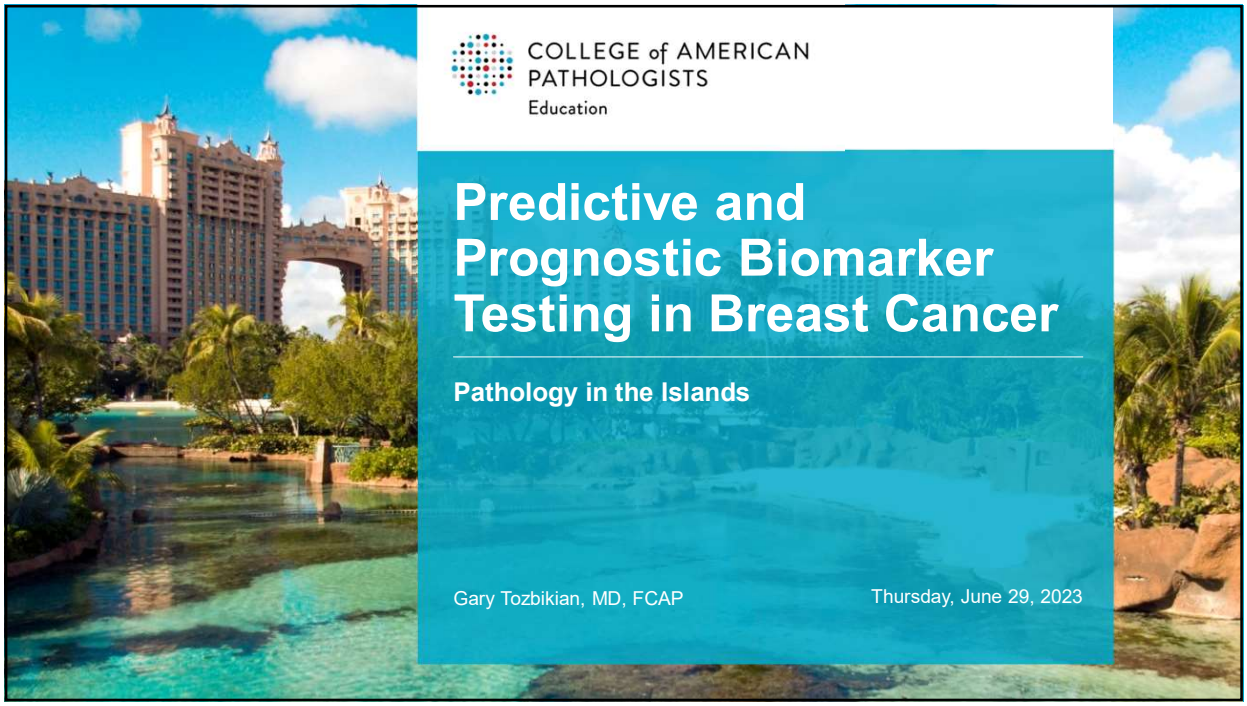
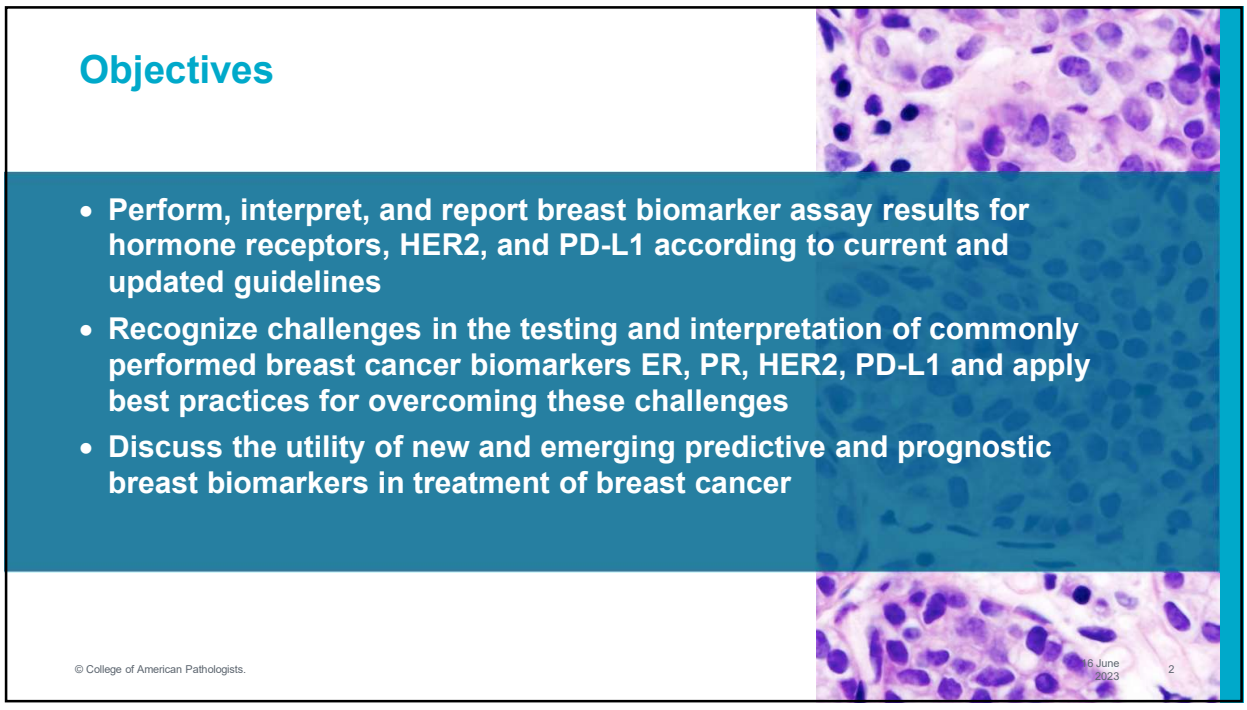


1



2

Outline

- Standard Breast Biomarkers (ER/PR/HER2)
  - ER-low breast cancer
  - HER2-low breast cancer
- Immunohistochemical (IHC) Companion Diagnostic Assays
  - PD-L1
  - Ki-67
  - MMR
- Single-gene, Multi-gene, and Next Generation Sequencing (NGS) assays
  - PIK3CA mutations
  - ESR1 mutations
  - NTRK fusions
  - Oncotype DX, Mammaprint/Blueprint, Biotheranostics Breast Cancer Index (BCI), Foundation One

3

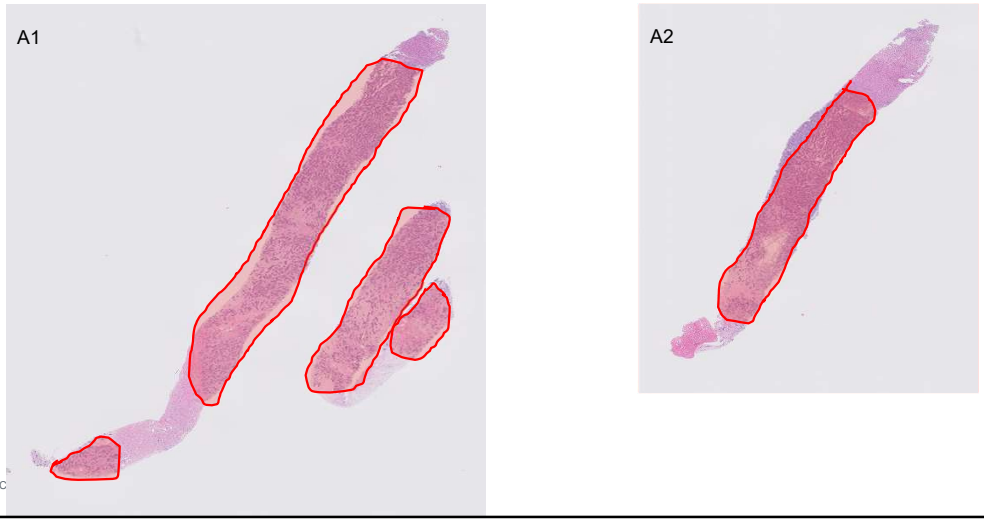
Surgical pathologists play an key role in ordering and interpreting biomarker tests

- Understand each assay's specific indications, specimen requirements, and technical pitfalls
- Select the correct specimen/tissue block for testing, considering tumor cellularity and tissue adequacy
- Act as a tissue steward, balancing and anticipating potential needs for other ancillary testing (e.g. molecular)

4

Example: Liver Bx with metastatic BC

Which block should be selected for biomarker testing: Block A1 or A2?



5

Surgical pathologists play an key role in ordering and interpreting biomarker tests

- **Provide accurate interpretation of assay results**
- **Quality control, flagging technical factors that impact the validity of the results (e.g. prolonged cold ischemic time)**
- **Report results in accordance with guideline recommendations**
- **Communicate and coordinate with broader clinical team (e.g. QNS, discordant, indeterminate results)**

16 June 2023 6

6

Estrogen Receptor (ER) and Progesterone Receptor (PR)

7

Estrogen Receptor-α (ER)

- **Function and biology:**
  - Part of the steroid receptor family, is a ligand dependent transcription factor, cell cycle regulator
  - Activated by hormone estradiol, after binding translocates to the nucleus and activates transcription of genes that stimulate growth of breast epithelial cells
- **Overview of clinical usage:**
  - ER is assessed by IHC, which is sensitive, specific, and inexpensive
  - Positivity defined as ≥1% nuclear positivity
  - Provides both predictive and prognostic information
  - Mandatory and routinely assessed on all primary invasive cancers and DCIS, and recurrences/metastatic lesions

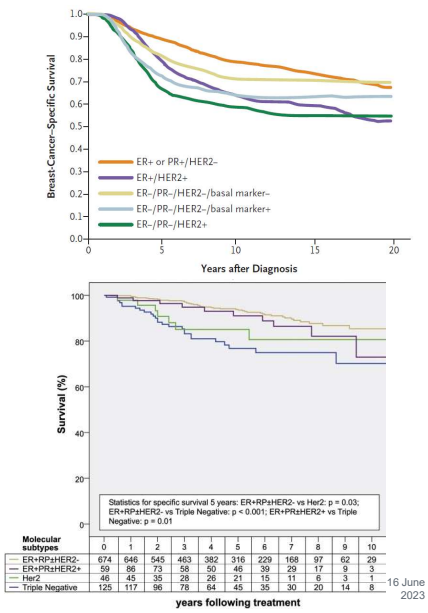
Future Oncol. (2014) 10(14), 2293–2301
Br J Cancer. 2020;123(8):1223-1227.
Breast Cancer Res. 2007;9(1):R6.
Mol Cell Endocrinol. 2008;290(1-2):24-30.
J Natl Cancer Inst. 2011;103(18):1397-1402.
Breast Cancer Res Treat. 1981;1(1):37-41.

