

REENGINEERING THE APPROACH TO METASTATIC BREAST CANCER

A Stepwise Guide to HER2 Testing and Management for HER2-Low and -Ultralow Disease

PART 3: Mastering HER2-low and Ultralow Metastatic Breast Cancer: Precise Testing for Targeted Therapeutic Selection

Supported by educational grants from AstraZeneca Pharmaceuticals and Daiichi Sankyo, Inc.

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Project Overview

- HER2-directed therapies transformed the treatment of breast cancer (BC). Initially, this benefit was limited to ~15% of patients with clear HER2 amplification or overexpression; however, it is now recognized that patients previously classified as “HER2-negative” may still derive meaningful benefit from antibody–drug conjugates (ADCs).

- This emerging understanding challenges traditional binary HER2 classification and underscores new diagnostic and therapeutic complexities. With the recognition of HER2-low and now HER2-ultralow disease categories, oncologists, pathologists, and multidisciplinary care teams face evolving questions about testing accuracy, interpretation, and therapeutic decision-making.

PURPOSE: Provide practical guidance on the identification and interpretation of HER2-low/-ultralow metastatic BC (mBC) using real world examples of how select institutions have optimized their testing and reporting algorithms

3-PART ACTIVITY SERIES

ACTIVITY 1 – Unveiling HER2-low and -ultralow Expression in mBC: Implications for the Multidisciplinary Care Team

ACTIVITY 2 – What’s Your Protocol? Streamlining Oncology/Pathology Collaboration Throughout the BC Journey

ACTIVITY 3 – Mastering HER2-low and -ultralow mBC: Precise Testing for Targeted Therapeutic Selection

THIS ACTIVITY

HER2: Human epidermal growth factor receptor 2

MEDICAL EDUCATION RESOURCES EFFICIENT

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Disclosures

DR MEISEL

DISCLOSES THE FOLLOWING:

- **ADVISORY BOARD:**
  AstraZeneca, Olema Oncology,
  Pfizer Inc., and Sermonix Pharmaceuticals
- **RESEARCH:**
  AstraZeneca, Olema Oncology,
  Sermonix Pharmaceuticals
- **CONSULTING FEES:**
  AstraZeneca, Genetech, Inc., GSK, Novartis
  Pharmaceuticals Corporation, Olema Oncology,
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- **RESEARCH SUPPORT:**
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- **CONSULTING FEES:**
  Cancer Expert Now, LLC

Learning Objectives

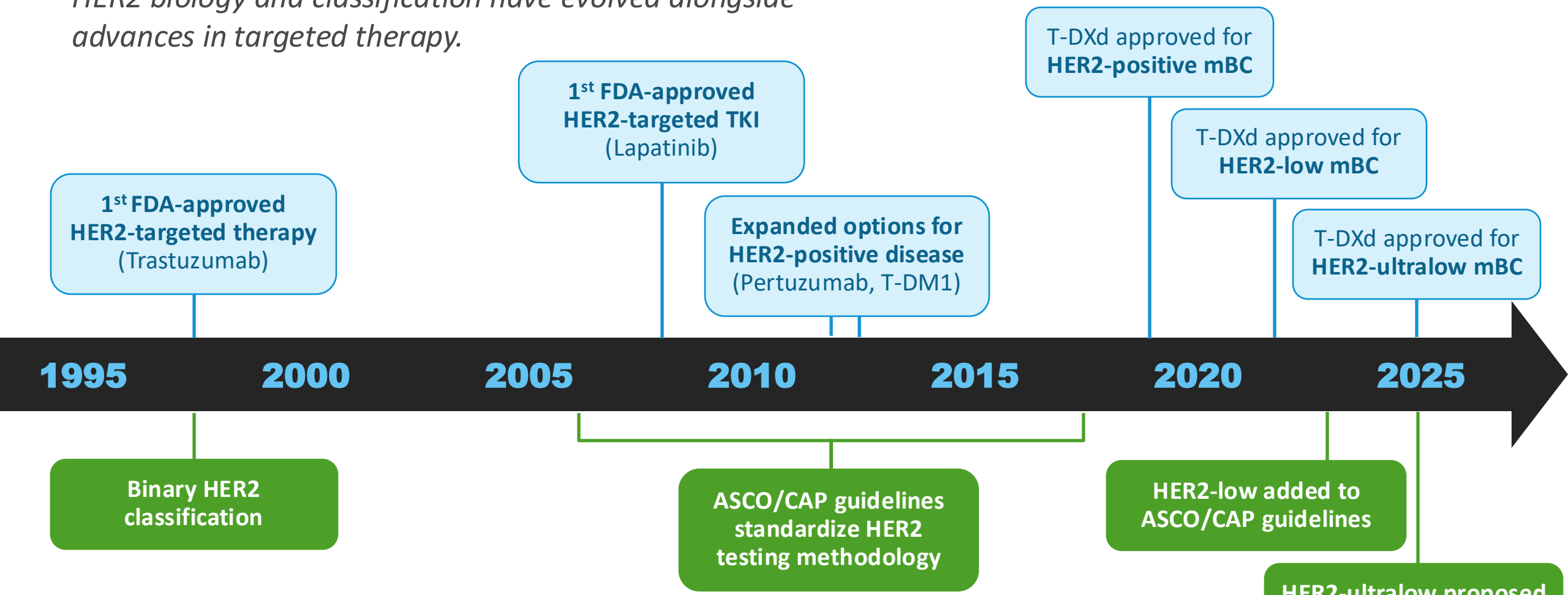
1. Apply the most up-to-date IHC scoring methodology to accurately determine HER2 status (e.g. low/ultralow vs. negative) in primary or metastatic BC specimens based on staining intensity, proportion, location, completeness, and percentage of tumor cells stained

2. Implement best practices for reporting HER2 IHC scoring results to the multidisciplinary BC care team using clinically relevant quantitative and qualitative terminology — including detailed descriptions of staining patterns — to help guide therapeutic selection

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EVOLUTION OF HER2 IN MBC

HER2 biology and classification have evolved alongside advances in targeted therapy.



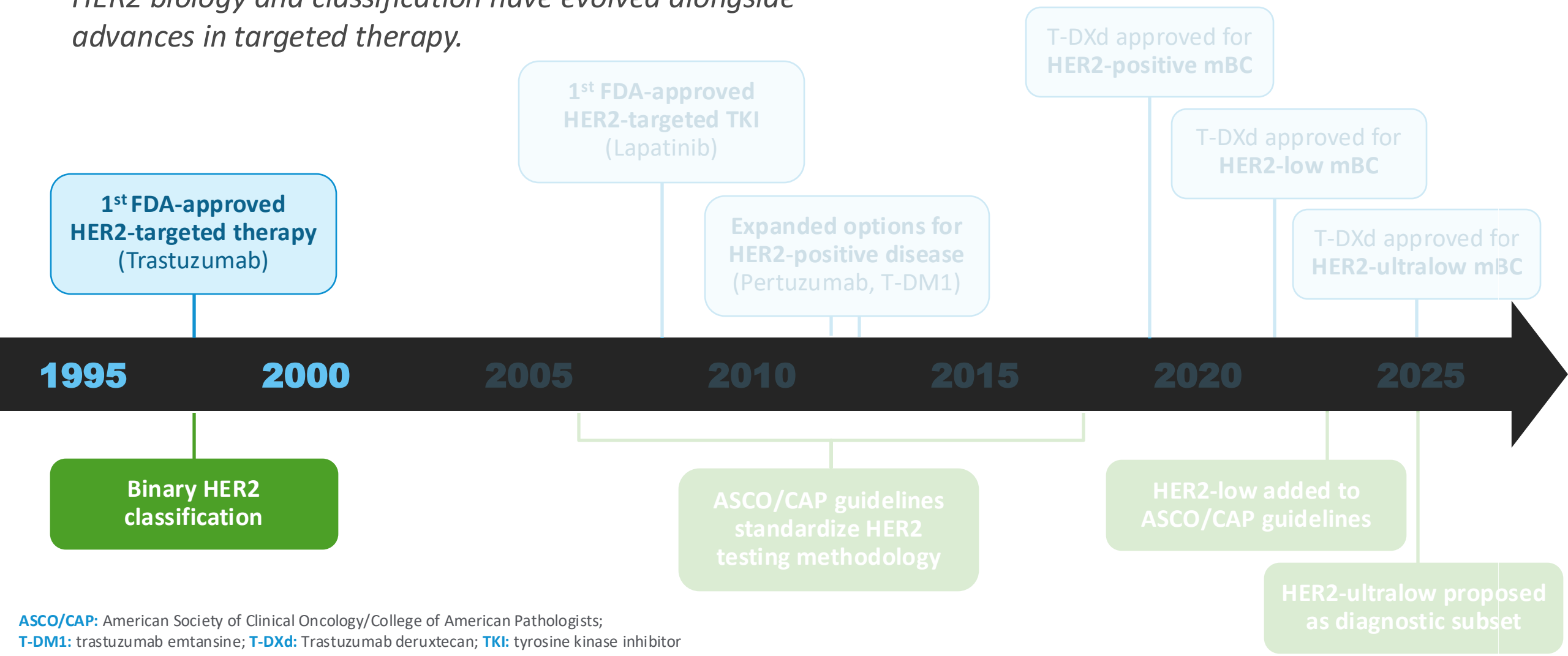
ASCO/CAP: American Society of Clinical Oncology/College of American Pathologists;
T-DM1: trastuzumab emtansine; **T-DXd:** Trastuzumab deruxtecan; **TKI:** tyrosine kinase inhibitor

