Breast Biomarker Testing and What to do with HER2

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Learning Objectives

- Understand the current ASCO/CAP biomarker guidelines
- Be familiar with the expected biomarker expression patterns for histologic types and grades of breast cancer
- Become familiar with evolving landscape of interpretation of HER2 assays
- Recognize the indications and importance of multigene assays in breast cancer treatment decision making
- Become familiar with which ancillary tests are indicated in the advanced or metastatic setting

Breast Cancer Treatment

- Ancillary testing is required to determine effective treatment options for patients with breast cancer
- Largely dependent on ER, PR and HER2 status
- Other contributing factors include size, grade, lymph node status and LVI (also age and co-morbidities)
- Results of multigene assays (e.g. Mammaprint, OncotypeDx)
- AJCC 8th Edition added clinical and pathologic prognostic staging which includes results of ancillary tests

When T is…	And N is…	And M is…	Then the stage group is…
Tis	N0	M0	0
T1	N0	M0	IA
T0	N1mi	M0	IB
T1	N1mi	M0	IB
T0	N1	M0	IIA
T1	N1	M0	IIA
T2	N0	M0	IIA
T2	N1	M0	IIB
T3	N0	M0	IIB
T0	N2	M0	IIIA
T1	N2	M0	IIIA
T2	N2	M0	IIIA
T3	N1	M0	IIIA
T3	N2	M0	IIIA
T4	N0	M0	IIIB
T4	N1	M0	IIIB
T4	N2	M0	IIIB
Any T	N3	M0	IIIC
Any T	Any N	M1	IV

When TNM is…	And Grade is…	And HER2 Status is…	And ER Status is…	And PR Status is…	Then the Clinical Prognostic Stage Group is…
T0 N1** M0 T1* N1** M0 T2 N0 M0	1	Positive	Positive	Positive	IB
				Negative	IIA
			Negative	Positive	IIA
				Negative	IIA
		Negative	Positive	Positive	IB
				Negative	IIA
			Negative	Positive	IIA
				Negative	IIA
	2	Positive	Positive	Positive	IB
				Negative	IIA
			Negative	Positive	IIA
				Negative	IIA
		Negative	Positive	Positive	IB
				Negative	IIA
			Negative	Positive	IIA
				Negative	IIB
	3	Positive	Positive	Positive	IB
				Negative	IIA
			Negative	Positive	IIA
				Negative	IIA
		Negative	Positive	Positive	IIA
				Negative	IIB
			Negative	Positive	IIB
				Negative	IIB

AJCC 8th Edition

Genomic Profile for Pathologic Prognostic Staging

When Oncotype Dx Score is less than 11…

And TNM is…	And Grade is…	And HER2 Status is…	And ER Status is…	And PR Status is…	Then the Pathological Prognostic Stage Group is…
T1 N0 M0 T2 N0 M0	Any	Negative	Positive	Any	IA

Notes

1. Obtaining genomic profiles is NOT required for assigning Pathological Prognostic Stage. However genomic profiles may be performed for use in determining appropriate treatment. If the OncotypeDx® test is performed in cases with a T1N0M0 or T2N0M0 cancer that is HER2-negative and ER-positive, and the recurrence score is less than 11, the case should be assigned Pathological Prognostic Stage Group IA.

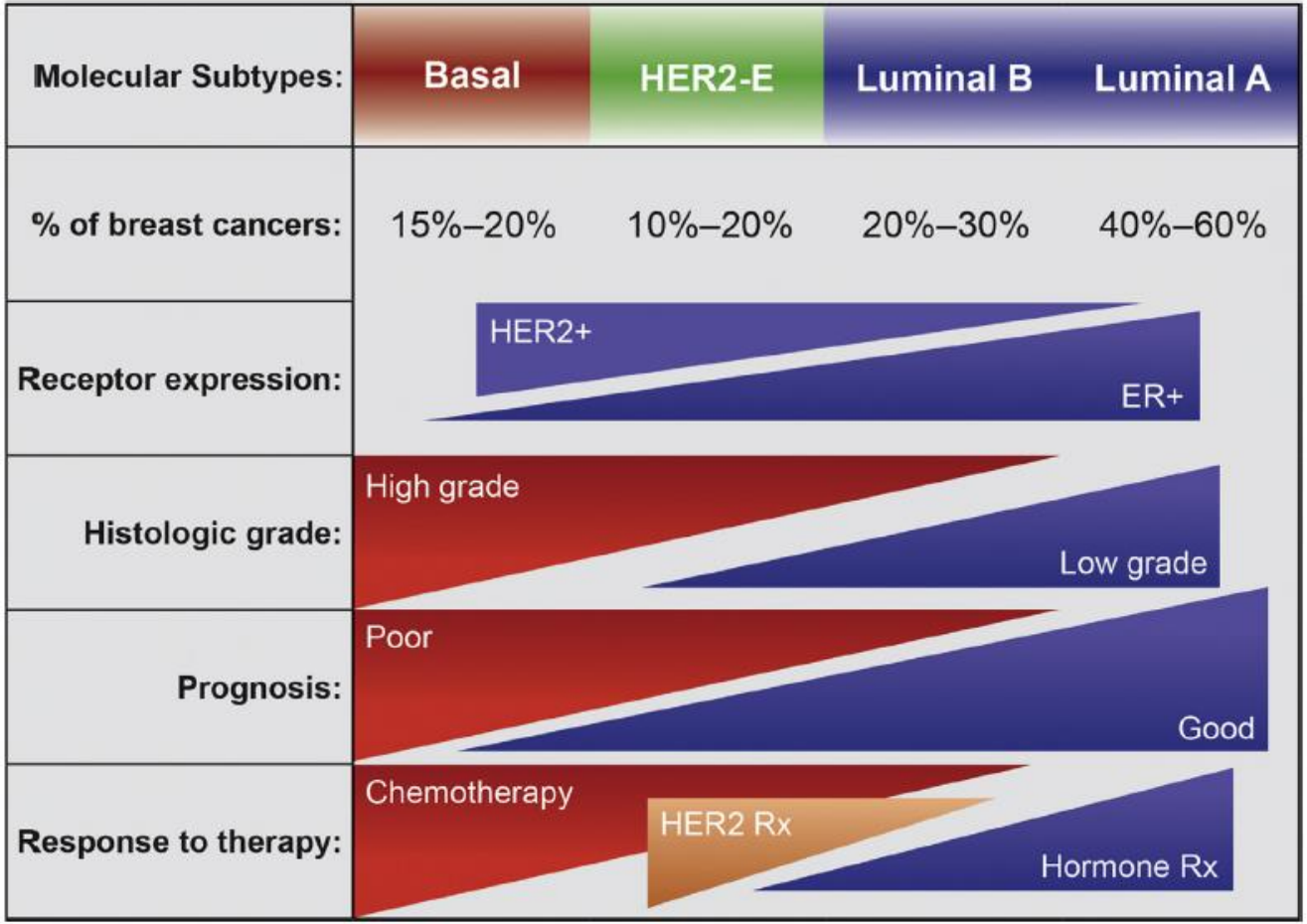
Breast Cancer Treatment

NCCN and St Gallen treatment recommendations organized by HR and HER2 status:

- HR+, HER2-
- HR+, HER2+
- HR-, HER2+
- HR-, HER2-

Molecular data support similar treatment groups, though correlation with IHC is imperfect

New HER2 low group has implications for “triple negative group”