8

Estrogen Receptor-α (ER)

- **Clinical significance:**
  - Key factor in selection of BC therapy
  - Predictive of response to endocrine therapy (ET) in primary and metastatic BC
  - Effect of ET is proportional to ER expression
  - Prognostic, correlates with grade, subtype, stage, and outcome
  - Surrogate for luminal molecular intrinsic subtype

Breast cancer specific survival by IHC subtype



Clin Cancer Res. 2008;14(5):1368-1376.
Breast. 2012 Jun;21(3):366-73.

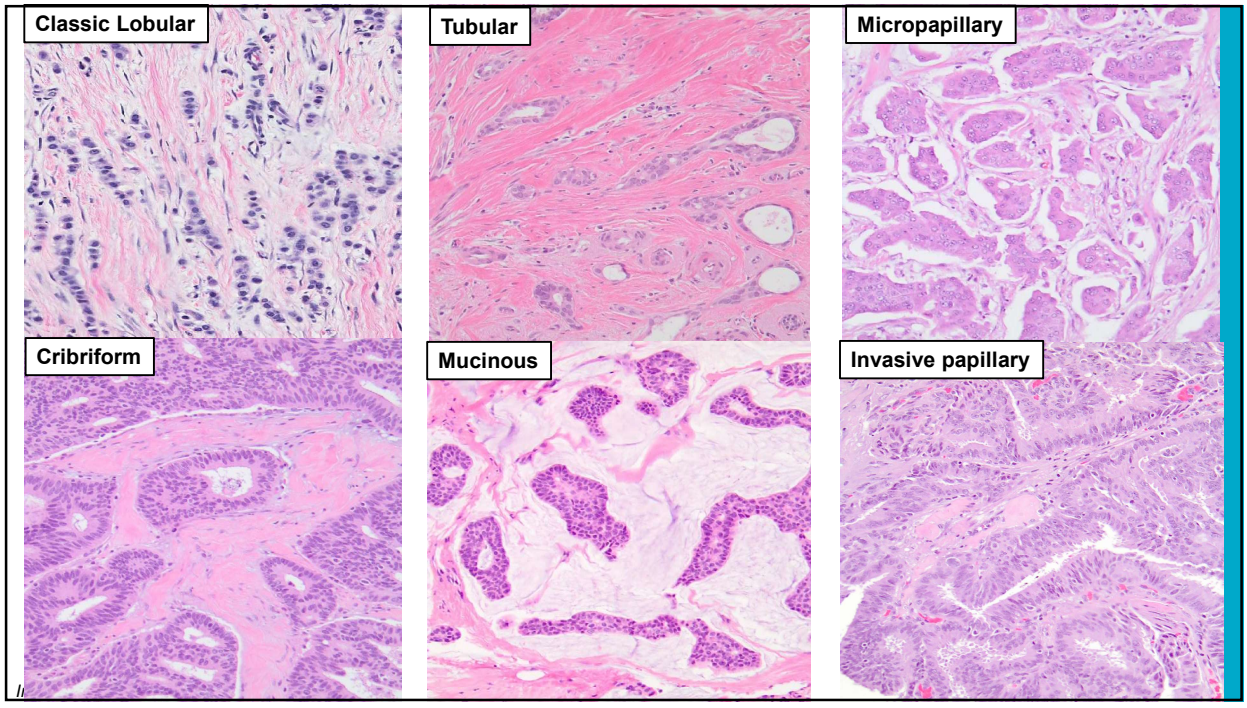
9

Estrogen Receptor-α (ER)

- **Clinicopathologic features of ER+ breast cancer:**
  - 75-80% of all invasive breast cancers (BC) express ER
  - Independent predictor of OS and DSS
  - Older age, earlier stage, less LN involvement
  - Lower histologic grade in IBC (NST type)
  - Associated with special BC subtypes (e.g. cribriform, lobular, mucinous, tubular, papillary, micropapillary)
  - More indolent but often with late recurrence in long-term survivors

Breast Cancer Res. 2007;9(1):R6.
Cancer. 2005;103(11):2241-2251.
Breast Cancer Res Treat. 2002;74(3):199-211.
Int J Cancer. 2019;145(12):3207-3217.

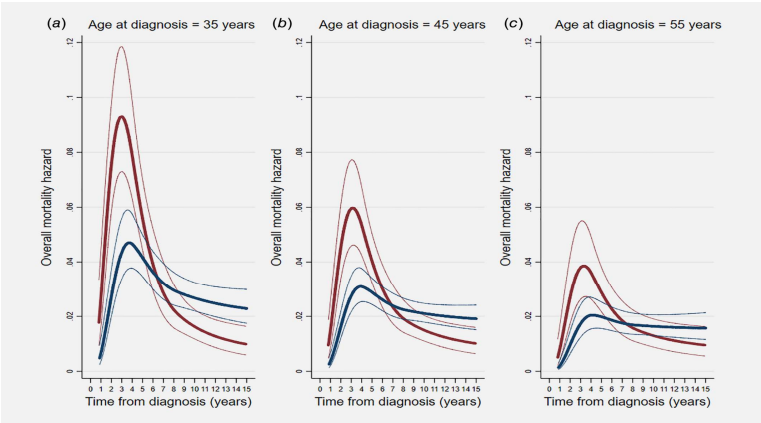
10



11

Long-term cumulative recurrence risk in ER+ BC

Mortality hazard after breast cancer as a function of time since diagnosis by ER status and age at diagnosis



ER+: blue line
ER-: red line

Int J Cancer. 2019;145(12):3207-3217.

12

Estrogen Receptor-α (ER)

- **Assessment and Reporting ER/PR:**
  - ER and PR are assessed by % of positively stained nuclei AND intensity of staining (ASCO/CAP and International Collaboration on Cancer Reporting (ICCR)
  - Other composite scoring systems (e.g. Allred Score) are optional
  - Only invasive cells included (in-situ and non-neoplastic cells must be avoided)
  - ER/PR is sensitive to pre-analytic factors, e.g. cold ischemia time (≤1 hr), fixation media (NBF) and time (6-72 hrs)
  - To ensure performance, laboratories must participate in internal and external validation, and proficiency testing

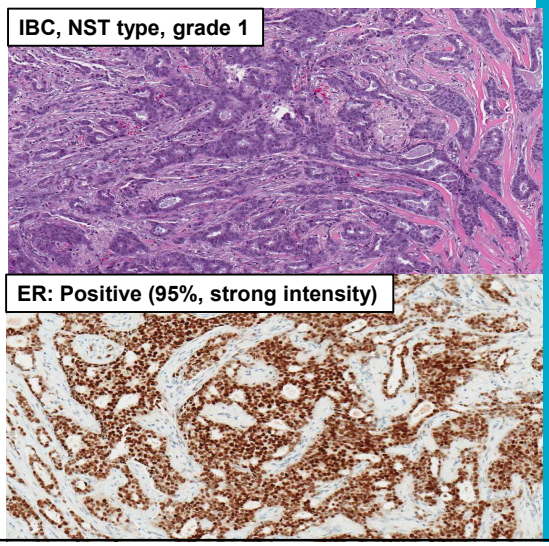
Am J Clin Pathol. 2003;120(1):86-92.
J Clin Oncol. 2010 Jul 20;28(21):3543
Arch Pathol Lab Med. 2010;134(6):930-935

13

ASCO/CAP Guideline Recommendations for Reporting Hormone Receptors (HR) in BC

Estrogen Receptor
___ Positive (>10%)
Low Positive (1-10%)
Negative (<1%)
___ % of nuclei staining
___ Intensity (weak, moderate, strong)

Progesterone Receptor
___ Positive (>1%)
Negative (<1%)
___ % of nuclei staining
___ Intensity (weak, moderate, strong)



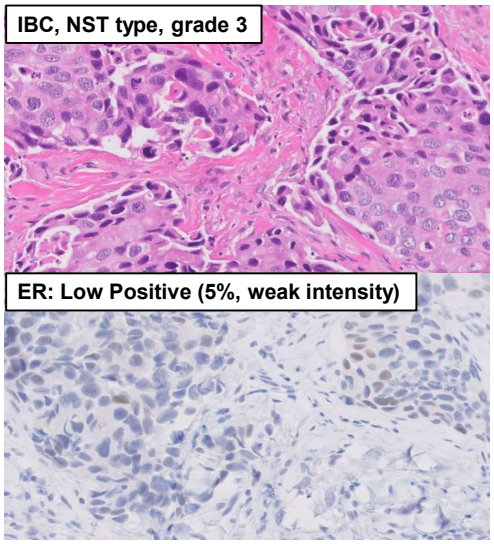
J Clin Oncol . 2020 Apr 20;38(12):1346-1366.