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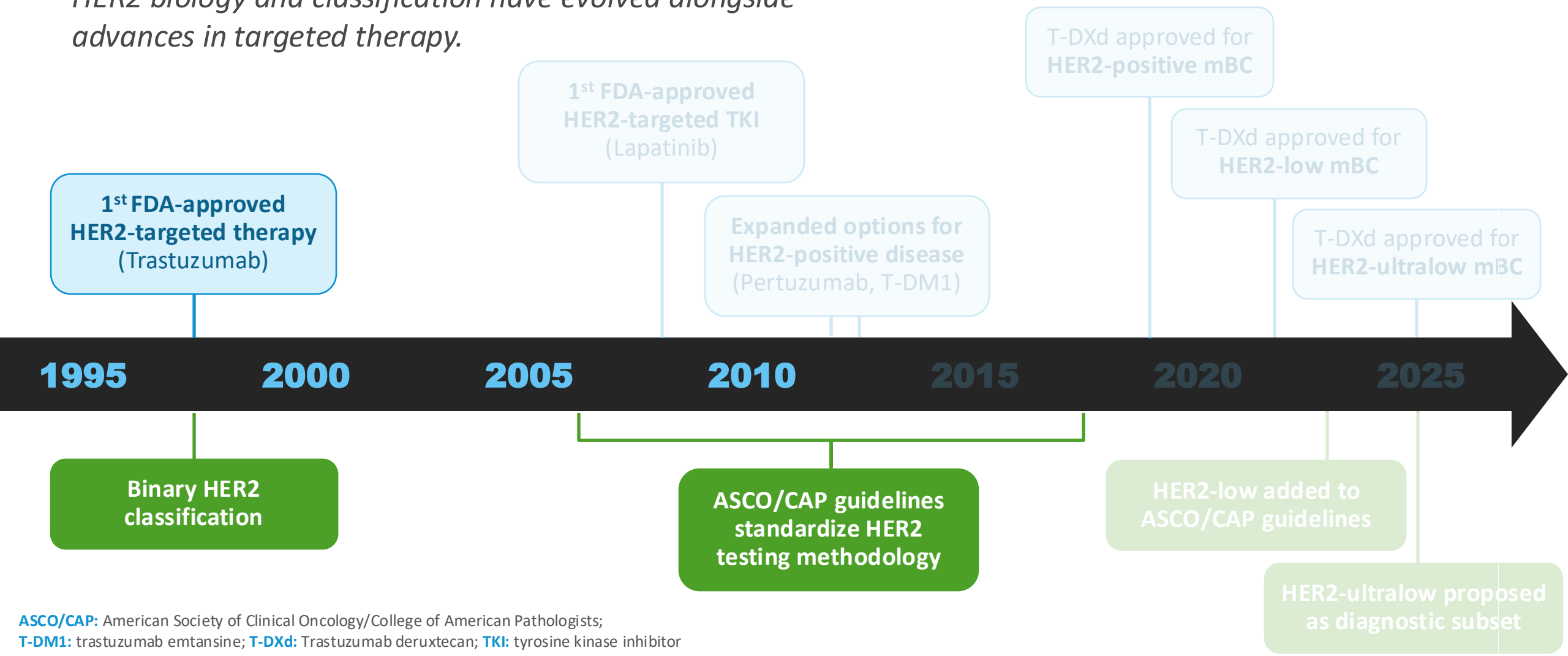
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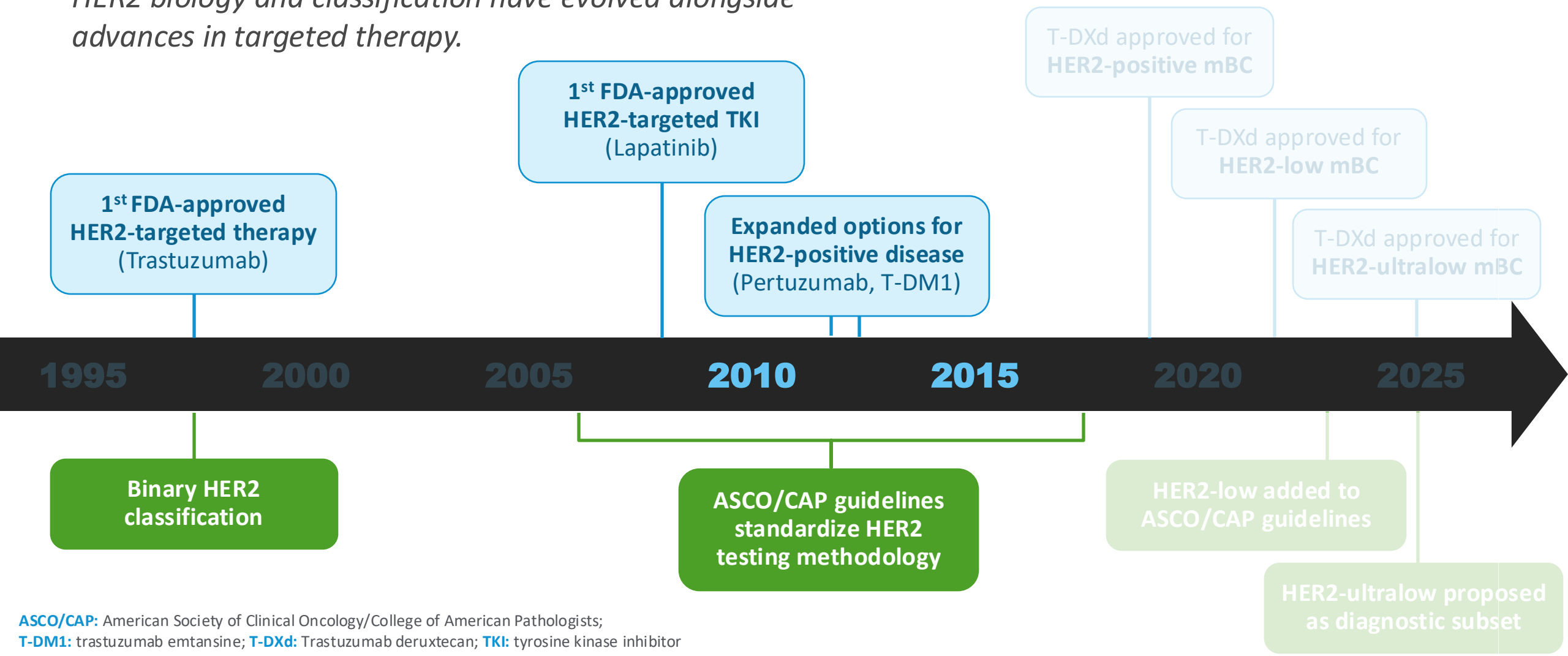
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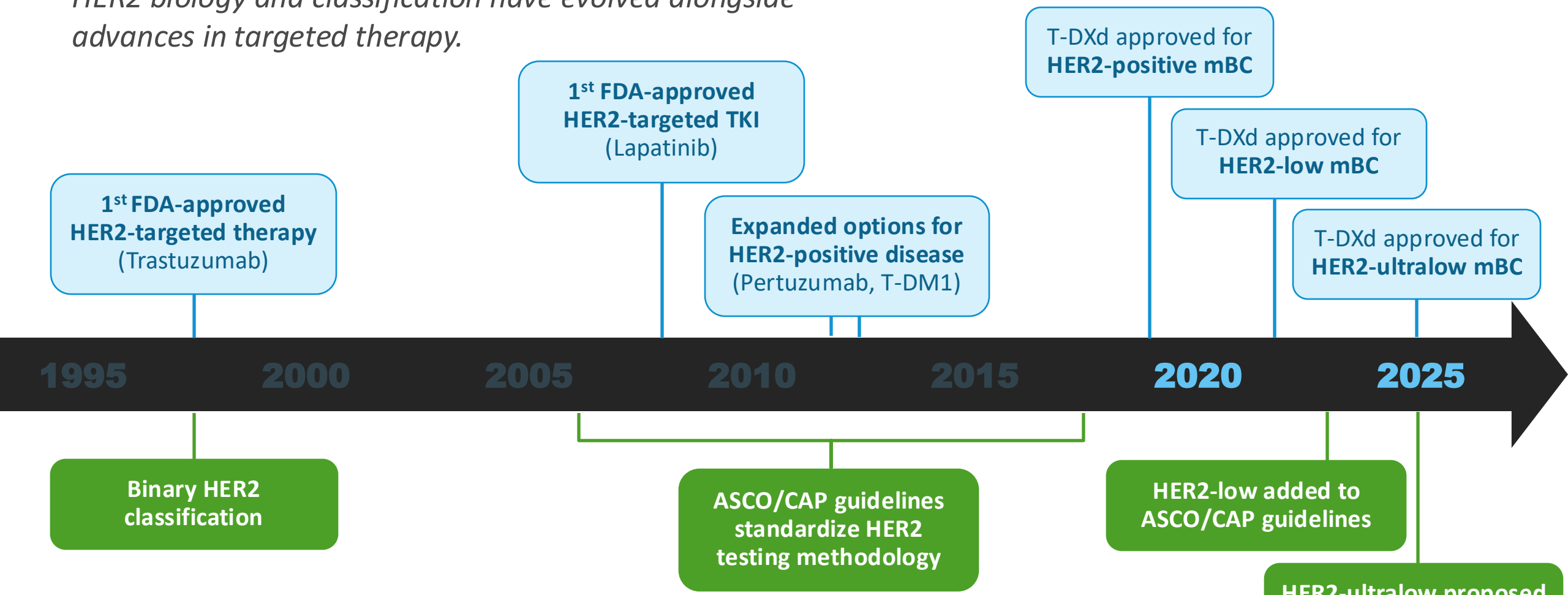
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IMPLICATIONS

WHY HER2-LOW/ ULTRALOW TESTING MATTERS IN MBC

CLINICAL CONTEXT

- Many HR+/HER2– mBC patients relapse after endocrine therapy without targeted options.
- HER2-low (IHC 1+ or 2+/ISH–) and ultralow (IHC 0 with faint staining ≤10%) categories now identify new candidates for HER2-targeted ADCs.