Allison, Surg Pathol Clin, 2018

Ancillary Testing: Further Refinements

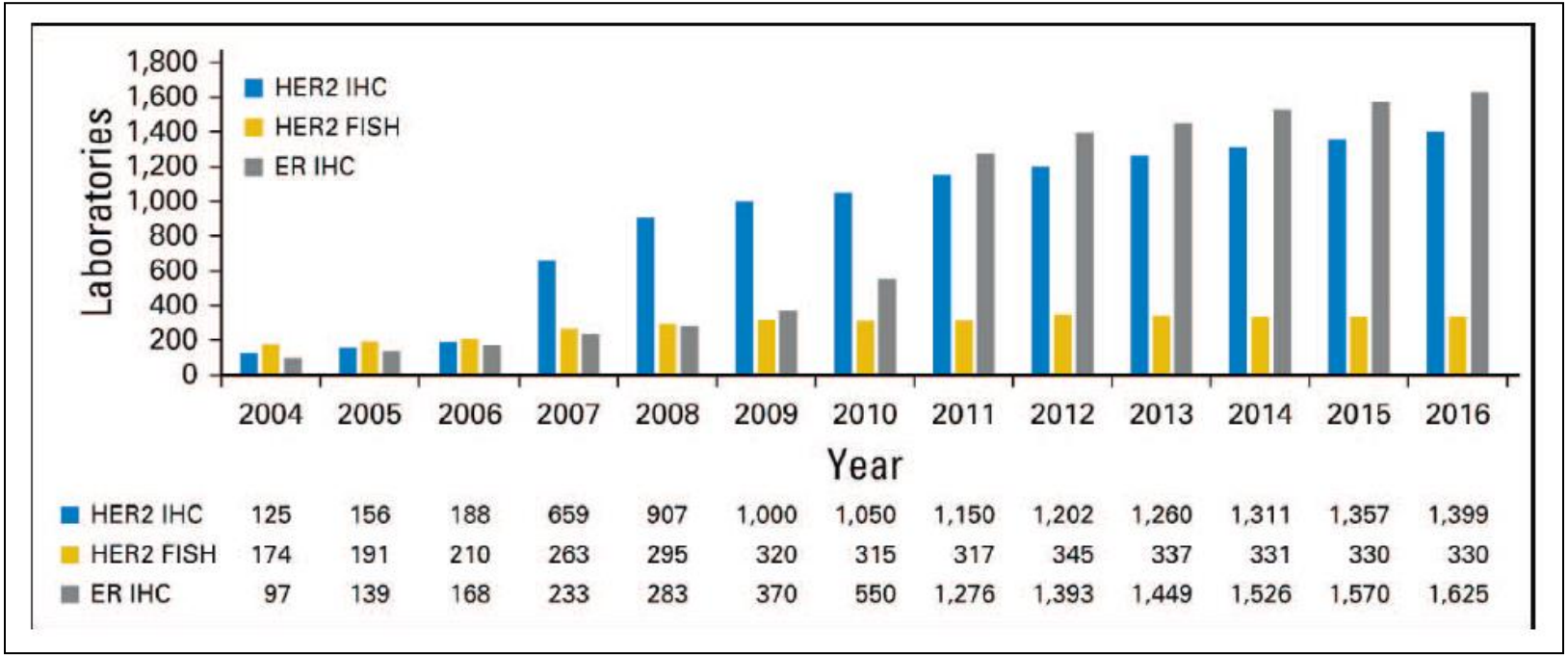
- ER, PR and HER2
- ER low positive tumors
- ER positive, node positive tumors, Ki-67 high
- HER2 low tumors
- Molecular assays guide need for chemotherapy in ER+ tumors with low nodal disease burden

  (and ?tumors with Ki-67 index between 5-30%)

ER, PR and HER2

- High stakes tests
- Not only provide overall treatment and prognostic groupings, also determine specific targeted therapies
- Consequences of errors are significant
  - Deprive potentially responsive patients of treatment
  - Treat potentially unresponsive patients with possibility of treatment related toxicities/side effects
- Large scale errors have been made
- ASCO/CAP Guidelines have led to quality improvement and standardization of reporting

Proficiency Testing



	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
HER2 IHC	125	156	188	659	907	1,000	1,050	1,150	1,202	1,260	1,311	1,357	1,399
HER2 FISH	174	191	210	263	295	320	315	317	345	337	331	330	330
ER IHC	97	139	168	233	283	370	550	1,276	1,393	1,449	1,526	1,570	1,625