14

ER-low positive breast cancer

- ER-low category introduced in the 2020 ASCO/CAP Guidelines
- Defined as 1-10% nuclear staining
  - Higher grade and worse outcomes compared to ER positive (>10%) BC
  - Reduced response to endocrine therapy (ET)
  - Molecular profiles and clinical behavior more similar to ER negative BC

- Additional confirmation procedures required:
  - Review/report status of external/internal controls
  - QA review with 2nd pathologist
  - Compare with prior ER/PR results (if available)
  - Consider repeat ER on different block/specimen

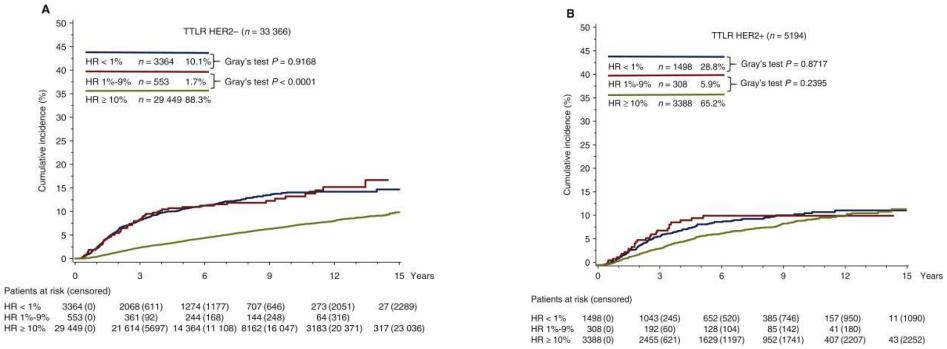


J Clin Oncol. 2020;38(12):1346-1366.
Br J Cancer. 2020;123(8):1223-1227.
Ann Oncol. 2021;32(11):1410-1424.

15

Worse outcome in ER-low positive BC compared to strongly HR positive BC

Cumulative incidence of time to local recurrence for patients with HER2-negative (A) and HER2-positive tumors (B), stratified by hormone receptor (HR) status

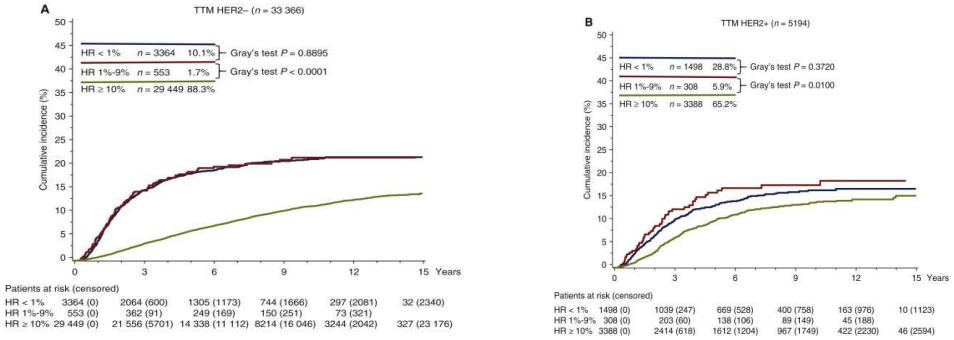


Ann Oncol. 2021;32(11):1410-1424.

16

Worse outcome in ER-low positive BC compared to strongly HR positive BC

Cumulative incidence of time to distance metastasis for patients with HER2-negative (A) and HER2-positive tumors (B), stratified by hormone receptor (HR) status



Ann Oncol. 2021;32(11):1410-1424.

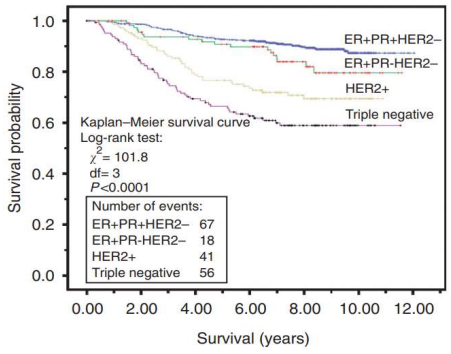
17

Progesterone Receptor

- **60-70% of invasive breast cancer express PR**
- **PR expression is present in 80% of ER+ BC**
- **ER+/PR- tumors (compared to ER+/PR+) frequently higher grade and stage, HER2+**
- **Less predictive value for response to ET (compared to ER)**
- **PR is an independent prognostic factor, loss of PR associated with reduced OS/DSS/DFS**

- **Mandatory and routinely assessed on all:**
  - **Primary invasive cancers**
  - **Recurrences and metastases**
  - **DCIS (optional)**

Survival (BCSS) and disease-free survival (DFS) in BC stratified by biomarker expression



Br J Cancer. 2014;110(3):565-572.

J Clin Oncol. 2007;25:4772-4778
Endocrinology. 1978;103(5):1742-1751

18

Human Epidermal Growth Factor Receptor 2 (HER2)

19

HER2

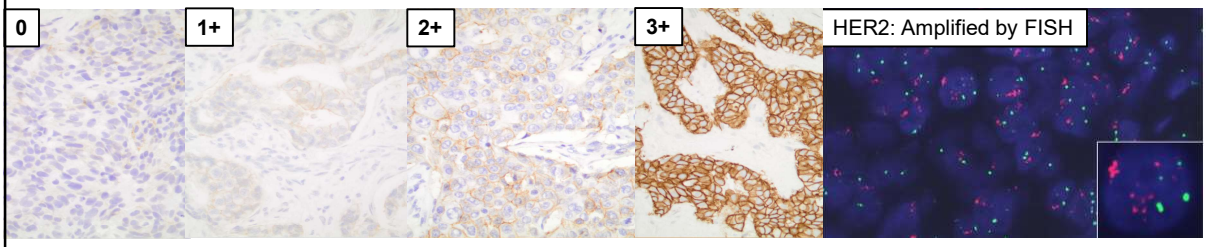
- **Function and biology:**
  - Part of ERBB family of cell surface receptors
    - Receptor tyrosine kinase expressed on surface of breast epithelial cells
    - HER2 signaling activates multiple major signaling pathways (MAPK, PI3K/AKT, etc) that promote cellular proliferation and anti-apoptosis
  - In the majority with HER2 gene amplification associated with increased protein expression
  - Positive in 14.9% of BC (NCI based on SEER reporting)
  - Associated with higher grade, more advanced stage
- **Overview of clinical usage:**
  - Provides both predictive and prognostic information
  - Mandatory and routinely done on all primary and recurrent/metastatic BC, not done for DCIS

 J Natl Cancer Inst. 2014 Apr 28;106(5) *J Clin Oncol 2013;31:3997-4013*

20

HER2 assessment

- FDA approved methods include immunohistochemistry (IHC) and/or in situ hybridization (ISH)
- FDA also approved NGS (Foundation One CDx) to detect ERBB2 amplification for treatment with HER2-targetted tx
- Somatic activating mutations in ERBB2 kinase domains occur in ~3% of metastatic BC (undetectable by IHC or ISH)
  - Response to HER2-directed tx can be variable
  - Some mutations (e.g. L755S, V8421) shown to promote resistance to HER2 tx



J Oncol. 2020;2020:6375956.
Breast Cancer Res Treat 2017.166:339–349

New Biotechnology. 2012 (29); 6:665-681

21

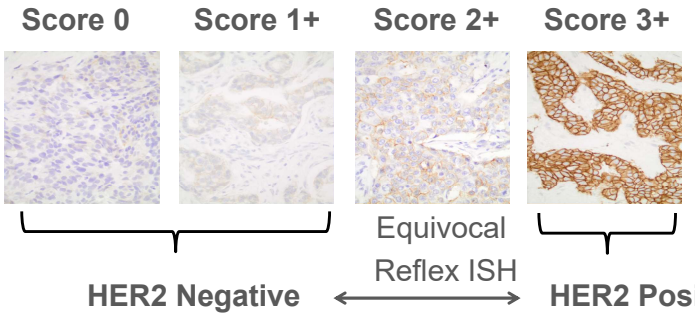
ASCO/CAP HER2 Guideline Recommendations Update (6/7/2023)

Interpretation	HER2 IHC score	IHC Scoring Criteria	
Negative	0	No staining visible	Faint/barely perceptible incomplete membrane staining in ≤10% of tumor cells
Negative	1+	Faint/ barely perceptible incomplete membrane staining in >10% of tumor cells	
Equivocal	2+	Weak to moderate complete membrane staining observed in >10% of tumor cells	
Positive	3+	Intense, complete membrane staining observed in >10% of tumor cells	

Wolff AC, et al. J Clin Oncol. 2023;JCO2202864.

22

The ASCO/CAP HER2 Guidelines take a dichotomous approach to HER2 classification

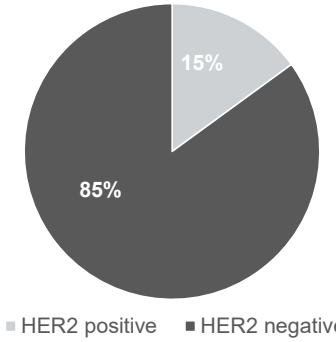


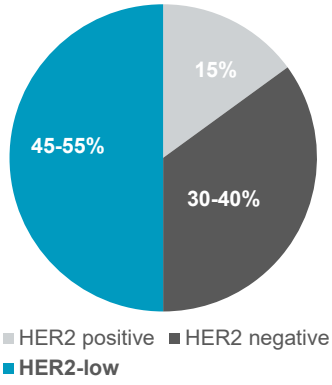
HER2 status is classified as either:
Positive: IHC 3+ or 2+/ISH+
Negative: IHC 2+/ISH-, IHC 1+, or IHC 0

Wolff AC, et al. J Clin Oncol. 2023;JCO2202864. *J Clin Oncol. 2015;33(24):2695-2704.*

23

"HER2-low BC" has recently emerged as an important therapeutic group





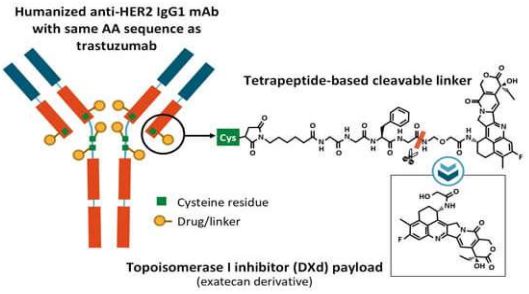
- **HER2-low BC is defined as HER2 IHC 1+ or 2+ and negative ISH**
- **45-55% of all primary BC are HER2-low**
- **Heterogeneous group of tumors, frequently ER+ and Luminal subtype**

J Clin Oncol. 2020;38(17):1951–1962 *NPJ Breast Cancer.* 2021;7:1-13. *J Clin Oncol.* 2020;38:1951-1962. *N Engl J Med.* 2022;387