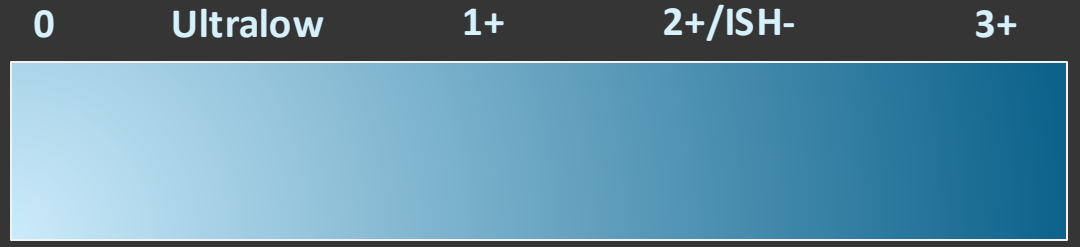
PRACTICE IMPACT

80% Up to ~80% of HR+/HER2– tumors fall into these lower-expression groups.

U.S. FDA approval of T-DXd for HER2-low (2022) and HER2-ultralow (2025) expands therapeutic eligibility.

Accurate IHC interpretation is now critical to match patients with evolving HER2-directed therapies.

HER2 EXPRESSION SPECTRUM



FDA: U.S. Food and Drug Administration; **IHC:** immunohistochemistry; **ISH:** in-situ hybridization

1. Huppert LA, et al. *CA Cancer J Clin*. 2023;73(5):480-515.
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DESTINY-BREAST06

DB-06 STUDY DESIGN

- 866 patients with HR+ mBC with HER2-low expression (IHC 1+ or 2+ and ISH negative) or ultralow HER2 expression (IHC 0) with membrane staining who received 1+ lines of ET and no previous chemotherapy
- Randomized 1:1 to T-DXd or physician's choice of chemotherapy

	T-DXd	CT	*P* value/HR
	HER2-LOW		
Median PFS (mo)	13.2	8.1	***P* <0.001**
12 mo OS (%)	87.6	81.7	**HR 0.83**
	HER2-ULTRALOW		
Median PFS (mo)	13.2	8.3	**HR 0.78**
12 mo OS (%)	84.0	78.7	**HR 0.75**

HER2-low and ultralow testing now directly influences treatment decisions.
These evolving thresholds also introduce new challenges in staining, scoring, and reporting accuracy.

CT: chemotherapy; **ET:** endocrine therapy; **OS:** overall survival; **PFS:** progression-free survival

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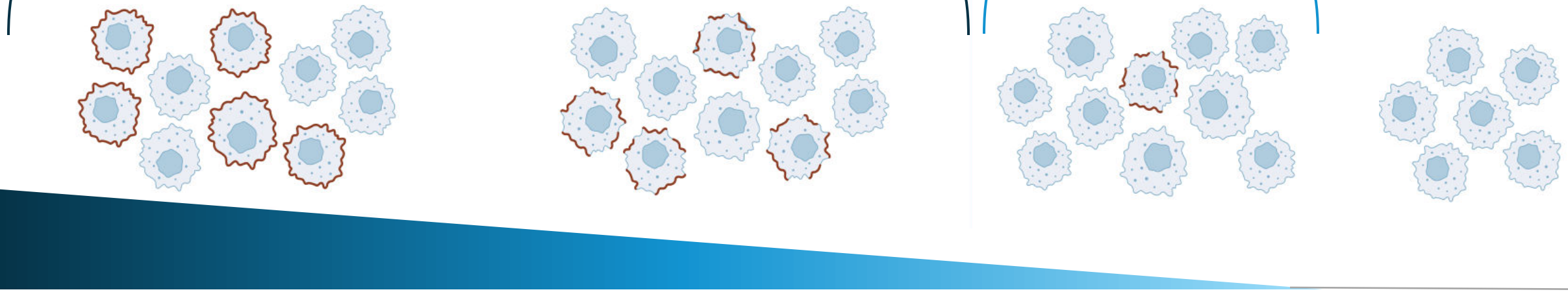
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DEFINING HER2-LOW AND -ULTRALOW IN mBC

MULTICENTER RESCORED COHORT

HER2-LOW
(~55% of tumors)

HER2-ULTRALOW
(~11% of tumors)



ICH 2+/ISH–
Weak-to-moderate complete membrane staining, >10% tumor cells

ICH 1+
Faint, incomplete membrane staining, >10% tumor cells

Faint, incomplete membrane staining, ***≤10% tumor cells***

ICH 0
Absent/ no observable staining

*IHC **2+ requires reflex ISH** (2+/ISH+ = HER2-positive)*

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IHC: Immunohistochemistry; **ISH:** in situ hybridization
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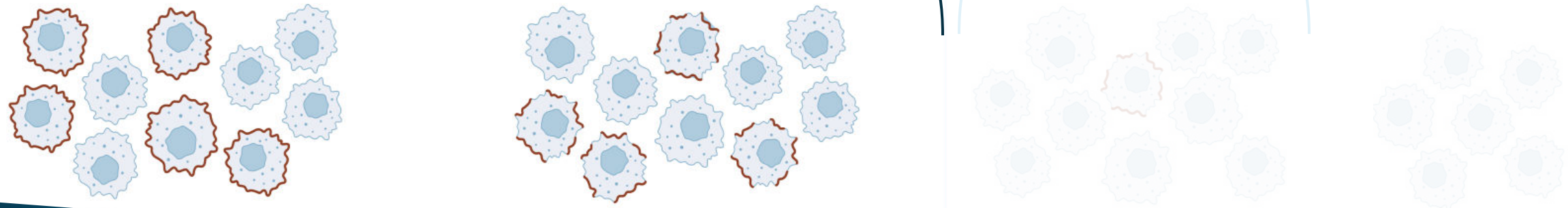
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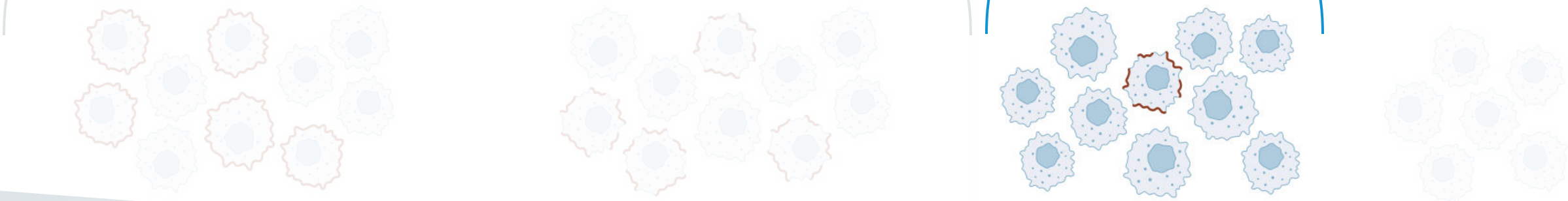
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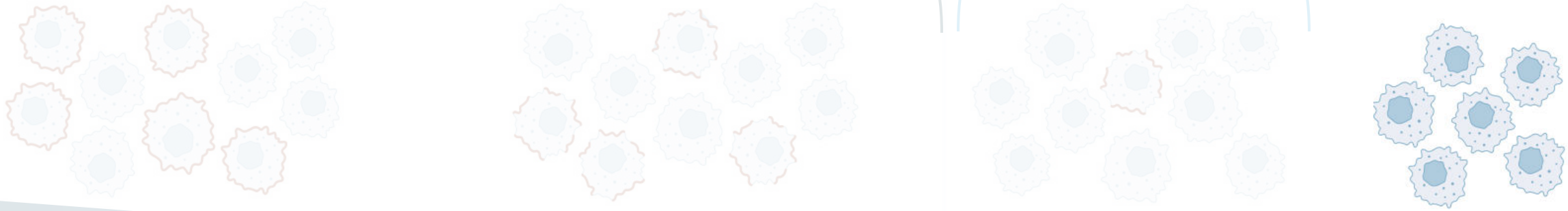
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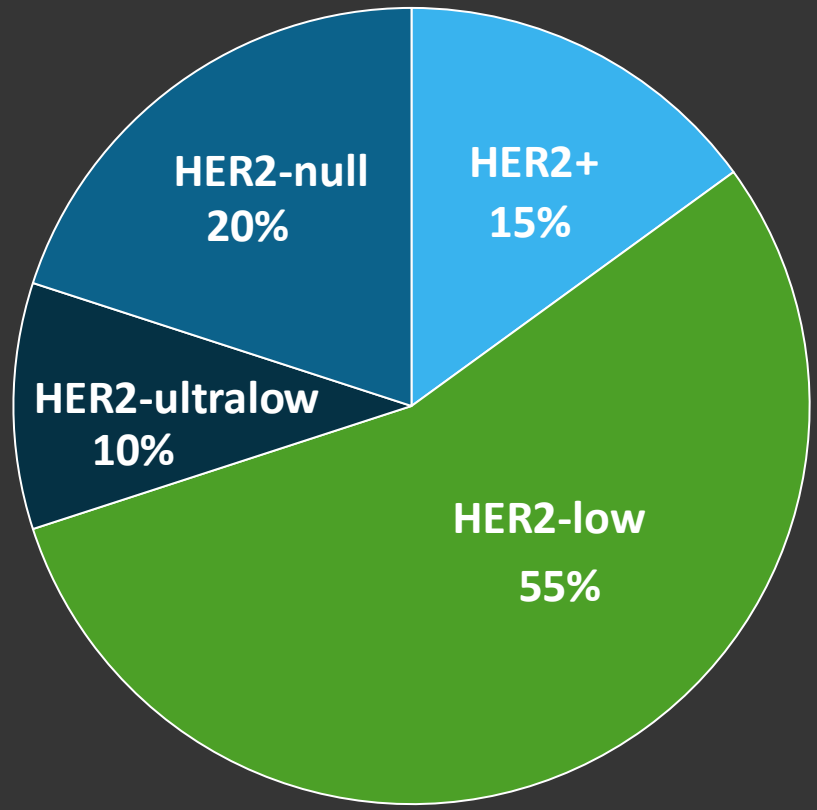
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HER2(ULTRA)-LOW BC
EPIDEMIOLOGY & CLINICOPATHOLOGY