Wolff, Arch Pathol Lab Med, 2018

Estrogen Receptor Testing

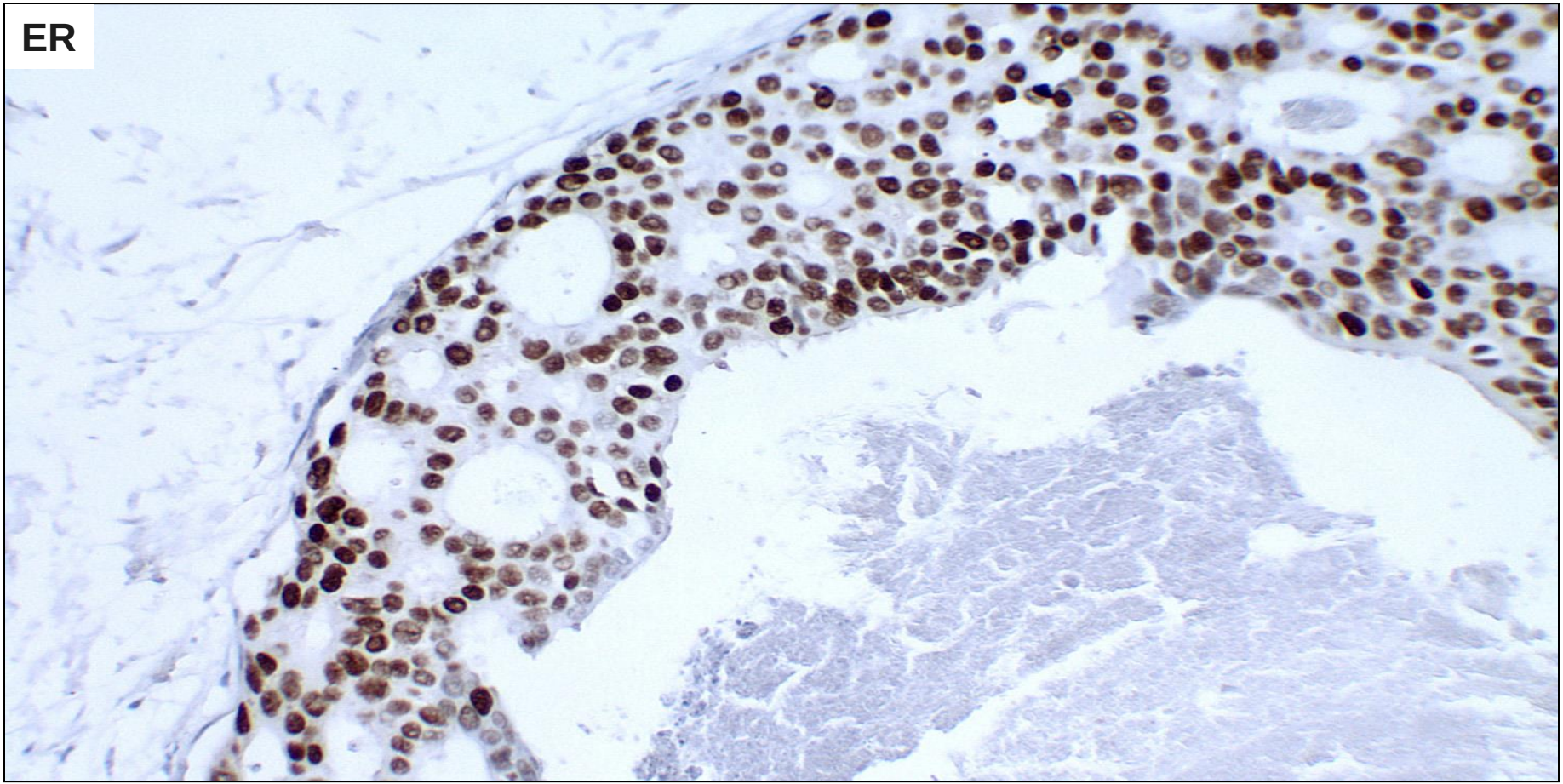
Estrogen Receptor

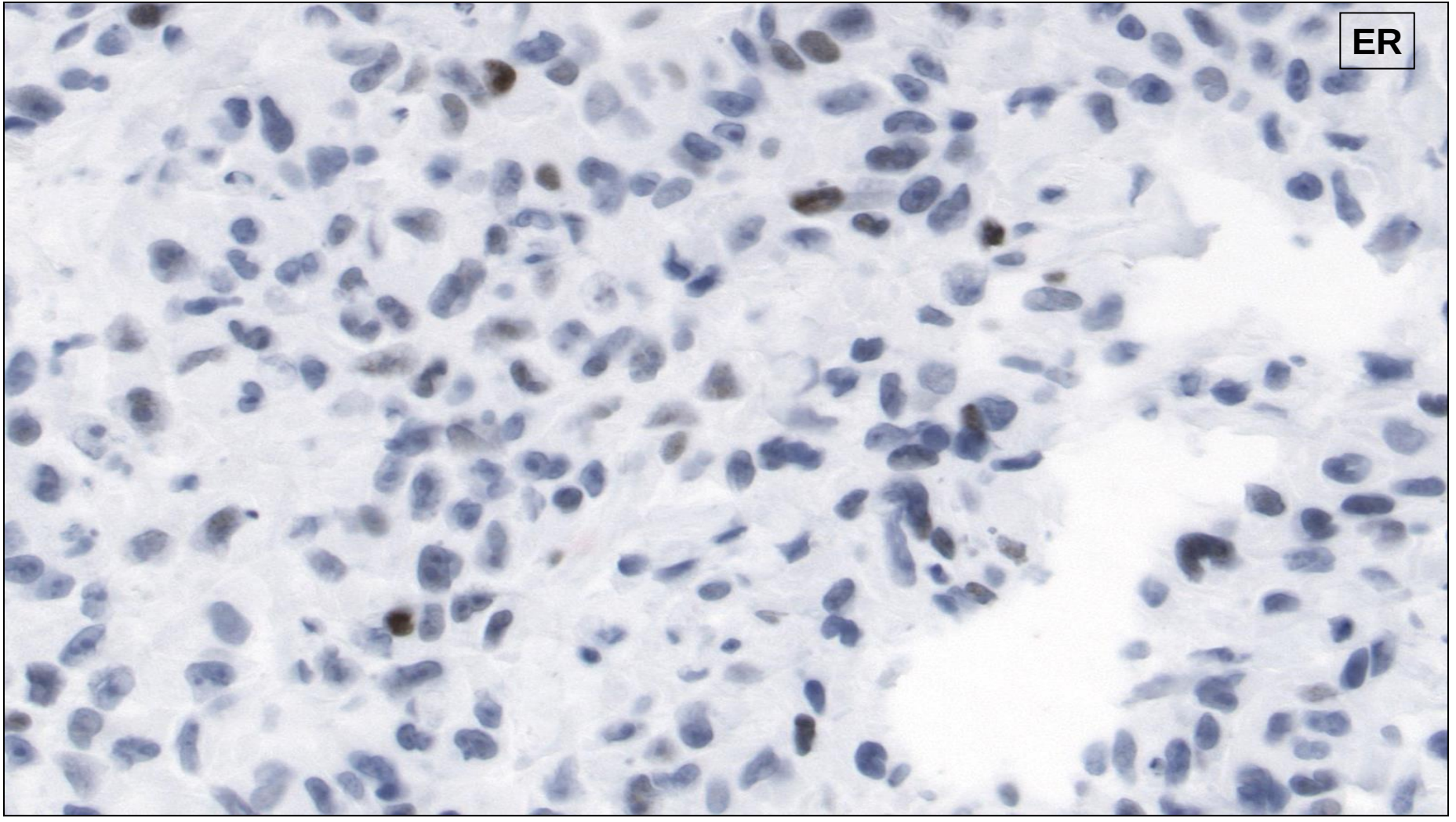
- Nuclear receptor, activated upon binding to estrogen (17-beta-estradiol)
- Role in normal breast development, differentiation and lactation
- ERα encoded by *ESR1* on chromosome 6
- ERβ encoded by *ESR2* on chromosome 14
- ER IHC antibodies recognize ERα

Estrogen Receptor IHC Issues

Multiple sources of variability exist in any given laboratory

- Pre-analytic variables (e.g. cold ischemic and fixation times)
- Choice of antibody
- Antigen retrieval techniques
- Use of controls
- Interpretation/scoring (?cut points too high or too low)