24

DESTINY-Breast04 established HER2-low BC as a relevant therapeutic subgroup

- Phase 3, multicenter, randomized study comparing efficacy/safety of trastuzumab deruxtecan (T-DXd) vs single agent chemotherapy
- 557 adults with unresectable or metastatic HER2-low BC
- HER2 IHC (4B5) centrally assessed using the 2018 ASCO/CAP criteria



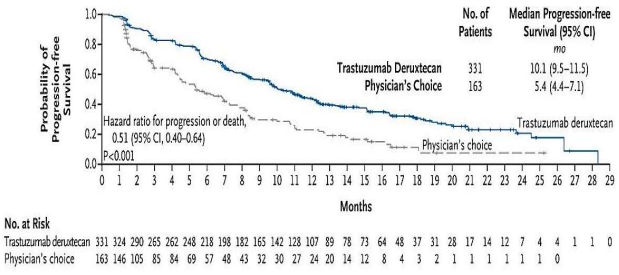
Chem Pharm Bull (Tokyo). 2019;67(3):173-185
N Engl J Med. 2022;387

25

DESTINY-Breast04 established HER2-low BC as an new therapeutic subgroup

- Median progression free survival (mPFS) nearly doubled:
  - 9.9 mo in the T-DXd arm vs. 5.1 mo in the chemotherapy arm
  - In the ER+/HER2-low population, mPFS was 10.1 mo vs. 5.4 mo
- 50% reduction in risk of death/disease progression with T-DXd vs. chemotherapy in overall population

Progression free survival in HR+ cohort



Chem Pharm Bull (Tokyo). 2019;67(3):173-185
N Engl J Med. 2022;387

26

Rapid changes in clinical practice guidelines in response to HER2-low BC

- **DESTINY-Breast04 data were presented** at the 2022 ASCO Annual Meeting Plenary Session (6/5/2022)
- **NCCN Clinical Practice Guidelines in Oncology:** recommend T-DXd for patients with recurrent unresectable or stage IV (M1) BC which is HER2-low (6/21/22)
- **ASCO Guideline Rapid Recommendation Update:** patients with HER2-low BC who have received at least one prior chemotherapy for metastatic disease, and if HR+ are refractory to endocrine therapy, should be offered treatment with T-DXd (8/4/22)
- **FDA approves T-DXd for HER2-low breast cancer:** patients with unresectable or metastatic HER2-low BC who received prior chemotherapy in the metastatic setting or recurred within 6 mo of completing adjuvant chemotherapy (8/5/22)

Cancers 2021;13(5):1015.
N Engl J Med 2022;387(1):9-20

27

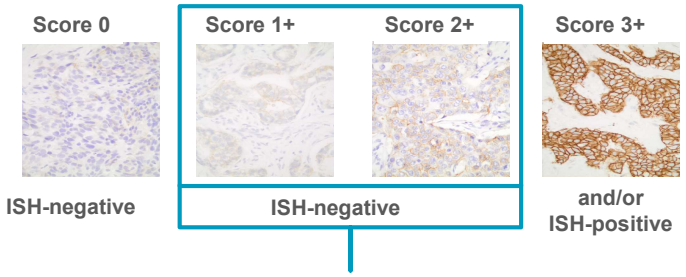
Rapid changes in clinical practice guidelines in response to HER2-low BC

- **FDA approves HER2 (4B5) as the companion diagnostic (CDx) for T-DXd (10/3/22)**
- **CAP Cancer Protocols (Version 1.5.0.1) updated the breast biomarker protocols to include notes for HER2-low BC expression (3/22/2023)**

Cancers 2021;13(5):1015.
N Engl J Med 2022;387(1):9-20

28

Updated CAP Biomarker Reporting Protocol acknowledges HER2-low expression (3/22/2023)



- **CAP Cancer Protocol (V.1.5.0.1)** updated the breast biomarker protocol to include notes for HER2-low expression:
- ***Breast cancers with HER2 IHC score 1+ or HER2 IHC score 2+ and a negative ISH result are eligible for clinically appropriate HER2-targeted therapy and may be reported as "HER2 Low"***

https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates

29

Rapid changes in clinical practice guidelines in response to HER2-low BC

- **FDA approves HER2 (4B5) as the companion diagnostic (CDx) for T-DXd (10/3/22)**
- **CAP Cancer Protocols (Version 1.5.0.1) updated the breast biomarker protocols to include notes for HER2-low BC expression (3/22/2023)**
- **ASCO/CAP HER2 Guideline Recommendations Update (6/7/2023)**

Wolff AC, et al. J Clin Oncol. 2023;JCO2202864.

Cancers 2021;13(5):1015.
N Engl J Med 2022;387(1):9-20

30

ASCO/CAP HER2 Guideline Recommendations Update (6/7/2023)

- **Acknowledges the clinical relevance of distinguishing HER2 IHC Score 0 from 1+**
- **No change to HER2 result categories (same as 2018 ASCO/CAP HER2 Guideline Recommendations)**
  - **HER2 IHC Score 1+ is still interpreted as "HER2 Negative"**

Wolff AC, et al. J Clin Oncol. 2023;JCO2202864.

31

ASCO/CAP HER2 Guideline Recommendations Update (6/7/2023)

- **Inclusion of a footnote in HER2 test reports to address HER2-low BC:**

Patients with breast cancers that are HER2 IHC 3+ or IHC 2+/ISH amplified may be eligible for several therapies that disrupt HER2 signaling pathways.

Invasive breast cancers that test 'HER2-negative' (IHC 0, 1+ or 2+/ISH not-amplified) are more specifically considered 'HER2-negative for protein overexpression/gene amplification' since non-overexpressed levels of the HER2 protein may be present in these cases.

Patients with breast cancers 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).*

Wolff AC, et al. J Clin Oncol. 2023;JCO2202864.

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ASCO/CAP HER2 Guideline Recommendations Update (6/7/2023)

- **Best Practices for distinguishing HER2 IHC Score 0 from 1+**

1. **Adherence to standardized ASCO/CAP Guidelines Scoring criteria**
2. **Using high power (40x) when discriminating 0 from 1+ staining**
3. **Considering 2nd pathologist review when results are close to the 0 vs. 1+ threshold**
4. **Use controls with a full range of expression (including 1+) to ensure the assay has an appropriate limit of detection**
5. **Careful attention to preanalytic conditions of samples from both primary and metastatic sites**

Wolff AC, et al. J Clin Oncol. 2023;JCO2202864.

33

ASCO/CAP HER2 Guideline Recommendations Update (6/7/2023)

- **Medical oncologists can also consider HER2 IHC results on prior or concurrent primary samples (or other metastatic sites)**
  - **There may be heterogeneity in HER2 expression levels between samples**
  - **Metastatic cancer tissue samples may suffer from preanalytic conditions that are not as well monitored as in primary breast tissue samples**

Wolff AC, et al. J Clin Oncol. 2023;JCO2202864.

34

Incorporating HER2-low into clinical practice raises several questions and challenges

- **Accuracy and reproducibility of IHC scoring by pathologists**
  - Strict adherence to scoring criteria
- **Optimal choice of specimen for testing**
  - Primary (core biopsy vs. resection) vs. metastasis
- **Testing algorithms to detect HER2 low breast cancer**
  - Need for repeat testing (intratumoral heterogeneity)
  - Archival HER2 results
- **Pre-analytics and Analytics**
  - Fixation and cold ischemia
  - Performance of different antibodies/assays (e.g. 4B5 vs. HercepTest)
  - Validation, external controls that cover the range of expression
- **Optimizing reporting**
  - Discrete reporting
  - Addressing HER2-low BC

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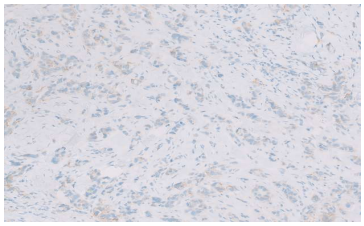
Differences in Staining Patterns and Intensity Between IHC 0 and 1+ Are Subtle

Scan the entire tumor at low power to assess for intratumoral heterogenity

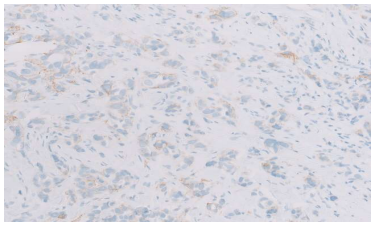
The right microscopic lens is also critical to accurately assess HER2 IHC score, as membrane staining is often not distinguishable at low magnifications:

- **HER2 IHC 3+ is readily recognizable at 2.5x or 5x**
- **HER2 IHC 2+ is visible at 10x but not at 5x**
- **HER2 IHC 1+ is not observable at 10x, and is best visualized at 40x**

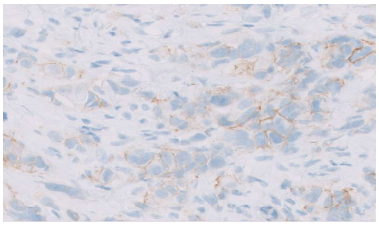
10x lens



20x lens



40x lens



40x magnification lens is required to discriminate HER2 0 versus 1+

Diagn Pathol. 2018;13:1-7.
NPJ Breast Cancer. 2021;7:1-13.

36

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Optimal choice of specimen for HER2 testing: Primary Tumor

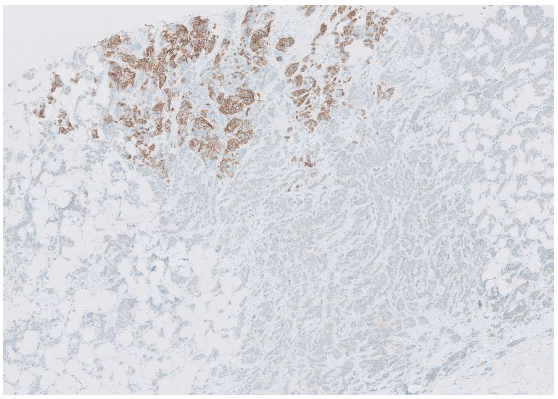
Core needle biopsies (CNB) have some limitations:

- They might not account for tumor heterogeneity
- They are prone to edge effects and crush artifacts

Resection specimens may provide a more representative assessment of HER2 expression

- Per current ASCO/CAP Guidelines, repeat HER2 testing on the excision is not mandatory if the initial result is negative (but is recommended in some clinical scenarios)
- They are prone to cold ischemic time and fixation artifact that may limited interpretation

Intratumoral heterogeneity for HER2 expression can cause discordant HER2 results



J Clin Pathol. 2015;68:93-99.
Diagn Pathol. 2012;6:60.