- Most traditionally “HER2-negative” BCs now fall within the HER2-low or HER2-ultralow spectrum
  - *Most of these patients may be eligible for HER2-targeted therapy*
- Most HER2-low tumors occur in HR^{+} disease

While the prognostic significance of HER2-low is unclear, the therapeutic implications emphasize the importance of accurate testing and classification.

APPROXIMATE DISTRIBUTION OF HER2 EXPRESSION IN BC



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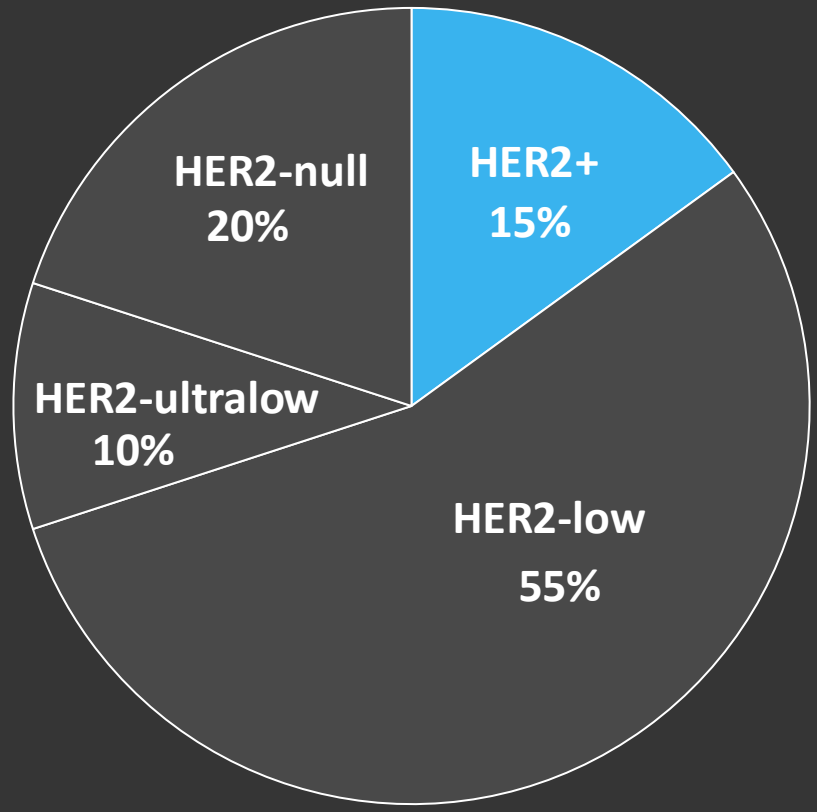
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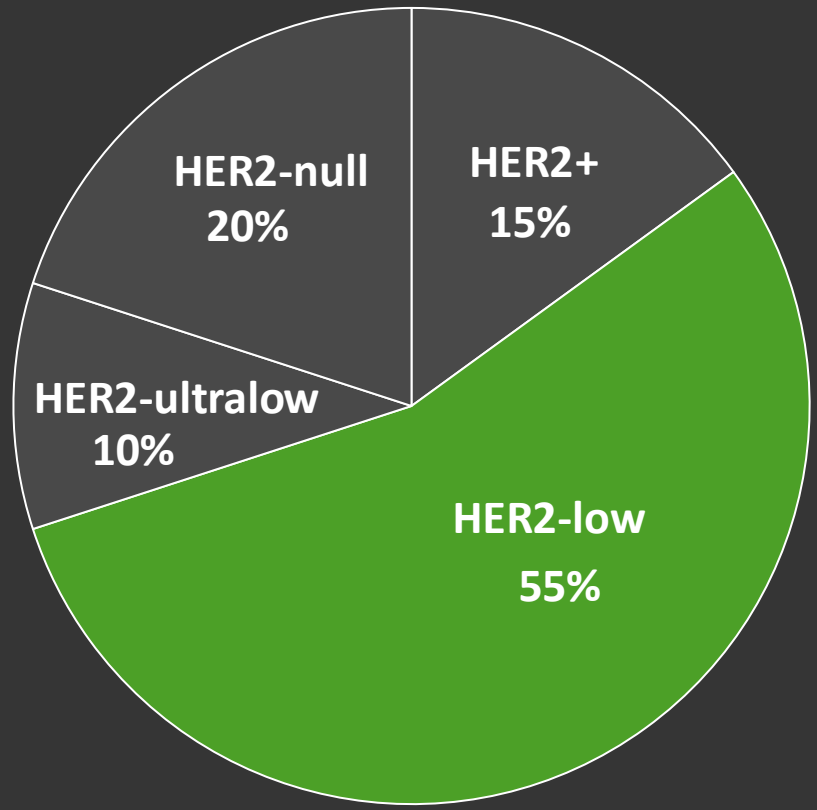
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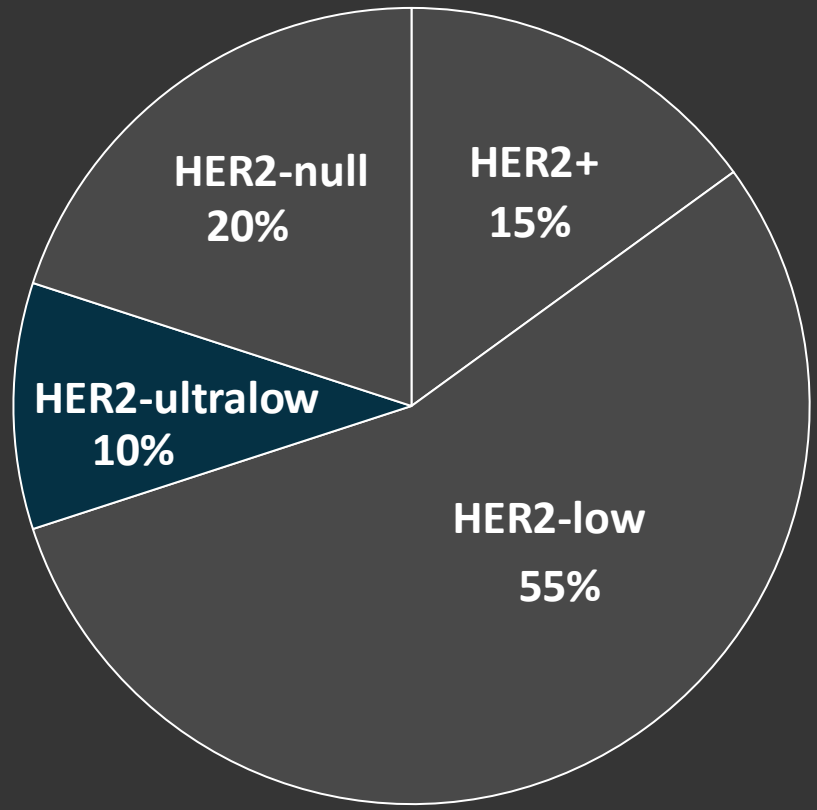
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6. Park WK, et al. *Cancers.* 2024;16(14):2566.