ER

Influence of Fixation Time

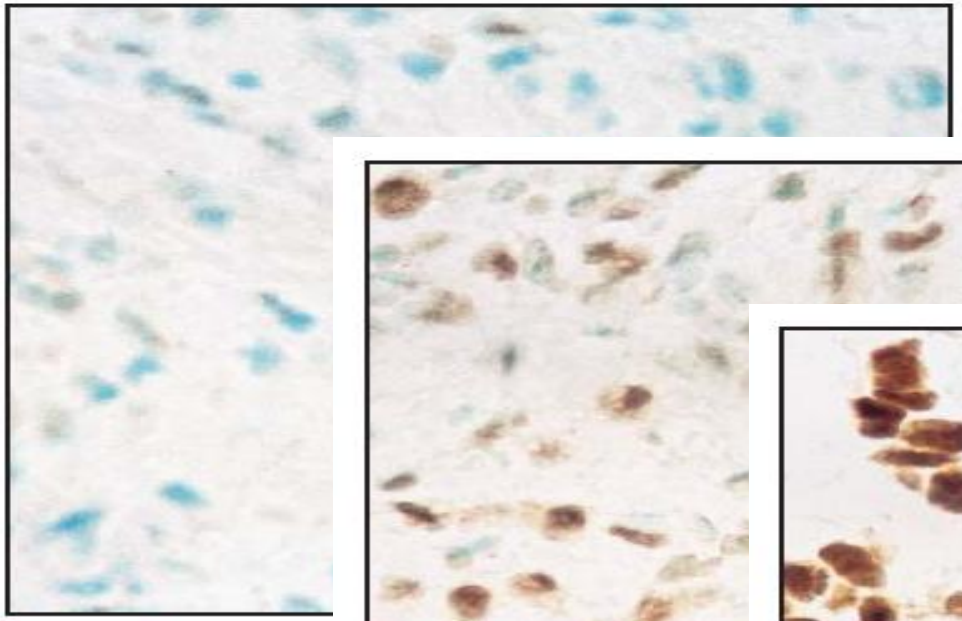


Image 1 Fixation, 3

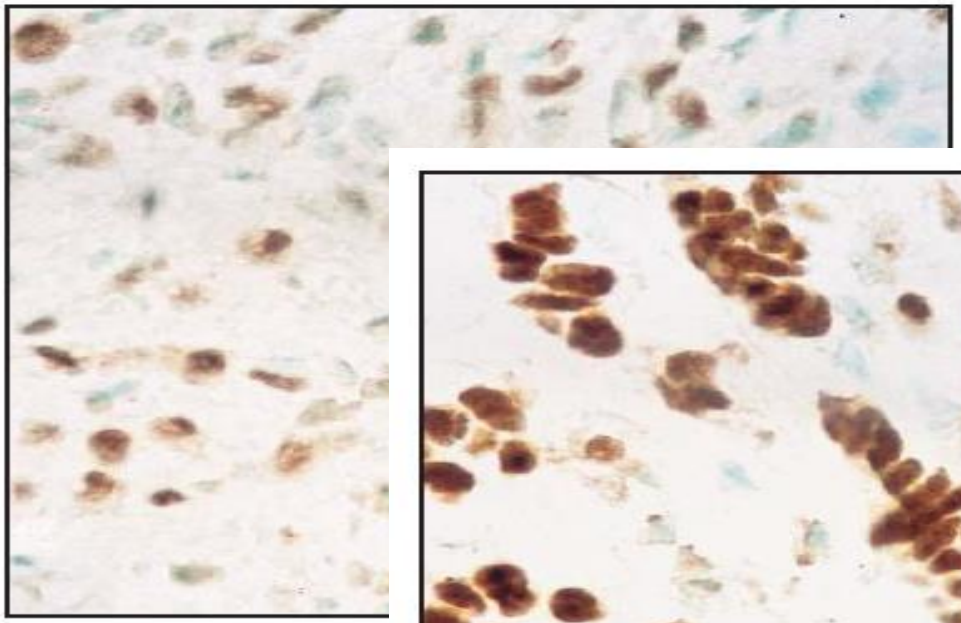


Image 2 Fixation, 6 h; a

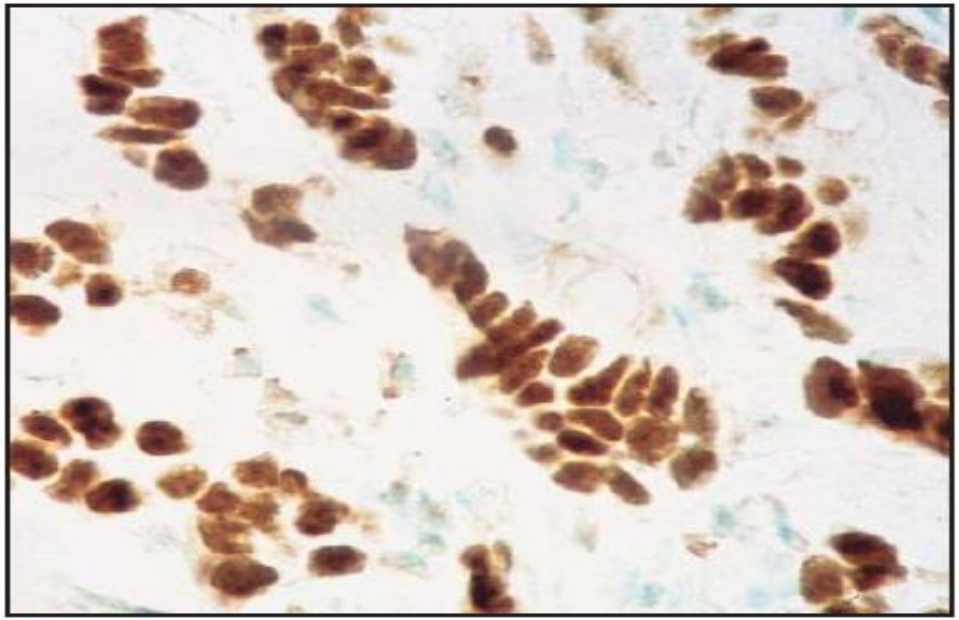
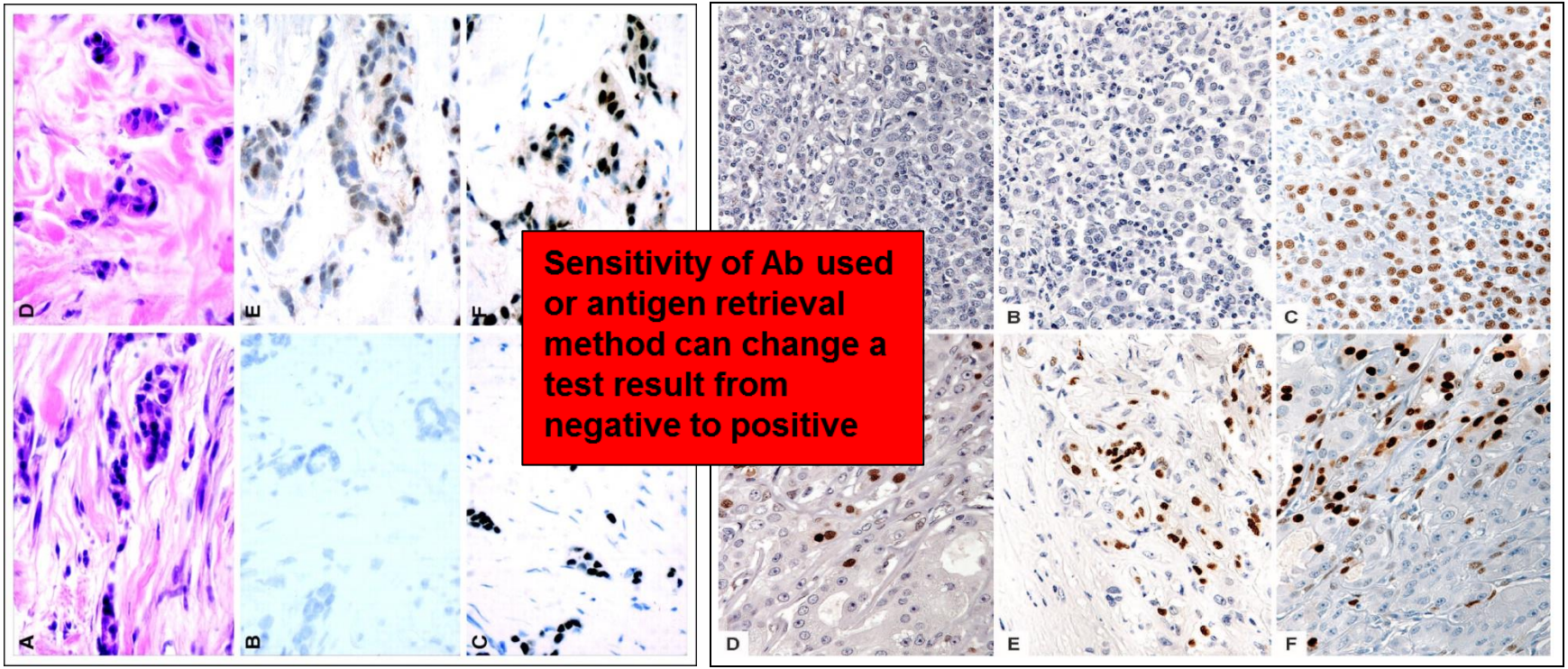


Image 3 Fixation, 8 h; antigen retrieval, 40 min.

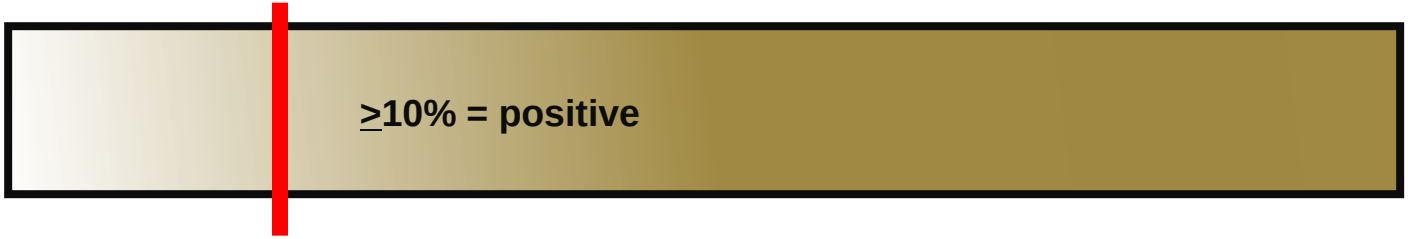
Goldstein, Am J Clin Pathol, 2003

Comparison of ER/PR Antibody Reagents



Cheang, 2005; Troxell, 2017

ER Interpretation/Scoring



Fewer positives
Pts potentially denied therapy

End up with a lot more positives!
Pts potentially treated with little benefit

2010

American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Unabridged Version)

Hammond, Arch Pathol Lab Med, 2010

Goal

Improve accuracy of hormone receptor testing and the utility of ER and PR as prognostic and predictive markers for assessing in situ and invasive breast carcinomas

Standardization

Accurate measurement of ER is critical for the care of all breast cancer patients

False Positive and Negative Results

False positive ER is very rare

- More likely due to misinterpretation of entrapped normal epithelium
- Overinterpretation of cytoplasmic staining
- Reporting the result for the control on the same slide as the carcinoma, instead of the carcinoma
- Transcribing error

False Positive and Negative Results

- False negative ER results are more common
- Most relate to issues discussed earlier
  - Cautery, decalcification procedures, prolonged ischemic time or poor fixation, technical issues, interpretation errors
- Tumor heterogeneity
- Transcribing error
- Check for normal internal control
- Correlate with histology

Estrogen Receptor in Breast Cancer

- ER is a weak prognostic factor
- But a strong predictive factor
- Thus women with ER+ cancers have a strong likelihood for responding to hormonal therapies