37

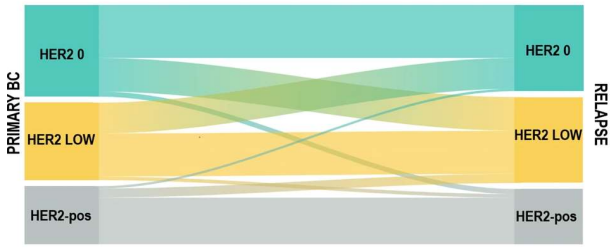
Optimal choice of specimen for HER2 testing: Metastatic Tumor

- Biomarker status can differ between primary and metastatic tumor
- A recent study in patients with matched primary and relapsed breast cancer samples (n=547)
- Prevalence of HER2-low expression:
  - 34.2% of primary tumors
  - 37.3% of metastases
- Overall discordance rate: 38%
  - 15% switched from HER2 0 to HER2-low
  - 14% switched from HER2-low to HER2 0

- **HER2-low expression is unstable during disease evolution**

- **Biopsy and HER2 reassessment of the metastasis (if the primary tumor is HER2 0) may open new therapeutic options in a relevant proportion of patients**

Evolution of HER2 expression from primary to recurrent breast cancer



		HER2 recurrence/metastasis N,%			Total
		0	Low	Positive	
HER2 primary BC N,%	0	132 (24.1)	83 (15.2)	13 (2.4)	228 (41.7)
	Low	77 (14.1)	101 (18.5)	9 (1.6)	187 (34.2)
	Positive	6 (1.1)	20 (3.7)	106 (19.4)	132 (24.1)
Total		215 (39.3)	204 (37.3)	128 (23.4)	547 (100)

Miglietta F, et al. Evolution of HER2-low expression from primary to recurrent breast cancer. NPJ Breast Cancer. 2021;7:137-021–00343-4

38

Testing algorithms need to be updated to detect HER2 low breast cancer

- **Need for repeat testing of the primary resection specimen?**
  - If the initial HER2 result from the core needle biopsy of the primary tumor is Score 0
  - Intratumoral heterogeneity for HER2 expression
  - Not currently mandated in the ASCO/CAP HER2 Guideline Recommendations
- **Interpretation of archival HER2 results**
  - Should archival HER2 IHC be re-reviewed for accuracy?
  - Need for repeat HER2 testing on archival specimens?

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Archival HER2 IHC results may not be accurate for HER2 low status

Retrospective study to estimate the prevalence of HER2-low breast cancer (BC) and describe its clinicopathological characteristics.

Giuseppe Viale, Naoki Niikura, Eriko Tokunaga, Olga Aleynikova, Naoki Hayashi, Joohyuk Sohn, Ciara O'Brien, Gavin Higgins, Della Varghese, Gareth D James, Akira Moh, Nana Scotto; European Institute of Oncology, University of Milan, Milan, Italy; Tokai University School of Medicine, Kanagawa, Japan; National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan; Segal Cancer Center/ Jewish General Hospital, McGill University, Montreal, QC, Canada; Department of Breast Surgical Oncology, St. Luke's International Hospital, Tokyo, Japan; Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, South Korea; The Christie NHS Foundation Trust, Manchester, United Kingdom; Victoria Cancer Biobank, Melbourne, Melbourne, Australia; AstraZeneca Pharmaceuticals LP, Gaithersburg, MD; Medical Statistics Consultancy Ltd, London, United Kingdom; Daiichi Sankyo Inc., Basking Ridge, NJ; AstraZeneca Pharmaceuticals, Cambridge, United Kingdom

Journal of Clinical Oncology®

Design:

- Multicenter, retrospective review of 233 pts from NCT04807595 with HER2 negative metastatic BC diagnosed from 2015-2017
- Archival HER2 IHC slides re-reviewed and scored by pathologists blinded to original score
- Readers were trained on HER2-low expression scoring prior to re-review
- Cases classified as HER2 negative vs. HER2-low
- Concordance between historical HER2 IHC scores and rescores assessed

Results:

- HER2-low prevalence: 63.2%
- **Concordance rate: 82%**

Journal of Clinical Oncology 2022 40:16_suppl, 1087-1087

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Potential analytical differences in HER2 IHC Assay Performance

- **Several FDA IHC assays are available to test for HER2 expression in breast cancer**
  - Use distinct antibodies, detection and retrieval systems, and have different performance characteristics
  - May display different specificity and sensitivity
  - While analytical validation and agreement has been well studied for these assays in identifying HER2 positive disease, less is known about their comparative performance in HER2 low expression

HER2 IHC Assay	Antibody/clone
Pathway anti-HER2/neu (Roche)	Rabbit monoclonal 4B5
HercepTest (Dako/Agilent)	Rabbit A0485
Bond Oracle HER2 IHC system (Leica)	Mouse CB11
InSite HER2/neu (BioGenex)	Mouse CB11

Am J Clin Pathol. 2015;144:686-703.
Breast Cancer Res. 2012.
J Clin Oncol. 2018;36:2105-2122.

41

Potential analytical differences in HER2 IHC Assay Performance

Comparison of HercepTest™ mAb pharmDx (Dako Omnis, GE001) with Ventana PATHWAY anti-HER-2/neu (4B5) in breast cancer: correlation with *HER2* amplification and HER2 low status

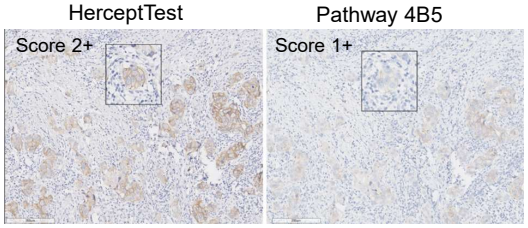
VIRCHOWS ARCHIV European Journal of Pathology

Josef Rüschoff[1] · Michael Friedrich[1] · Iris Nagelmeier[2] · Matthias Kirchner[2] · Lena M. Andresen[3] · Karin Salomon[3] · Bryce Portier[4] · Simone T. Sredni[4] · Hans Ulrich Schildhaus[1,2] · Bharat Jasani[1] · Marius Grzelinski[1] · Giuseppe Viale[5]

- Studied agreement between HercepTest and PATHWAY 4B5 in 119 cases with range of scores 0-3+
- Complete agreement was achieved in only 69.7% (83/119)
- Discordant cases were due to higher HER2-status scoring using the HercepTest

HercepTest (mAb)	PATHWAY 4B5 0	1+	2+	3+	Total
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

HercepTest Pathway 4B5



Virchows Arch. 2022;481(5):685-694.

42

Potential analytical differences in HER2 IHC Assay Performance

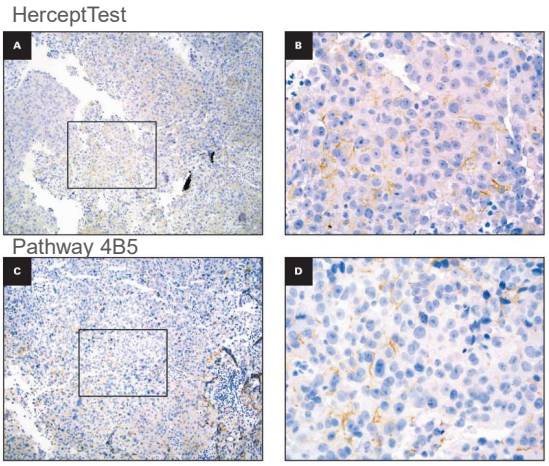
Interobserver and Interantibody Reproducibility of HER2 Immunohistochemical Scoring in an Enriched HER2-Low–Expressing Breast Cancer Cohort

AJCP American Journal of Clinical Pathology

- Studied agreement between HercepTest and Pathway 4B5 in 114 CNB cases enriched for HER2 low BC
- Independently scored by 6 breast pathologists familiar with HER2 low concept

- Antibody agreement for 0 vs. 1+
  - HercepTest: 78.1% (kappa: 0.53)
  - Pathway 4B5: 72.2% (kappa: 0.41)

– Conclusion: Notable interobserver and inter-antibody variation, especially in 0-1+ cases, although performance was superior to earlier studies

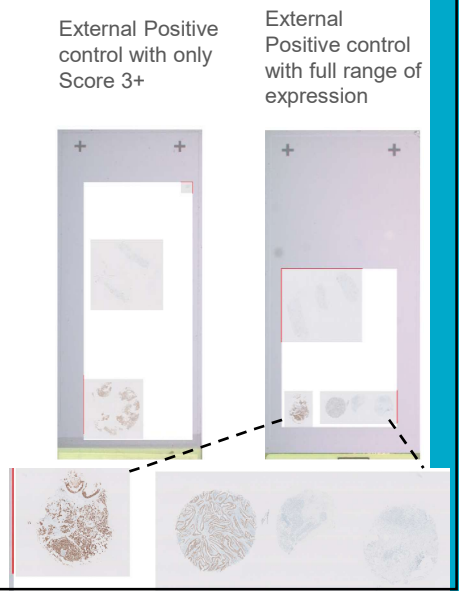


Am J Clin Pathol 2023;159:484-491

43

Optimizing specimens for HER2 testing

- **Need to optimize specimens for HER2 testing to ensure the HER2-low BC are captured**
- **Pre-analytic considerations for improving HER2 testing include:**
  - Standardization of fixation processes (10% neutral-buffered formalin solution and fixed for 6−72 hours)
  - Optimal tissue requirements (including documentation of cold ischemic time and fixation time)
  - On slide controls with full range of staining intensities
- **Validation procedures**
  - CAP requirements for predictive biomarkers requires 40 challenges (20+ and 20-)
  - Include challenges that represent the full spectrum of expression of HER2 observed in clinical practice
- **Improved QA/QC**
  - Peer consensus scoring near 0-1+ cutoff
  - Tracking HER2-low rates
  - Improved external proficiency testing



Arch Pathol Lab Med 2018;142:1364–1382.
Lab Med 2011;42:459–467.

