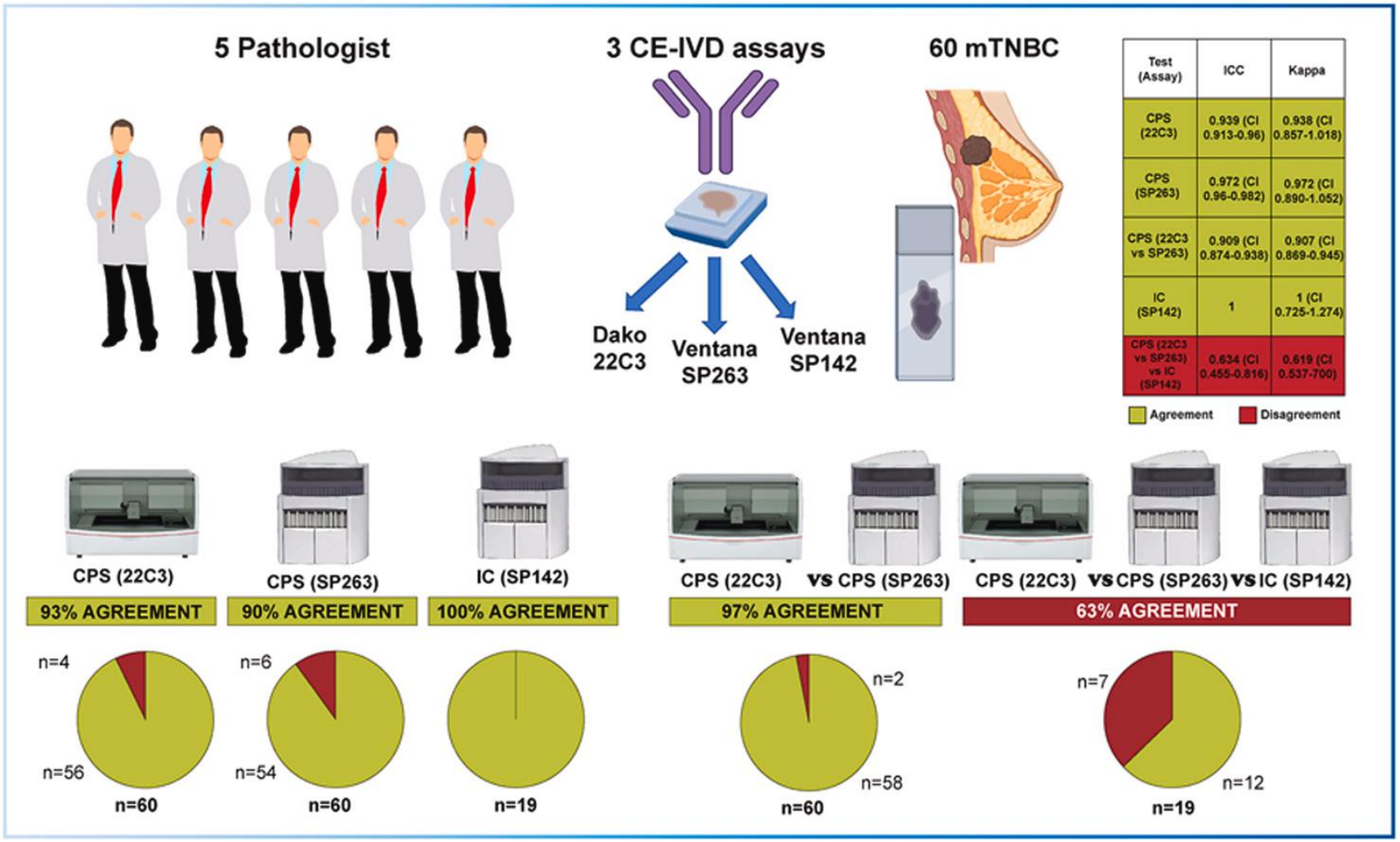
Validated assays for IC:
- Ventana SP142

ecancermedicalscience

Biomarkers for precision immunotherapy in the metastatic setting: hope or reality?

Elham Sajjadi[1,2], Konstantinos Venetis[1,2], Cristian Scatena[3] and Nicola Fusco[1,2]

PD-L1 TEST ANALYTICAL VALIDATION IN mTNBC



- In mTNBC, CPS can be reliably assessed either by 22C3 (which was used in the KEYNOTE studies) or SP263, providing the use of the dedicated platform (i.e. Dako and Ventana).
- CPS and IC are not interchangeable tests in mTNBC
- PD-L1 test in mTNBC is reproducible when assessed by specifically trained pathologists using CE-IVD assays, i.e. 22C3 and SP263 for CPS and SP142 for IC score.

Human Pathology 144 (2024) 22–27
Contents lists available at ScienceDirect
Human Pathology
journal homepage: www.elsevier.com/locate/humpath

PD-L1 testing in metastatic triple-negative breast cancer: Interobserver and interplatform reproducibility of CE-IVD assays for CPS and IC scores

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Thank you

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