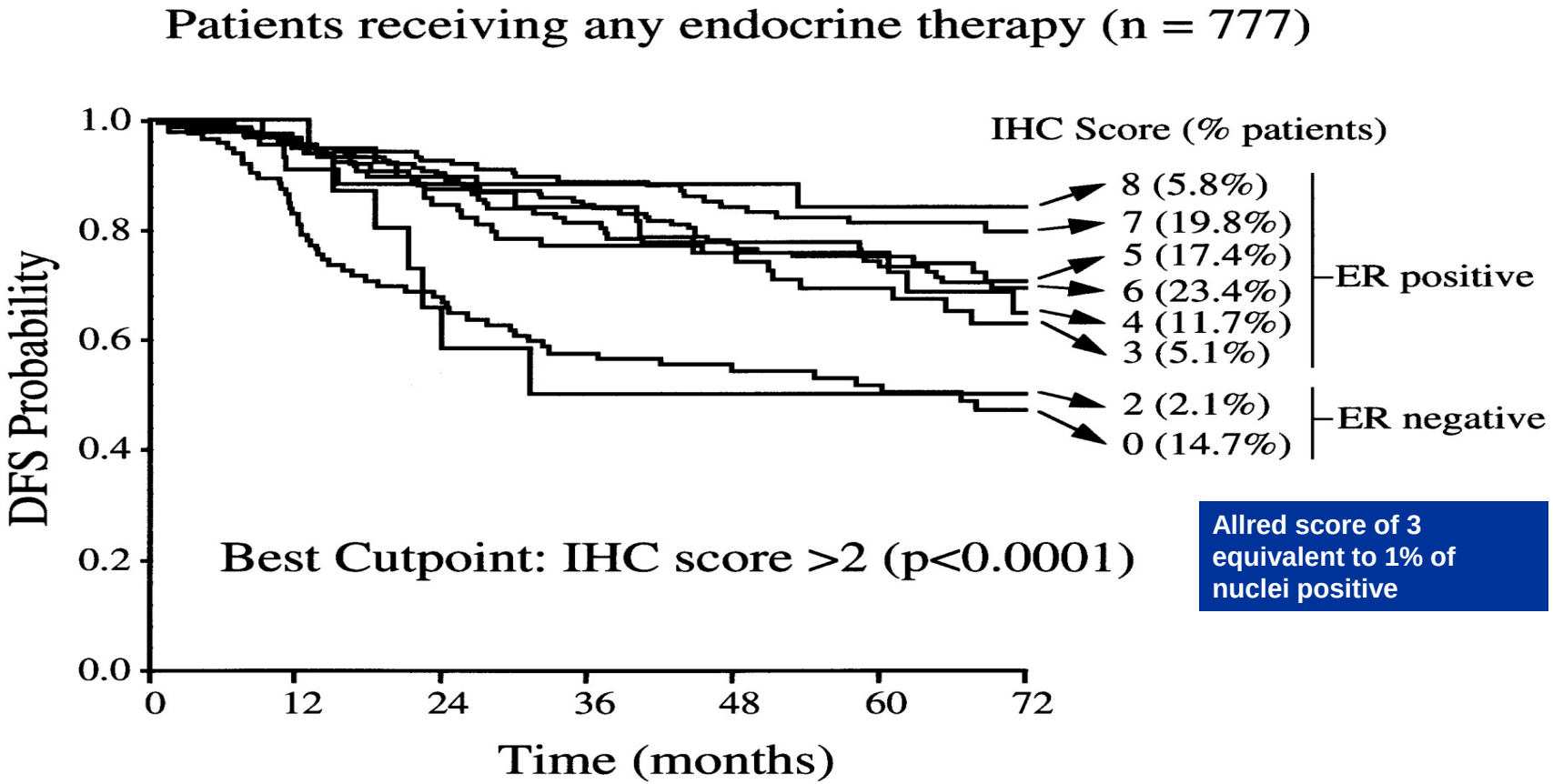
Quantification of ER

Why quantify?

"The percentage of stained tumor cells may provide valuable predictive and prognostic information to inform treatment strategies"

ASCO/CAP Guidelines, 2010

ER Level and Disease-free Survival



Allred score of 3 equivalent to 1% of nuclei positive

Harvey J M et al. JCO 1999;17:1474-1474

JOURNAL OF CLINICAL ONCOLOGY

Categories of Endocrine Responsiveness

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Highly endocrine responsive:

- Tumors express high levels of both HRs in the majority of cells

Incompletely endocrine responsive:

- Some expression of HRs but at lower levels or lacking either ER or PR

Endocrine non-responsive:

- Tumors having no detectable expression of steroid hormone receptors

Goldhirsch, St. Gallen Conference 2007, Ann Oncol

Quantification of ER

- Overall survival
- Disease-free survival
- Recurrence/relapse-free survival
- 5 year-survival
- Response to endocrine therapy
- Time to recurrence

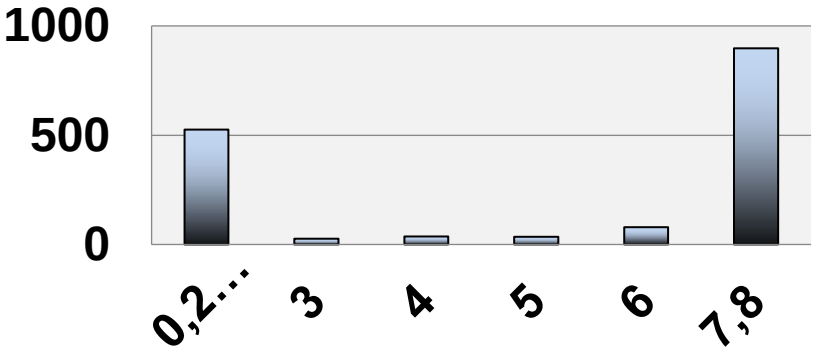
All positively associated with ER levels

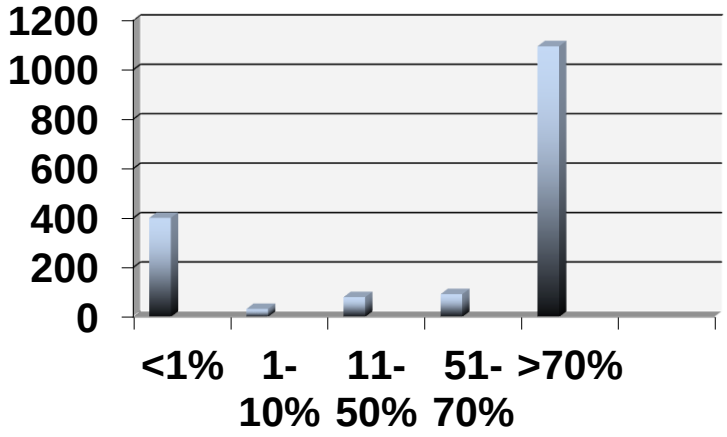
Cowen PN, 1990, Histopathology
Esteban JM, 1994, J Cell Biochem Suppl
Elledge RM, 2000 In J Cancer
Stendahl M, 2006, Clin Cancer Res
Yamashita H, 2006, Breast Cancer
Dowsett M, 2008, JCO

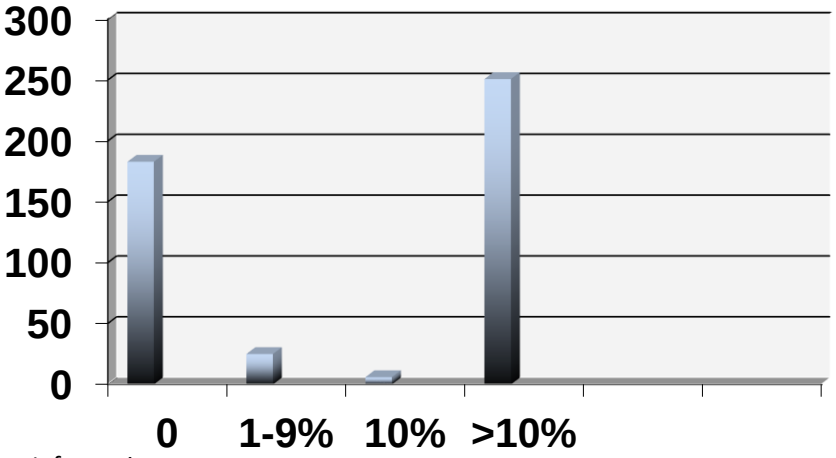
Does IHC Permit Reliable Quantification of ER?