44

Reporting HER2 IHC Results:

- HER2 IHC results be reported discretely in a standardized fashion (0 to 3+)
- Avoid using imprecise range of scores: e.g. “Negative: 0 to 1+”
- Reporting HER2 results in outline/synoptic style of report is helpful for improving readability
- Document the methods: primary antibody used, formalin fixation and cold ischemic times

HER2 Score by IHC
Negative (0)
Negative (1+)
Equivocal (2+)
Positive (3+)
Cannot be determined (indeterminate) Explain:
Not performed

- **Recommended notes for HER2-low expression:**

CAP Biomarker Protocols (Version 1.5.0.1):

Breast cancers with HER2 IHC score 1+ or HER2 IHC score 2+ and a negative ISH result are eligible for clinically appropriate HER2-targeted therapy and may be reported as “HER2 Low”.

2023 ASCO/CAP Update:

Patients with breast cancers 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).”

https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates

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Programmed Death Ligand-1 (PD-L1)

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IHC assays for immune checkpoint inhibitors eligibility in BC (PD-L1)

- Programmed death ligand 1 (PD-L1) is an immune checkpoint protein expressed on activated immune cells and tumor cells
- Co-inhibitory factor that binds receptors programmed cell death 1 (PD-1) and B7-1 on the surface of activated T cells to regulate the immune response and limit autoimmunity
- Expression of PD-L1 in cancer is an adaptive immune resistance mechanism to avoid T cell mediated anticancer immune response

 Ann Oncol. 2021;32(8):994-1004

J Cancer Epidemiol Biomarkers Prev 2014;23:2965
Cancer Immunol Res 2014;2:361)

47

IHC assays for immune checkpoint inhibitors eligibility in BC (PD-L1)

- Selective blockade of the PD-1/PD-L1 axis is used as a therapeutic strategy in the treatment of multiple cancers to prevent T cell inhibition and reactivate T cell mediated tumor cell killing
- PD-L1 (22C3) is the FDA approved companion diagnostic (CDx) to pembrolizumab a humanized monoclonal PD-1 blocking antibody
- PD-L1 (SP142) is the FDA approved CDx to atezolizumab + nab-paclitaxel, however FDA approval withdrawn for TNBC indication in 2021, when followup studies did not show benefit

 Ann Oncol. 2021;32(8):994-1004

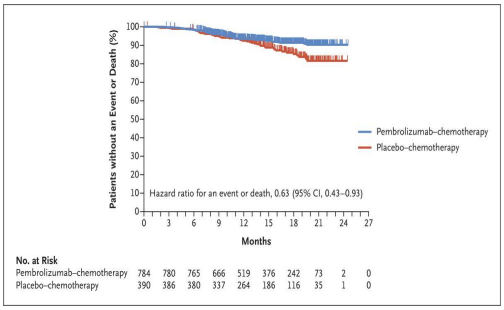
J Cancer Epidemiol Biomarkers Prev 2014;23:2965
Cancer Immunol Res 2014;2:361)

48

PD-L1 (22C3) Assessment

- FDA approval for pembrolizumab for **advanced/metastatic triple negative BC** based on results of KEYNOTE-355 (2020)
  - TNBC: 28% with CPS ≥ 10
  - mPFS of 9.7 vs. 5.6 months
  - **Positive PDL1 result required**
- FDA approved combination pembrolizumab + chemotherapy as neoadjuvant therapy followed by adjuvant pembrolizumab for pts with **high-risk, early-stage TNBC** based on results of KEYNOTE-522 (2021)
  - Higher pCR rates (64.8% vs. 51.2%)
  - Lower rates of disease progression (7.4% vs. 12.8%)
  - **PD-L1 testing not required**

KM estimates of Event-free Survival, According to Trial Group in the Intention-to-Treat Population (Keynote-522)



J Clin Oncol 2020;38:1000
N Engl J Med. 2020;382(9):810-821.

49

PD-L1 (22C3) Assessment

Interpretation:

- Staining can be present in both immune cell (IC) and tumor cell (TC) components
- In TNBC, both TC and IC staining is counted towards scoring
  - IC staining characteristics: membranous or cytoplasmic is often indistinguishable (both included)
  - TC staining characteristics: membranous, linear, partial to complete circumferential staining pattern
- Any degree of intensity staining in IC counts towards scoring
- Only staining in lymphocytes and macrophages is counted
- Staining in neutrophils, eosinophils and plasma cells is not counted
- Avoid scoring in necrotic areas

J Clin Oncol 2020;38:1000

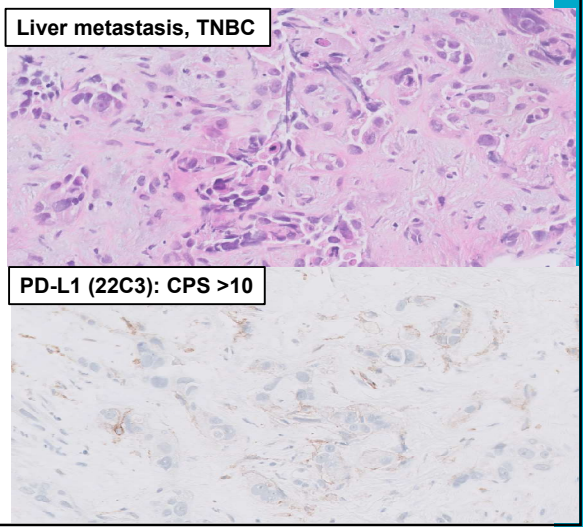
50

PD-L1 (22C3) Assessment in TNBC

Interpretation:

- Expression determined by using combined positive score (CPS)
- Specimen should be considered to have PDL1 expression if CPS is ≥ 10

$$\text{CPS} = \frac{\text{\# of PDL1 staining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total \# of viable tumor cells}} \times 100$$



J Clin Oncol 2020;38:1000

51

Ki67

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Ki67 IHC assessment in BC

- Nuclear protein marker of cell proliferation
- Numerous studies correlate increased proliferation index with high grade, ER negativity, chemotherapy sensitivity, worse prognosis in BC
- Widespread adoption of Ki67 limited due to **poor inter-observer and inter-laboratory variability**, lack of consensus on scoring methodology and cutoff

J Natl Cancer Inst. 2013;105(24):1897-1906
J Natl Cancer Inst. 2021;113(7):808-819
Lancet Oncol. 2010;11(2):174-183.
Mod Pathol. 2010;23(2):251-259.

J Clin Oncol. 2005;23(28):7212-7220.
Lancet Oncol. 2020;21(11):1443-1454
J Clin Oncol. 2009;27(17):2809-2815

53

Ki67 IHC assessment in BC

- International Ki67 BC Working Group (IKWG) recommendations (2021):
  - **Ki67 is highly sensitive to pre-analytic factors**, recommend adherence to ASCO/CAP specimen handling guidelines for ER/PR/HER2
  - **New standardized visual scoring method**
  - Conclude Ki67 has **limited clinical utility**: namely to eliminate need for multigene assay testing in pts with early stage BC (ER+, pT1-2, pN0-1) when Ki67 are ≤5% or ≥30% (lack of sufficient observer concordance at intermediate levels)

J Natl Cancer Inst. 2013;105(24):1897-1906
J Natl Cancer Inst. 2021;113(7):808-819
Lancet Oncol. 2010;11(2):174-183.
Mod Pathol. 2010;23(2):251-259.

J Clin Oncol. 2005;23(28):7212-7220.
Lancet Oncol. 2020;21(11):1443-1454
J Clin Oncol. 2009;27(17):2809-2815

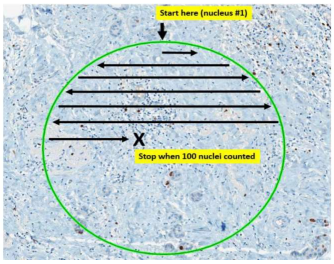
54

IKWG scoring method for Ki67 in BC

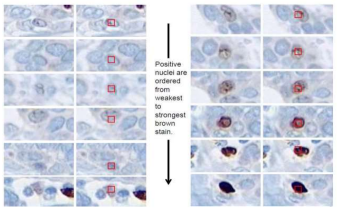
1. Complete online calibration exercise www.ki67inbreastcancerwg.org/
2. Install online scoring app (software program)
3. Review the Ki67 stained slide and input estimates of the percentage area as each "field type" (negligible, low, medium or high)
4. Score 100 nuclei as positive or negative in each "field type" using typewriter method (total 400)
5. App calculates a "weighted global score" which is the Ki67 index

- Advantages:
  - Achieved an intraclass correlation coefficient (ICC): 0.87
- Disadvantages:
  - Requires a minimum 9 minutes per case, training on a calibration set, installation of software
  - Low kappa values (0.6) in the 10-20% range

Typeriter counting pattern



Positive: any definite brown staining in the nucleus of invasive BC cell above the surrounding background cytoplasm and extracellular matrix



J Natl Cancer Inst. 2021;113(7):808-819

55

Is digital image analysis and computer assisted scoring the solution?