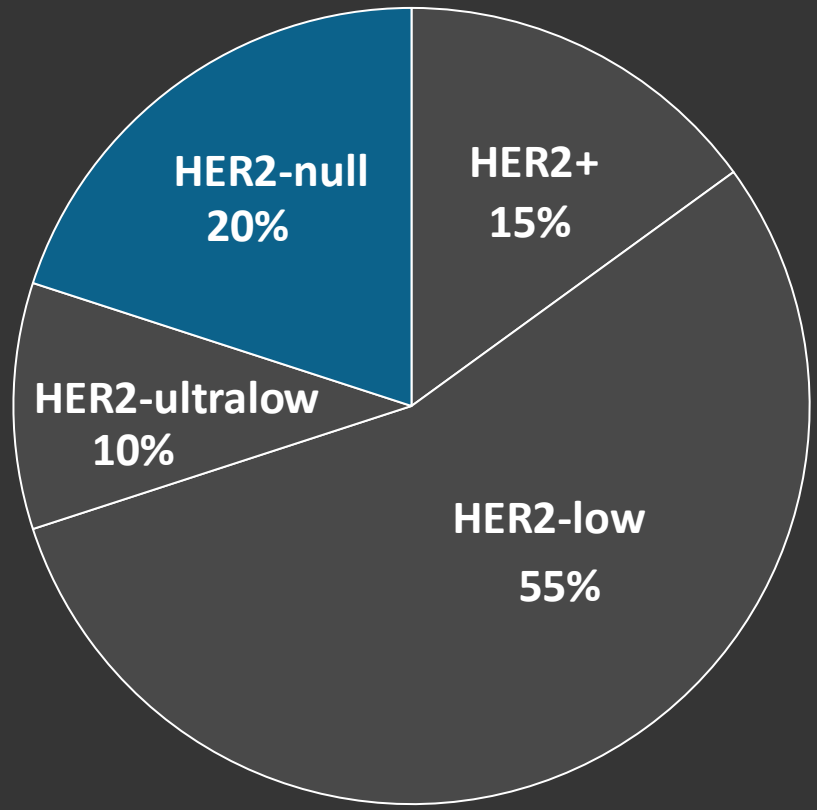
EFFICIENT

HER2(ULTRA)-LOW BC
EPIDEMIOLOGY & CLINICOPATHOLOGY

- Most traditionally “HER2-negative” BCs now fall within the HER2-low or HER2-ultralow spectrum
  - *Most of these patients may be eligible for HER2-targeted therapy*
- Most HER2-low tumors occur in HR^{+} disease

While the prognostic significance of HER2-low is unclear, the therapeutic implications emphasize the importance of accurate testing and classification.

APPROXIMATE DISTRIBUTION OF HER2 EXPRESSION IN BC



1. Zhang H, et al. *Mod Pathol.* 2022;35(8):1075-1082. **2.** Lv H, et al. *Breast Cancer Res.* 2025;27(1):45.
3. National Cancer Institute (NCI). NCI Website. 2023. https://seer.cancer.gov/statfacts/html/breast-subtypes.html.
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BARRIERS TO ACCURATE TESTING & REPORTING

HER2-LOW/ULTRALOW MBC

BARRIER	IMPLICATION
Reproducibility of IHC 0 vs 1+	High interobserver variability; frequent misclassification[1,2]
Inconsistent HER2 categories across time/sites	Reclassification is difficult; older reports may not align with current HER2-low definitions[3]
Reporting & EHR access issues	HER2 status often missing/indeterminate in EHR; delays in treatment decisions[4]
Sensitivity of current testing	IHC may not detect low-level HER2; requires more precise or quantitative methods[5]
Need for advanced tools	AI/digital pathology could reduce variability and enhance accuracy, but not widely implemented[6]

AI: artificial intelligence; **EHR:** electronic health records

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HER2 ANALYSIS

STAINING TECHNIQUE
(ASCO/CAP)

SPECIMEN PREPARATION
Fixation, sectioning, antigen retrieval

PRIMARY ANTIBODY BINDING
HER2-specific antibody

SECONDARY ANTIBODY & DETECTION
Chromogen visualization

COUNTERSTAINING & INTERPRETATION
Scoring: 0, 1+, 2+, 3+

CHALLENGES

STAINING PROCESS/INTERPRETATION

PRE-ANALYTICAL VARIABILITY
Fixation, tissue processing

TECHNICAL ARTIFACTS
Background, edge effects

SCORING SUBJECTIVITY
0 vs 1+ variability

BORDERLINE/EQUIVOCAL CASES
2+ reflex ISH testing

HETEROGENEITY
Patchy/basolateral staining

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WHICH IHC ANTIBODY/ SYSTEM TO USE FOR HER2

BACKGROUND

- Commercial HER2 IHC assays (e.g., PATHWAY 4B5, HercepTest) can yield variable staining intensity and classification results.
- Differences in antibody clone, detection chemistry, and platform automation may influence HER2 0, 1+, and 2+ interpretation.

COMPARISON OF HER2 SCORINGS FROM INDICATED IHC ASSAYS

		PATHWAY 4B5				
		0	1+	2+	3+	Total
Hercep Test (mAb)	0	35	0	0	0	35
	1+	17	8	0	0	25
	2+	4	12	13	1	30
	3+	0	0	2	27	29
	Total	56	20	15	28	119

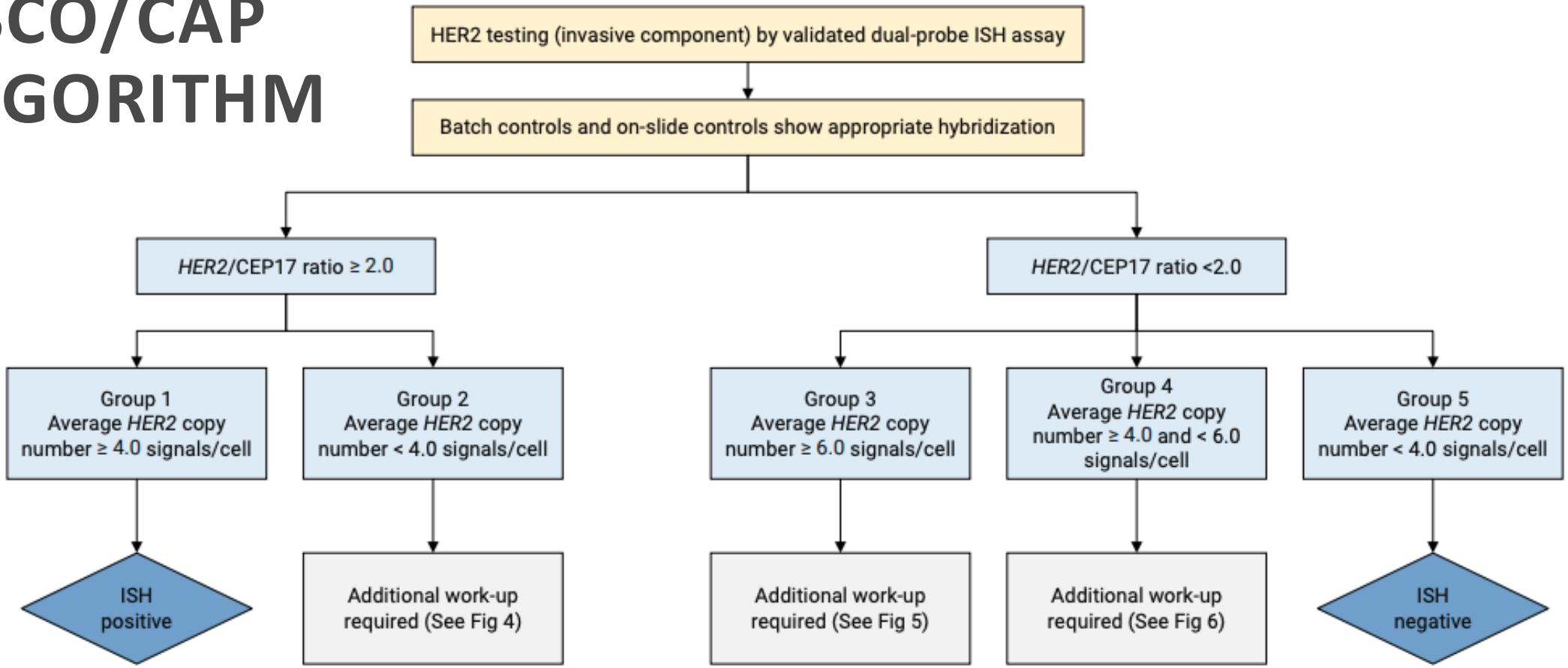
KEY FINDINGS

- **55% of HercepTest 2+ cases (16 of 29) were reclassified as 0 or 1+ by PATHWAY 4B5.**
- PATHWAY 4B5 missed two FISH-positive tumors scored 1+ vs 2+ with HercepTest.
- Implication: assay choice can shift HER2-low/ultralow classification and affect reflex ISH rates.

FISH: fluorescence in situ hybridization

Rüschoff J, et al. *Virchows Arch*. 2022;481(5):685-694.

ASCO/CAP ALGORITHM



COMMON STAINING PITFALLS:

HIGH BACKGROUND CYTOPLASMIC STAINING • CRUSHED ARTIFACTS • WEAK STAINING INTENSITY • GRANULAR STAINING

Even with clear definitions, interpreting faint or incomplete staining can be challenging in daily practice.

1. Wolff AC, et al. *Arch Pathol Lab Med.* 2023;147(9): 993–1000. **2.** Marabi M, et al. *Hum Pathol.* 2025;162:105818.

EFFICIENT



FACULTY DISCUSSION

Does true HER2-0 breast cancer really exist, or do current assays fail to detect ultra-low expression that may still respond to T-DXd?