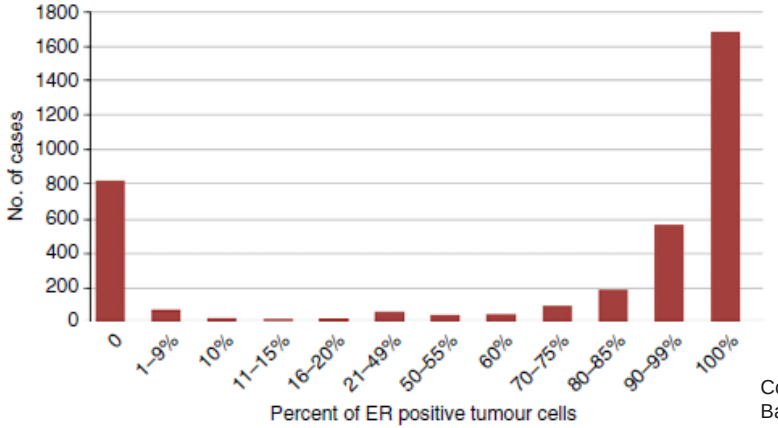
Current IHC methods utilize highly sensitive antibodies and detection systems and often employ signal enhancement

ER Distribution







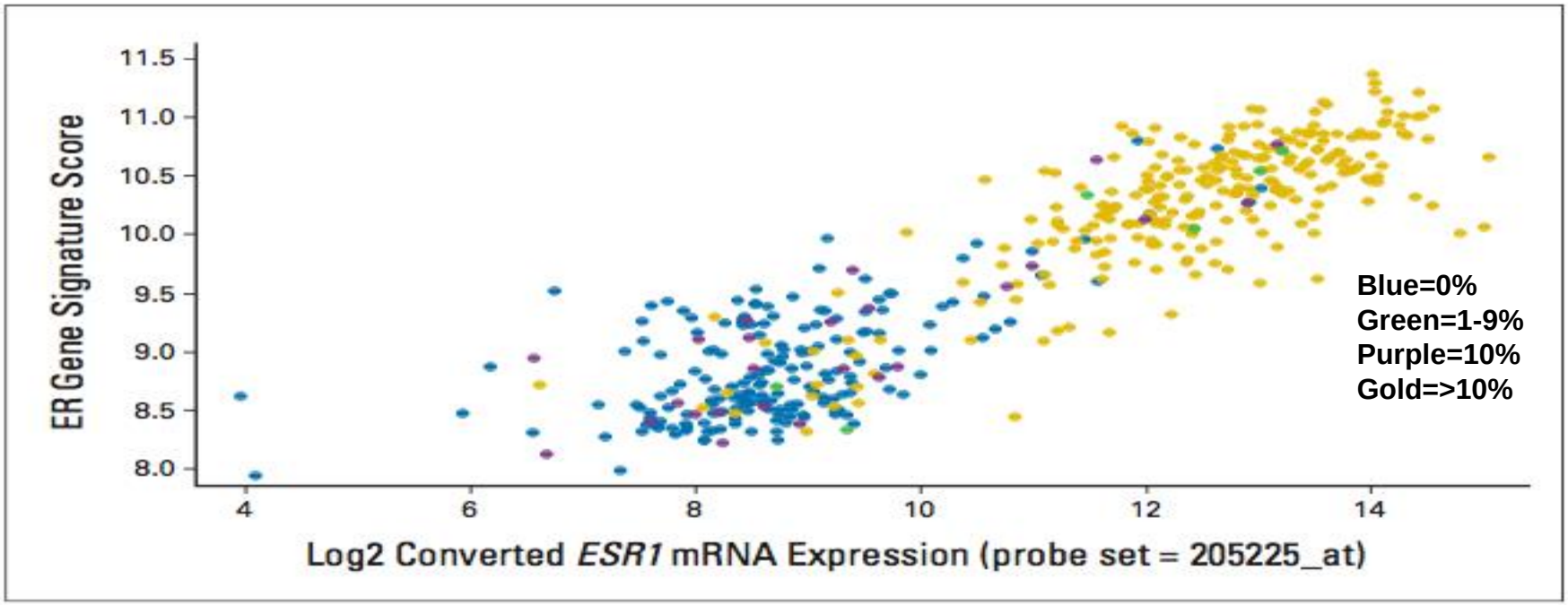


Collins, 2005
Badve, 2008
Iwamoto, 2012
Zhang, 2014
Muftah, 2017

Quantification of ER

- We know from ligand binding assay days that ER in breast cancer is a continuous variable
- ER is not biologically bimodal
- ?Need for alternative methodologies

Comparison of ER IHC, Gene Signature Score and mRNA Expression



Iwamoto, JCO, 2012

Quantification of ER

- IHC qualitative test
- Semi-quantitative at best
- Sensitivity of antibody used, or antigen retrieval method can change a test result from negative/borderline to positive, and leads to dichotomization of results
- But, while decision to treat or not is binary, the response to treatment is usually more of a spectrum
- IHC is the gold standard; ER negativity by mRNA testing does not negate an IHC ER+ result

Allison, JCO, 2020

Reporting of ER

- Report per current ASCO/CAP guidelines
- Positive: 1-100% of tumor cell nuclei stained
  - ER low positive 1-10%; include recommended comment
  - Confirmatory testing and/or adjudication for cases with weak staining or ≤10% of tumor cell nuclei staining
  - Report status of internal positive control for low positive group
- Negative: reported as either <1% or 0
- Be aware that results in the 1-5% range may vary by observer

Allison, JCO, 2020

Reporting of PR

- Same reporting criteria as ER
- Extremely rare for a tumor to be ER-/PR+, thus PR essentially prognostic/predictive in the ER+ disease
- ER+, PR low + or negative typically higher grade, more proliferative tumors (luminal B-like)
- Worse prognosis, poorer response to therapy
- Proposed mechanisms of PR loss include:
  - Abnormal ER alpha signaling pathways
  - Loss of PR gene
  - Downregulation by HER2

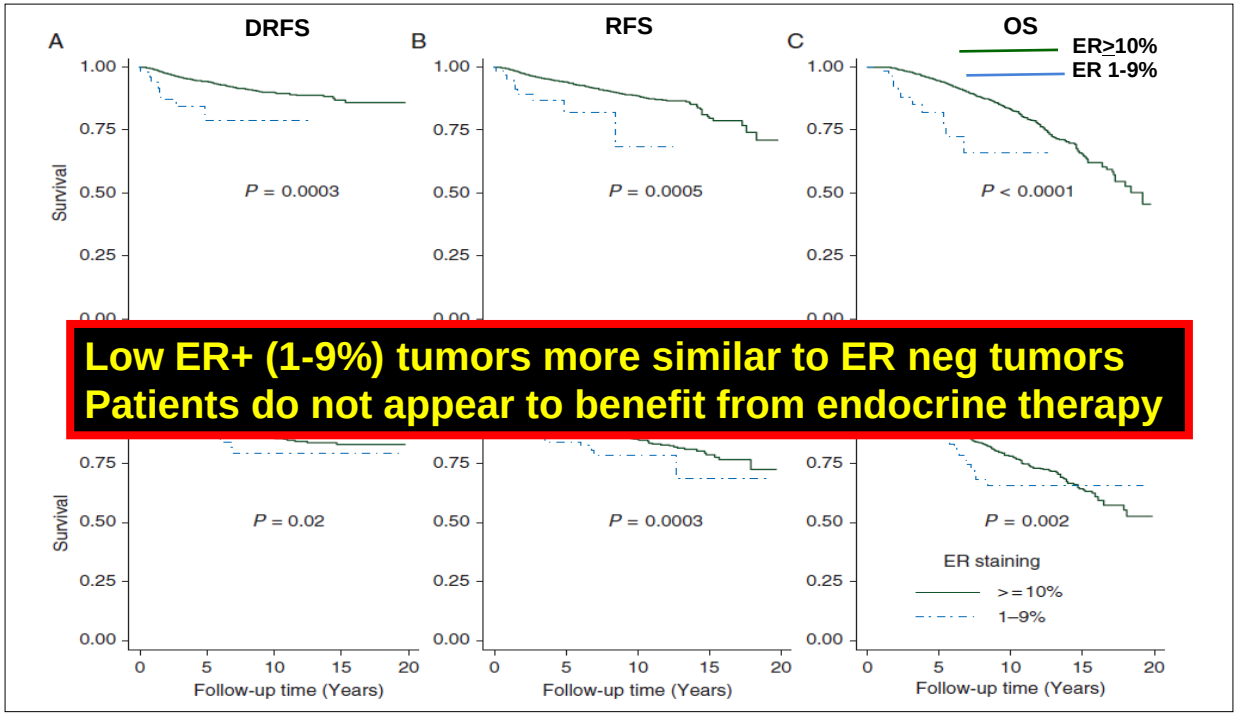
What about low ER group?