- Many different commercially available and open-source platforms offering automated Ki67 assessment
- The IKWG compared 10 different Ki67 software scoring platforms using 7 different scanners, achieved an the intraclass correlation coefficient (ICC): 0.83
- When 8 different testing laboratories used the same scanner/analysis system, the ICC increased to 0.89

Conclusions:

- Different scanners and analysis systems provide different Ki67 scores from one another
- Studies suggest need for inter-platform standardization
- Technology not yet widely adopted in clinical practice

J Natl Cancer Inst. 2021;113(7):808-819

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Ki67 IHC for abemaciclib eligibility

- Abemaciclib is a CDK4/6 inhibitor, in combination with ET was orginally FDA approved (Oct 2021) as adjuvant tx for used in BC patients based on the MonarchE trial
- Indications:
  - ER+/HER2-
  - High risk of recurrence:
    - ≥4 positive axillary LN or
    - 1-3 positive LN and tumor ≥5 cm or grade 3
  - And Ki67 ≥20%

- Ki67 IHC MIB-1 pharmDX (Dako Omnis) is the FDA-approved companion diagnostic (CDx) for abemaciclib
- User manual recommends visual scoring "multiple regions" in cases of "heterogeneity", requires assessment of 200 or more cells

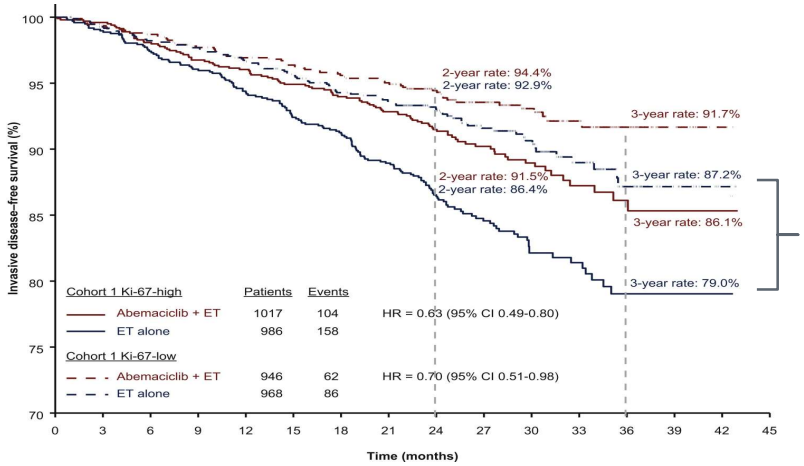
J Clin Oncol. 2021;39(6):685-693.
Ann Oncol. 2021;32(12):1571-1581.

J Clin Oncol. 2020;38(34):3987-3998.

57

Abemaciclib benefit observed regardless of Ki67, Ki67 high tumors had higher risk of recurrence

Kaplan-Meier curves of invasive disease-free survival in Ki-67 high versus Ki-67 low (MonarchE)



ET only

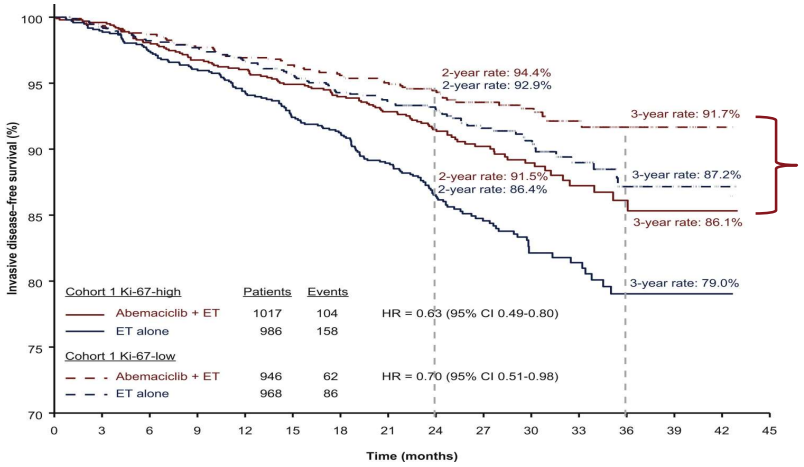
Ki67 low vs. Ki67 high

Ann Oncol. 2021;32(12):1571-1581.

58

Abemaciclib benefit observed regardless of Ki67, Ki67 high tumors had higher risk of recurrence

Kaplan-Meier curves of invasive disease-free survival in Ki-67 high versus Ki-67 low (MonarchE)



ET + Abemaciclib

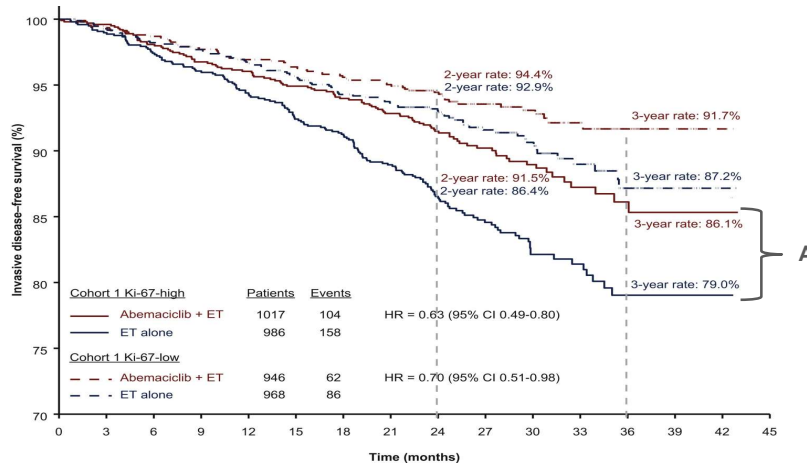
Ki67 low vs. Ki67 high

Ann Oncol. 2021;32(12):1571-1581.

59

Abemaciclib benefit observed regardless of Ki67, Ki67 high tumors had higher risk of recurrence

Kaplan-Meier curves of invasive disease-free survival in Ki-67 high versus Ki-67 low (MonarchE)



Ki67 high:

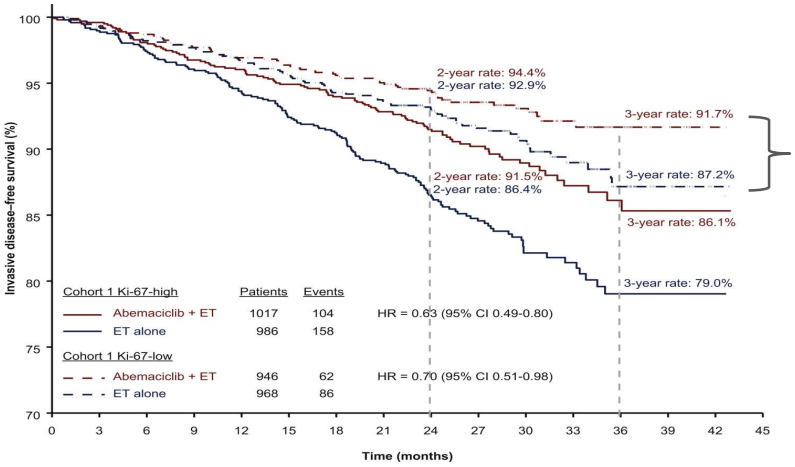
Abemaciclib+ET vs. ET only

Ann Oncol. 2021;32(12):1571-1581.

60

Abemaciclib benefit observed regardless of Ki67, Ki67 high tumors had higher risk of recurrence

Kaplan-Meier curves of invasive disease-free survival in Ki-67 high versus Ki-67 low (MonarchE)



Ki67 low:

Abemaciclib+ ET vs. ET only

Ann Oncol. 2021;32(12):1571-1581.

61

Ki67 questions and controversy

- In MonarchE, Ki67 was prognostic, but not predictive of greater relative benefit for addition of abemaciclib
- Observed a larger mean absolute benefit in Ki67 "high" group:
  - Ki67 ≥20%: 7.1%
  - Ki67 <20%: 4.5% (still significant and substantial)
- Concern over reproducibility of Ki67 in real-world conditions
  - Eligibility cutoff of 20% falls into range of low accuracy
  - Inherent subjectivity of visual scoring
  - FDA approved CDx requires a specific platform (Agilent/Dako Omnis) which may not be available in many labs
  - For reasons of cost/practicality many labs may run Ki67 on their readily available platform as an laboratory developed test, introduces further variability and potential inaccuracy
- **Update: On 3/3/2023 the FDA expanded the indication for abemaciclib, removing the Ki67 ≥20% requirement**

Ann Oncol. 2021;32(12):1571-1581.
J Clin Oncol. 2022;40(33):3796-3799.

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Mismatch Repair (MMR), microsatellite instability (MSI) and Tumor Mutational Burden (TMB)

63

Mismatch repair deficiency (MMR), microsatellite instability (MSI) and Tumor Mutational Burden (TMB) Testing in BC

- **Pembrolizumab** (anti-PD1 monoclonal Ab) is FDA (2017) approved as a tumor-agnostic therapy with unresectable/metastatic tumors that progress on initial therapy that are **MMR-deficient or MSI-High or TMB-high**
- **Dostarlimab-gxly** (anti-PD1 monoclonal Ab) is FDA (2021) approved for advanced/recurrent solid tumors that progress after initial treatment that are MMR-deficient solid tumors (GARNET Trial)
- FDA approved CDx for MMR deficiency is the Ventana MMR RxDx Panel (MLH1/MSH2/PMS2/MLH6)
- MSI status can be assessed by PCR or NGS
- FDA approved CDx for TMB is by NGS (Foundation One)

Arch Pathol Lab Med. 2022;146(10):1194-1210.

64

Mismatch repair deficiency (MMR), microsatellite instability (MSI) and Tumor Mutational Burden (TMB) Testing in BC

- Recent CAP Guidelines for MMR and MSI-testing do not provide specific recommendations for patients with BC due to paucity of data
- MMR-deficiency and MSI-High status is rare in breast cancer (<2%)
- Routine universal screening for MMR-deficiency, etc. is not recommended in BC due to low incidence
- Testing can be considered for BC patients with advanced stage/metastatic/progressive disease, and patients with no other treatment alternatives, being considered for immune checkpoint therapy

Arch Pathol Lab Med. 2022;146(10):1194-1210.
Breast Cancer Res Treat. 2020;182(3):765.
Nat Med. 2019;25(10):1526-1533.