EFFICIENT

CASE 1: DISCORDANT IHC RESULTS

HISTORY & PHYSICAL

Pathology Findings

Digital Specimens



56-year-old woman with HR+/HER2– mBC

- New liver lesions identified on imaging, consistent with progression
- Original diagnosis (4 years prior): invasive ductal carcinoma (IDC), HER2 IHC 0

PRIOR TREATMENT: surgery, adjuvant endocrine therapy, and chemotherapy after recurrence

Liver biopsy performed for confirmation and HER2 re-evaluation

CASE 1: DISCORDANT IHC RESULTS

History & Physical

PATHOLOGY FINDINGS

Digital Specimens



Primary breast tumor (archival core biopsy):
Reported as HER2 IHC 0

New metastatic liver biopsy (current specimen):
IHC shows **faint, incomplete membrane staining in ~15% of tumor cells**

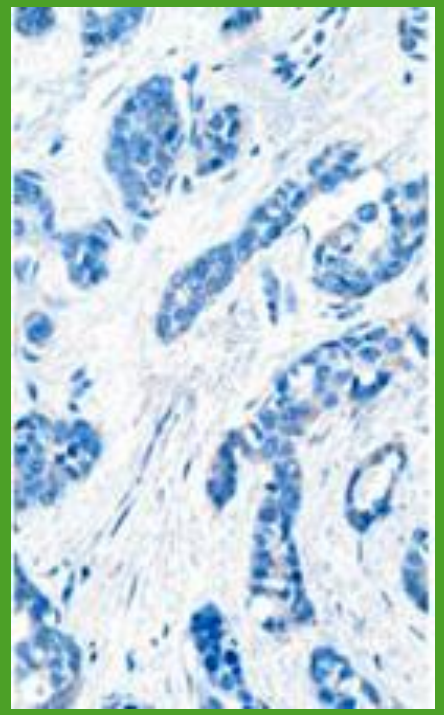
EFFICIENT

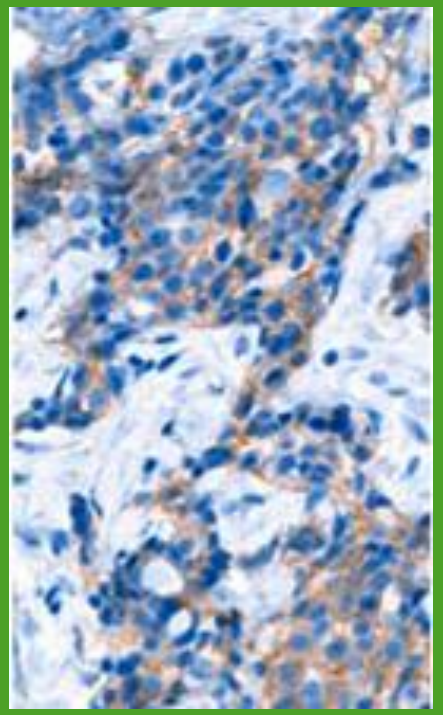
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History & Physical

Pathology Findings

DIGITAL SPECIMENS





WHAT EXPLAINS THE DISCORDANCE BETWEEN SAMPLES?

Sajjadi E, et al. *Front Mol Biosci*. 2023;10:1176309.

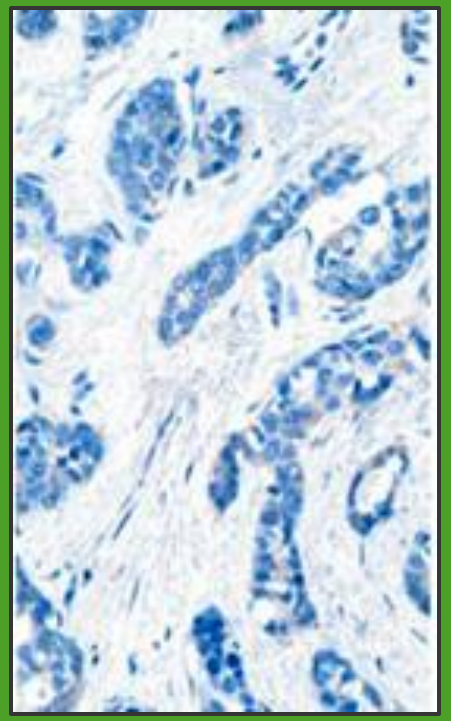
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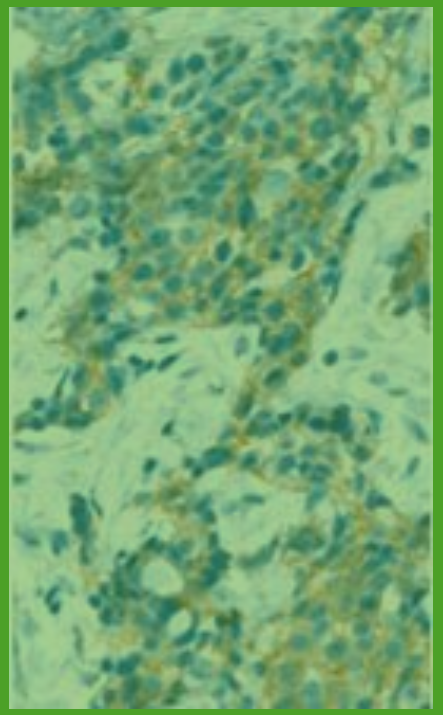
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EFFICIENT

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History & Physical

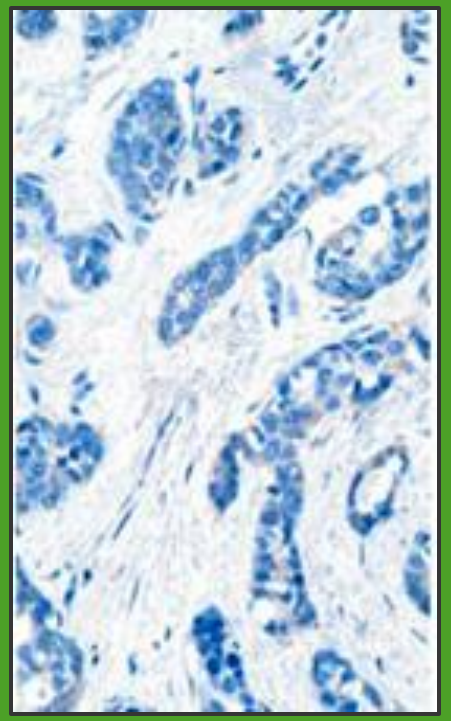
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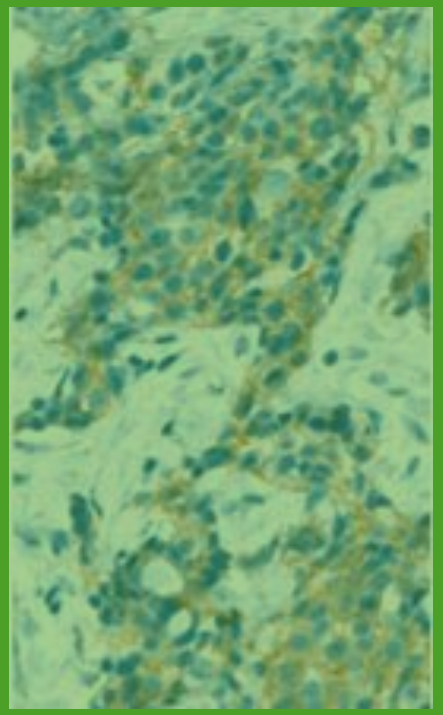
DIGITAL SPECIMENS

SCORE

0

(ultra-low)