Low ER positive group

- Appears to be a heterogeneous group for which benefit from ER targeted therapy will be difficult to determine
- Some studies indicate tumors are more similar to triple negative cancers (e.g. are basal-like by molecular profiling, are more likely to be *BRCA* mutation carriers, are less likely to respond to tamoxifen-as a group)

Which threshold for ER positivity? a retrospective study based on 9639 patients

M. Yi[1], L. Huo[2], K. B. Koenig[3], E. A. Mittendorf[1], F. Meric-Bernstam[1], H. M. Kuerer[1], I. Bedrosian[1], A. U. Buzdar[3], W. F. Symmans[2], J. R. Crow[1], M. Bender[1], R. R. Shah[1], G. N. Hortobagyi[3] & K. K. Hunt[1*]



Low ER+ (1-9%) tumors more similar to ER neg tumors
Patients do not appear to benefit from endocrine therapy

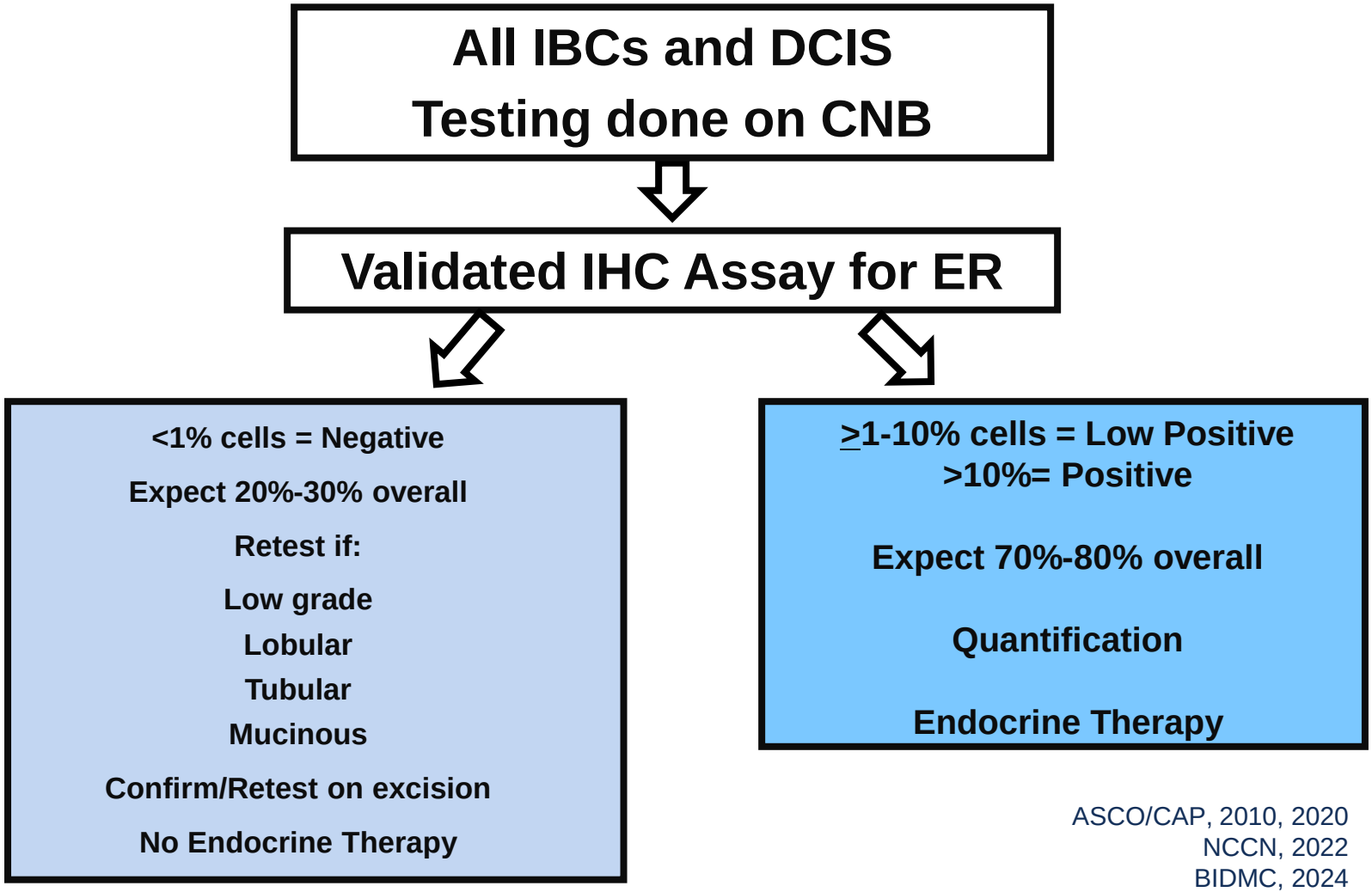
2.6% of tumors ER borderline (1-9%)

Endocrine Rx

No endocrine Rx

Ann Oncol, 2014

Heterogeneity suggests low ER+ group may need additional (molecular) testing to determine subtype/biology



ASCO/CAP, 2010, 2020
NCCN, 2022
BIDMC, 2024

Address Discordant Results

- Low grade invasive and special type cancers (eg, tubular, invasive cribriform) should be ER+
- Know the low-grade ER- cancers (eg, adenoid cystic, secretory, TCCRP)
- High grade carcinomas may be ER+ or negative
- Consider additional testing or review of morphology when result does not make sense

HER2 Testing

HER2 Receptor

- HER2 belongs to a family of growth factor receptors (HER1/EGFR, HER3 and HER4) located on the cell surface
- Responsible for cell development, proliferation and survival
- Upon activation, HER2 proteins dimerize activating intracellular signaling via MAP-kinase and PI3-kinase pathways
- HER2 gene amplification leads to HER2 overexpression on cell surface