65

Evaluating Mismatch Repair Status to Screen Clinical Advanced Breast Carcinomas for Immunotherapy: Experience From a Large Academic Institution

Vidya Arole[1,2], Saba Shafi[1,2], Bindu Challa[1,2], Anil V. Parwani[1,2], Gary Tozbikian[1,2], Zaibo Li[1,2]

Clinical Breast Cancer

- Study Design:
  - Assessed 163 clinically advanced breast cancers (144 metastatic, 14 locally recurrent, 5 primary)
  - Performed IHC (MMR) or NGS (MSI)
  - Correlated MMR/MSI status with clinicopathologic features
- Results:
  - 78% TNBC, 10% HER2+, 11% HR+
  - Only 1 case (0.6%) showed MMR deficient (loss of MLH1 and PMS2, no hypermethylation of MLH1 promoter)
  - All 127 TNBC and ER-low positive cases showed preserved MMR
- Conclusion:
  - Routine testing of MMR to screen BC patients for immunotherapy eligibility is not cost effective

Clin Breast Cancer. 2022;22(5):e680-e684.

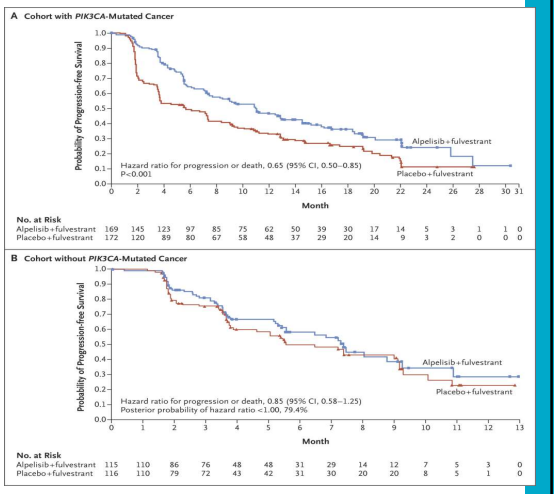
66

PIK3CA Mutation

67

PIK3CA mutations for alpelisib eligibility

- **Alpelisib** (PI3K inhibitor) in combination with fulvestrant as FDA approved (2019) for pts with **Hormone Receptor+, HER2- BC with PIK3CA mutations** who have progressed on/after ET
- Increased median PFS (11 vs. 5.7 months) (SOLAR-1 trial)
- **PIK3CA mutations detected in 40% of HR+ advanced/metastatic BC**
  - Associated with decreased DFS
  - Major cause of resistance to ET
  - Can be initially present in primary tumor or acquired after receiving ET
- Multiple FDA-approved CDx to detect PIK3CA variants by PCR (therascreen) or NGS (Foundation) can be performed on plasma specimens or FFPE



Breast Cancer Res. 2020;22(1):33.
N Engl J Med. 2019;380(20):1929-1940.

68

Estrogen Receptor-1 (ESR1)

69

ESR1 mutations in BC

- ESR1 encodes the ER-α receptor
- ESR1 mutation in ligand biding domain resulting in ligand independent receptor activity
- Implicated in ET resistance to tamoxifen and aromatase inhibitors
- Uncommon in primary BC (<2%)
- Acquired ESR1 mutation is detected in 25-30% recurrent / metastatic tumors after long-term ET
- Associated with poor survival
- Detection of ESR1 mutation may lead to alternative therapeutic approaches, e.g. selective estrogen receptor degraders (SERD) such as fulvestrant, however additional clinical trials needed
- Detected by NGS assays (e.g. Foundation), assays to detect ESR1 mutations in ctDNA in plasma by PCR under development

Breast. 2017; 31:244–59.
Nat Rev Clin Oncol. 2015; 12:573– 83.
Nat Genet. 2013;45(12):1415-1416.
BMJ Open. 2022;12(3):e055821.
Cancer. 2019;125(21):3714-3728.
Cancers (Basel). 2021;13(3):556.

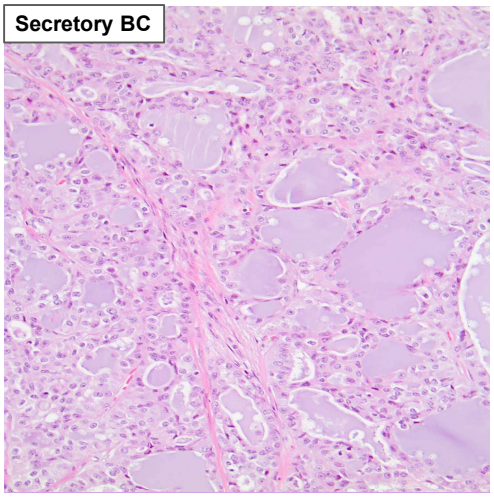
70

Neurotrophic Tyrosine Receptor Kinase (NTRK)

71

NRTK fusions in BC

- NTRK genes (NTRK1, NTRK2, NTRK3) encode receptor tyrosine kinases that can serve as oncogenic drivers as the result of gene fusions
- In BC, ETV6-NTRK3 fusion is detected in >90% of secretory BC
- FDA approved tyrosine kinase inhibitors (larotrectinbib in 2018 and entrectinib in 2019) for any solid tumors with NTRK gene fusion that has progressed under 1st line tx
- Detection by several methods (IHC, break-apart FISH, RT-PCR, RNA-sequencing, NGS)
- IHC has reported false negative rate of 45% (especially fusions with NTRK1 and NTRK2)



J Mol Diagn. 2019;21(4):553-571.
N Engl J Med. 2018;378(8):731-739.

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Oncotype DX ®

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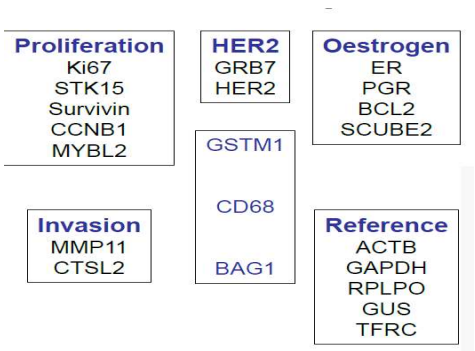
Oncotype DX ®

Exact Sciences, Madison WI, performed at Genomic Health

- **RT-PCR assay that examines weighted expression of 16 genes (5 reference genes)**
- **Provides a recurrence score (RS): which predicts the likelihood that chemotherapy (with ET) will benefit the patient**
- **Tissue requirements: FFPE, submission of highest grade component, >1 mm of invasion, sufficient tissue for fifteen 5µm unstained slides**



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Oncotype DX ®

Indications:

- NOT DONE for DCIS or microinvasion (now there is a Oncotype DCIS for XRT)
- NOT DONE for ER/PR negative patients
- NOT DONE for HER2 positive patients
- NOT DONE ≥4 LN+

- Oncotype DX incorporated into AJCC 8th edition Cancer Staging Manual for prognostic staging
- Not FDA-approved for HER2 clinical assessment due to discrepancies with IHC/FISH results, however any discrepancies should prompt pathologist re-review

Lancet Oncol. 2010;11:55-65.
J Clin Oncol. 2010;28:1829-1834.
N Engl J Med. 2021;385(25):2336-2347.

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Oncotype DX ®

Indications:

- Extensively validated in NSABP20, SWOG 8814 trials, RxPonder clinical trails where it demonstrated prognostic and predictive value

- Indications: ER+, HER2-, LN negative and LN positive (1-3 LN)

- For patients considered for de-escalation of therapy with low-RS

Lancet Oncol. 2010;11:55-65.
J Clin Oncol. 2010;28:1829-1834.
N Engl J Med. 2021;385(25):2336-2347.

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Oncotype DX ®

TAILORx trial:

- **Phase III trial, 10,273 patients (ER+/HER2-, axillary LN-)**
- **Significant (p=0.004) interaction with patient age and RS**

Age	RS 0-10	RS 11-15	RS 16-20	RS 21-25	RS 26-100
>50	No CT benefit				Large CT benefit
≤50	No CT benefit	1.6% CT benefit	6.5% CT benefit	Large CT benefit	

RxPonder Trial: (ER+/HER2-, 1-3 axillary LN+)

- Post-menopausal pts with RS of 0-25 had no significant benefit
- Pre-menopausal pts with RS of 0-25 benefited from the addition of chemotherapy

N Engl J Med. 2018 Jul 12;379(2):111-121
J Clin Oncol. 2022;40(16):1816-1837.

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Mammaprint/Blueprint®

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MammaPrint/BluePrint ®

(Agendia, Netherlands)

- **Prognostic test in Stage I-III BC pts with 0-3 positive LN, and pT size <5 cm MammaPrint measures 70 genes and provides a MammaPrint Index (MPI): Low or High risk of recurrence**
- **BluePrint measures 80 genes, categorizes tumor into LuminalA, LuminalB or Basal-like subtypes**
- **Tissue requirements: FFPE, >1 mm of invasion, 9 mm² area of tumor with 30% cellularity, ten 5µm unstained slides**

- **Most useful for older patients >50 years old with high clinical risk:**
  - **Low Genomic Risk – ET alone may be considered**
- **For younger patients ≤ 50 years old with high clinical risk:**
  - **ASCO does not recommend use of Mammaprint (MINDACT trial update reported benefit of adjuvant chemotherapy for these pts)**

N Engl J Med. 2016;375(8):717-729
Lancet Oncol. 2021;22(4):476-488.

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Breast Cancer Index ®

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Breast Cancer Index (BCI)

- Assesses benefit of extended ET beyond 5 years
- Indicated for HR+ BC, N0 to N1
- Predictive of likelihood benefit of extended ET
- Prognostic estimate of % risk of recurrence 5-10 years after diagnosis
- Some histologic subtypes are excluded (microinvasive ca, metaplastic, neuroendocrine ca, adenoid cystic ca, phyllodes tumor)
- Not validated on ER/PR negative BC, male BC, T4 disease, ≥pN2
- Specimen requirements: FFPE primary tumor, ≥40% tumor cellularity, 3-4 10 µm unstained slides

Ann Oncol. 2019 Nov; 30(11): 1776–1783
Clin Cancer Res. 2021;27(1):311-319.

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Closing Comments & Summary

- The therapeutic landscape in breast cancer continues to evolve rapidly, the demands on pathology to provide critical biomarker information to guide therapeutic decision-making continues grow in complexity
- Accurate and reliable biomarker testing is needed to identify patients who should be eligible for novel targeted therapies
- Pathologists play a central role in assuring the quality and accuracy of biomarker testing, interpreting results, and communicating with the clinical team

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Questions?

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COLLEGE of AMERICAN PATHOLOGISTS

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