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EFFICIENT

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History & Physical

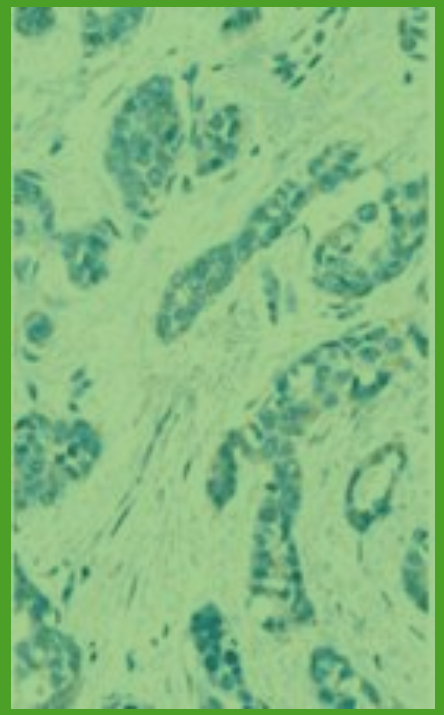
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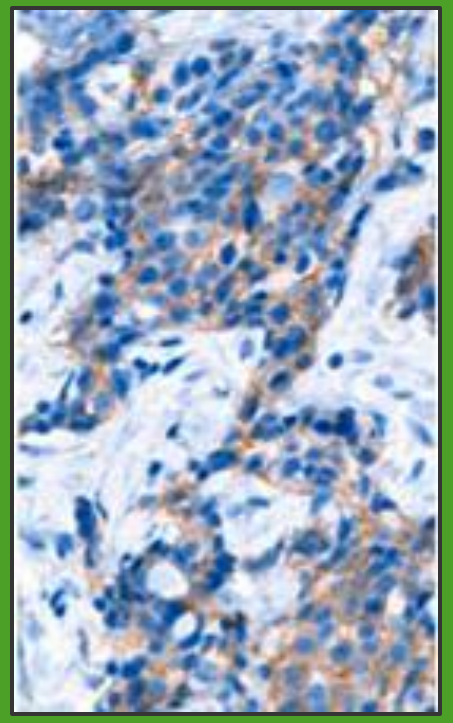
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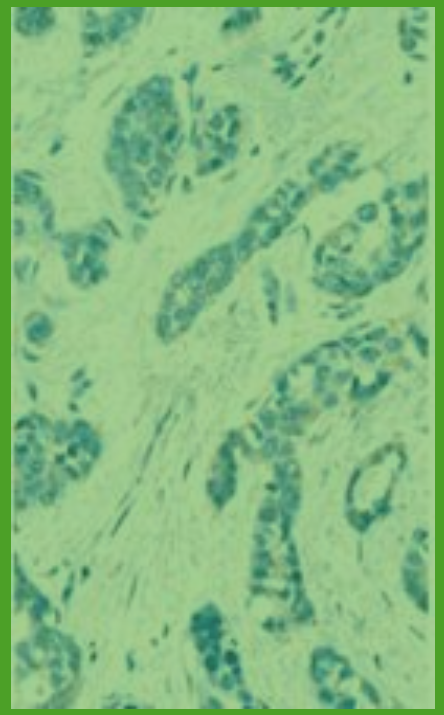
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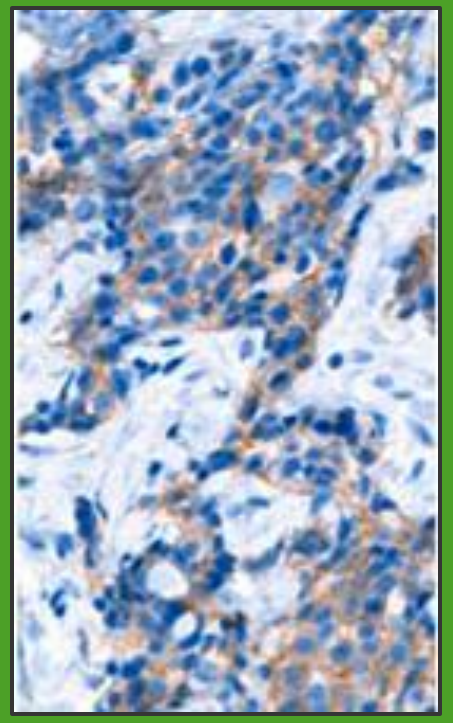
DIGITAL SPECIMENS

SCORE

0

(ultra-low)





SCORE

1+

(low)

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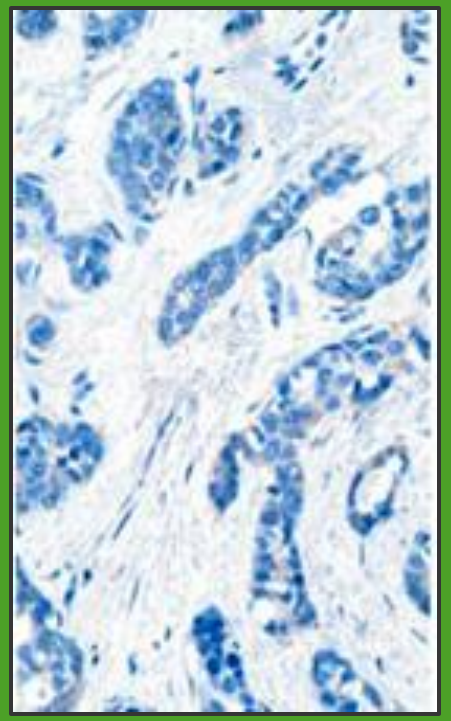
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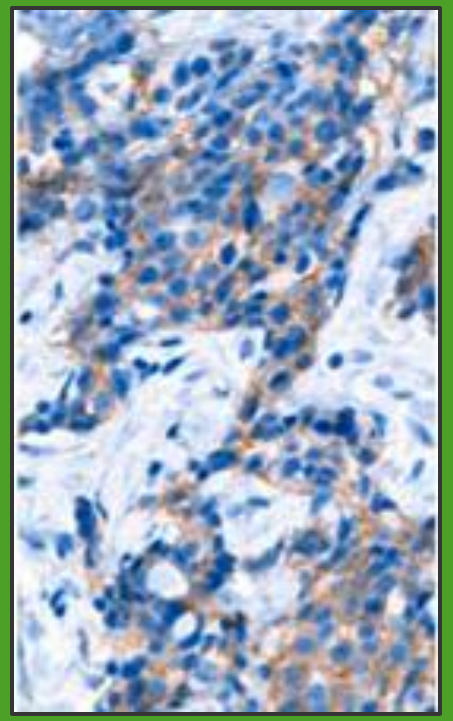
History & Physical

Pathology Findings

DIGITAL SPECIMENS

SCORE
0
(ultra-low)





SCORE
1+
(low)

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DISCORDANT HER2 STAINING
IMPLICATIONS

CLINICAL CHALLENGE

HER2 expression can differ between primary and metastatic sites or among tumor areas, creating discordant results.

IMPACT ON CARE

Discordance may alter eligibility for HER2-targeted ADCs, particularly in HER2-low or ultralow disease.

PATHOLOGIST'S ROLE

Ensure standardized scoring, clear reporting of which specimen was tested, and transparent communication with the oncology team.

ASCO/CAP guidelines call for repeat testing on metastatic lesions when feasible and reflex ISH for equivocal (IHC 2+) cases.

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FACULTY DISCUSSION

When scores differ between primary and metastatic samples, how do you communicate that with the oncologist? Which sample do you prioritize?

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REPORTING BEST PRACTICES
CAP GUIDANCE

1. **HER2 IHC 1+ or 0 results still interpreted as HER2-negative**
2. **Semiquantitative IHC score must always be reported**
3. **Context footnote required for HER2 testing reports**

DOCUMENTATION EXAMPLES

HER2-negative for protein overexpression (1+ staining present)

…Patients with BCs that are HER2 IHC 1+ or IHC 2+/ISH not-amplified may be eligible for a treatment that targets non-amplified/non-overexpressed levels of HER2 expression for cytotoxic drug delivery (IHC 0 results do not result in eligibility currently).

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CASE 2: INTRA-TUMOR STAINING HETEROGENEITY

HISTORY & PHYSICAL

Pathology Findings

Digital Specimen



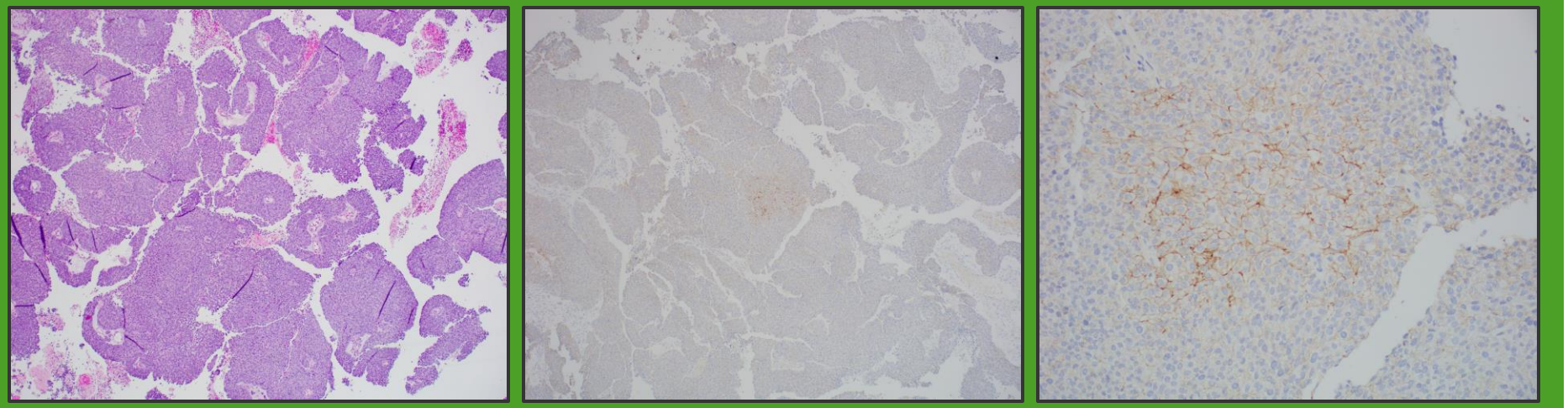
- 62-year-old woman with HR+/HER2– IDC
- Underwent lumpectomy and adjuvant endocrine therapy 3 years ago
- Presents with biopsy-confirmed local recurrence in the breast
- HER2 testing performed on recurrent specimen to reassess expression pattern

CASE 2: INTRA-TUMOR STAINING HETEROGENEITY

History & Physical

Pathology Findings

DIGITAL SPECIMENS



IHC RESULTS:

90% of invasive tumor cells: faint, incomplete membrane staining (IHC 1+)

10%: weak, complete membrane staining (IHC 2+)

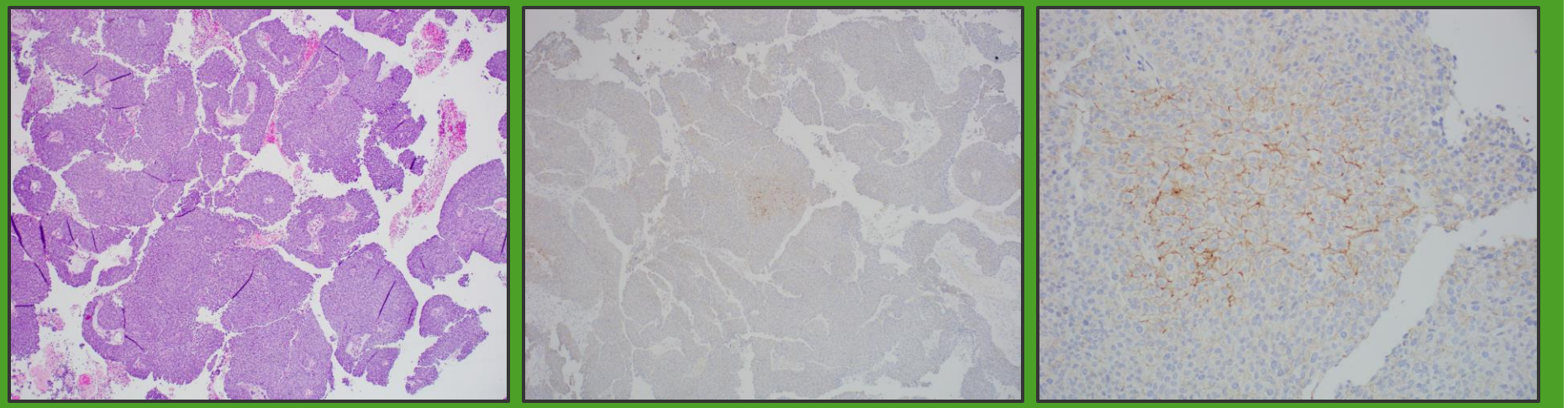
Images courtesy of Dr. Xiaoxian (Bill) Li

EFFICIENT

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RECOMMENDATION: REFLEX FISH TO EXAMINE HER2 AMPLIFICATION

Images courtesy of Dr. Xiaoxian (Bill) Li

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INTRA-TUMOR STAINING HETEROGENEITY

IMPLICATIONS

CLINICAL CHALLENGE

HER2 expression may vary across regions, leading to inconsistent IHC scoring.

Suboptimal intra-observer agreement distinguishing IHC 0 vs 1+.

IMPACT ON CARE

Can blur distinctions between HER2-low and ultralow, affecting treatment eligibility and reproducibility across laboratories.

PATHOLOGIST'S ROLE

Clearly document heterogeneous staining patterns in reports.

When feasible, evaluate multiple blocks or areas to capture variability.

Intra-tumor heterogeneity highlights the need for standardized scoring methods and, where possible, objective digital tools to support consistency.

1. Tarantino P, et al. *J Clin Oncol.* 2020;38(17):1951-1962. **2.** Liu Y. *Transl Breast Cancer Res.* 2023;30:4:15. **3.** Wolff AC, et al. *J Clin Oncol.* 2023;41(22):3867-3872.
4. Schettini F, et al. *Breast.* 2021;59:339-350. **5.** Fernandez AI, et al. *JAMA Oncol.* 2022;8(4):1-4.

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INTRA-TUMOR STAINING HETEROGENEITY

IMPLICATIONS

CLINICAL CHALLENGE

HER2 expression may vary across regions, leading to inconsistent IHC scoring.

Suboptimal intra-observer agreement distinguishing IHC 0 vs 1+.

IMPACT ON CARE

Can blur distinctions between HER2-low and ultralow, affecting treatment eligibility and reproducibility across laboratories.

PATHOLOGIST'S ROLE

Clearly document heterogeneous staining patterns in reports.

When feasible, evaluate multiple blocks or areas to capture variability.

Intra-tumor heterogeneity highlights the need for standardized scoring methods and, where possible, objective digital tools to support consistency.

1. Tarantino P, et al. *J Clin Oncol.* 2020;38(17):1951-1962. **2.** Liu Y. *Transl Breast Cancer Res.* 2023;30:4:15. **3.** Wolff AC, et al. *J Clin Oncol.* 2023;41(22):3867-3872.
4. Schettini F, et al. *Breast.* 2021;59:339-350. **5.** Fernandez AI, et al. *JAMA Oncol.* 2022;8(4):1-4.

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FACULTY DISCUSSION

When HER2 staining is heterogenous within a sample, how do you communicate that with the oncologist?

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PRACTICAL PROTOCOL

MODIFIED KI-67 METHOD

STEP 1: WHOLE SLIDE

Evaluate the **whole slide at 10x** to identify IHC staining heterogeneity

1

STEP 2: REGIONS, HIGH POWER FIELD

Evaluate each region at **high power (40x)** and estimate the percentage of membranous staining using **visual assessment** (eyeballing)

2

STEP 3: GLOBAL %

Calculate the overall (global) HER2 membranous staining across all regions

3

Courtesy of Dr. Xiaoxian (Bill) Li

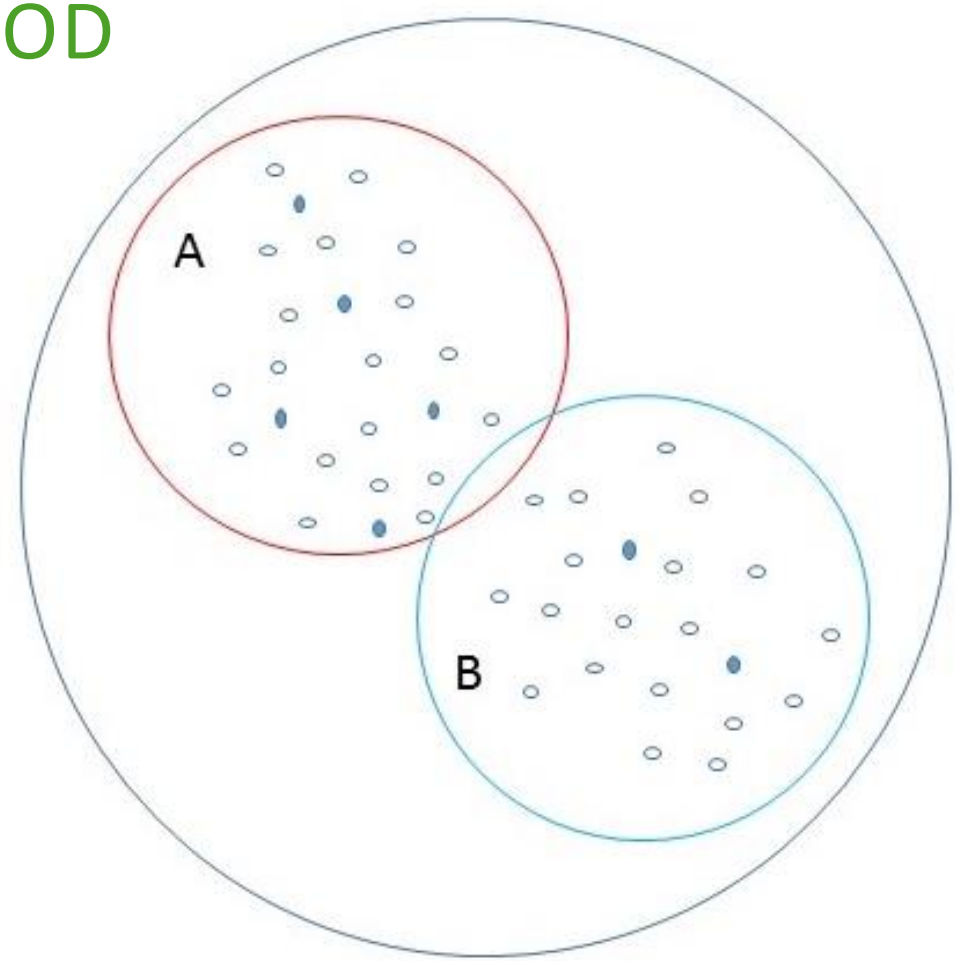
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CALCULATING GLOBAL HER2 STAINING

APPLYING THE MODIFIED KI-67 METHOD

	PERCENT POSITIVE CELLS		PERCENT TOTAL TUMOR AREA		PRODUCT
REGION A	20%	X	50%	=	10%
REGION B	10%	X	50%	=	5%

Global percentage
of HER2 membranous staining:
10% + **5%** = **15%**



Weighted average reflects overall HER2 expression across heterogeneous tumor regions

Courtesy of Dr. Xiaoxian (Bill) Li

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FACULTY DISCUSSION

How might digital or AI-assisted tools support consistency and quality assurance in HER2 IHC interpretation?



KEY TAKEAWAYS

- Accurate identification of HER2-low/ultralow disease requires careful **standardized interpretation of IHC**.
- **Discordance** between primary and metastatic lesions should be clearly documented as it can directly impact treatment decisions.
- **Intra-tumor heterogeneity** can complicate HER2 scoring and may warrant additional testing.
- **ASCO/CAP guidelines** provide a structured framework for reflex ISH testing, reporting and communication with oncology teams.
- **Digital and AI-assisted tools** can further enhance scoring consistency and support reproducibility across laboratories.

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