2018

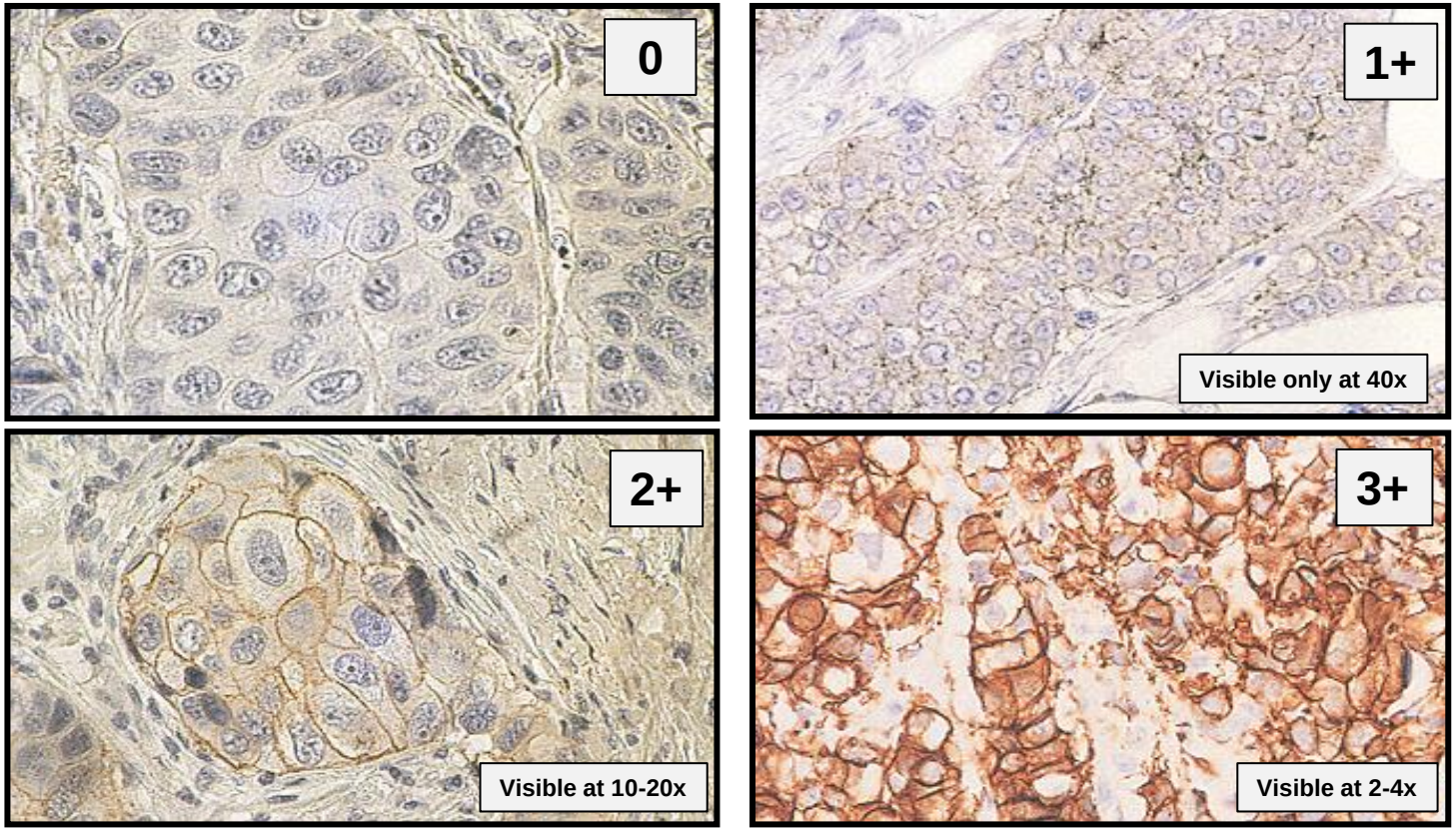
Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update

Antonio C. Wolff, M. Elizabeth Hale Hammond, Kimberly H. Allison, Brittany E. Harvey, Pamela B. Mangu, John M.S. Bartlett, Michael Bilous, Ian O. Ellis, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, Lisa M. McShane, Mitchell Dowsett

Wolff, Arch Pathol Lab Med, 2018

HER2 Scoring: HercepTest



HER2

- Current guidelines mandate additional testing with ISH for all equivocal (2+) cases
- Patients treated based on positive result (IHC 3+, or IHC 2+/FISH+)
- Newer trials indicating benefit among patients with HER2 low positive disease (IHC 1+/2+, ISH negative) with T-DXd, an antibody drug conjugate (ADC) containing trastuzumab and deruxtecan (topoisomerase I inhibitor)

Then everything changed on June 5, 2022

HER2

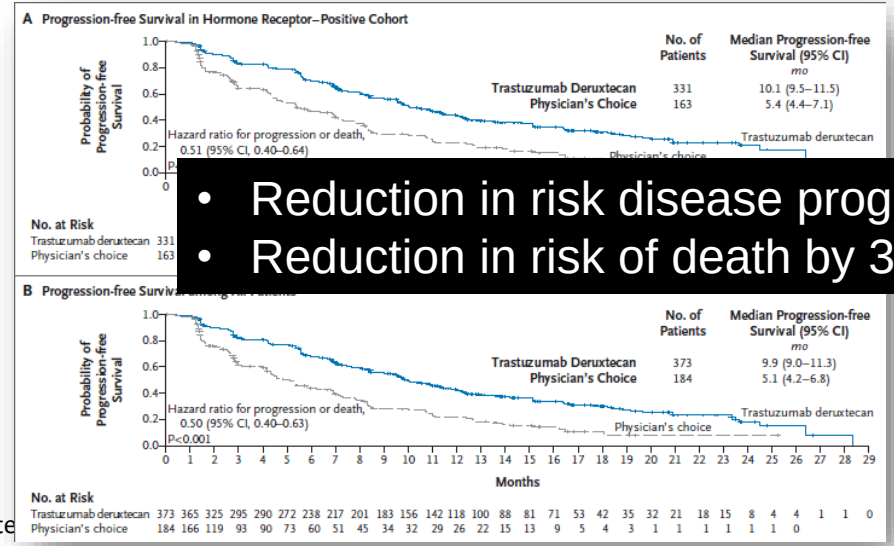
- Newer trials indicating benefit among patients with HER2 “low” (positive) disease (defined as IHC 1+/2+, ISH negative) with T-DXd, a novel HER2-targeted ADC designed to deliver a topoisomerase I inhibitor payload to HER2-expressing cancer cells

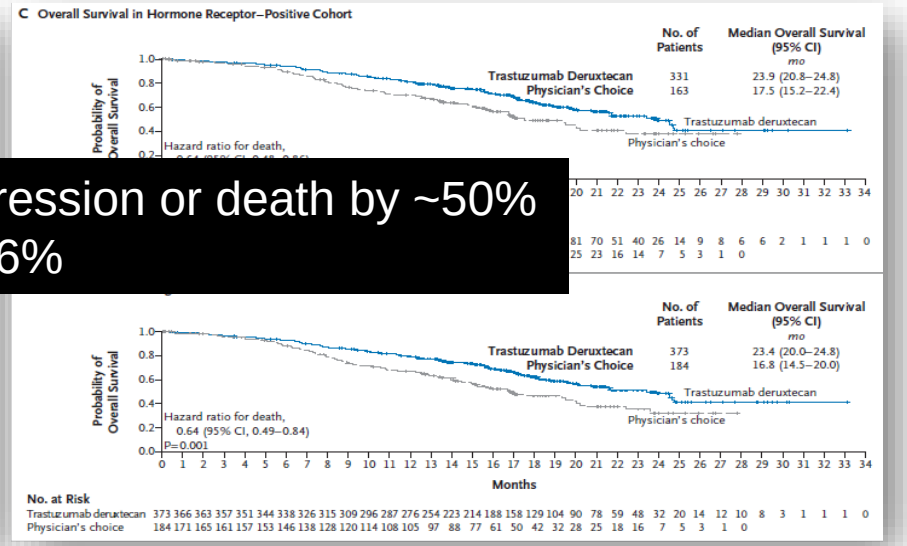
Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer

S. Modi, W. Jacot, T. Yamashita, J. Sohn, M. Vidal, E. Tokunaga, J. Tsurutani, N.T. Ueno, A. Prat, Y.S. Chae, K.S. Lee, N. Niikura, Y.H. Park, B. Xu, X. Wang, M. Gil-Gil, W. Li, J.-Y. Pierga, S.-A. Im, H.C.F. Moore, H.S. Rugo, R. Yerushalmi, F. Zagouri, A. Gombos, S.-B. Kim, Q. Liu, T. Luo, C. Saura, P. Schmid, T. Sun, D. Gambhire, L. Yung, Y. Wang, J. Singh, P. Vitazka, G. Meinhardt, N. Harbeck, and D.A. Cameron

NEJM, published online June 5, 2022

Destiny-Breast04 (DB-04)





- Reduction in risk disease progression or death by ~50%
- Reduction in risk of death by 36%

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

American Society of Clinical Oncology–College of American Pathologists Guideline Update

Antonio C. Wolff, MD; Mark R. Somerfield, PhD; Mitchell Dowsett, PhD; M. Elizabeth H. Hammond, MD; D
Lisa M. McShane, PhD; Thomas J. Saphner, MD; Patricia A. Spears, BS; Kimberly H. A

# of Clinical Oncology–College of ologists Guideline Update for Human al Growth Factor Receptor 2 Testing in Breast Cancer

How Low Can HER2 Go?

Stuart J. Schnitt, MD; Paolo Tarantino, MD; Laura C. Collins, MD

n Factor Receptor 2 "Low" in ast Cancer in 2023

Shabnam Jaffer, MD

AFFIRMED PRIOR GUIDELINE

June, 2023

T-DXd, an antibody drug conjugate (ADC) containing trastuzumab and deruxtecan (topoisomerase I inhibitor)

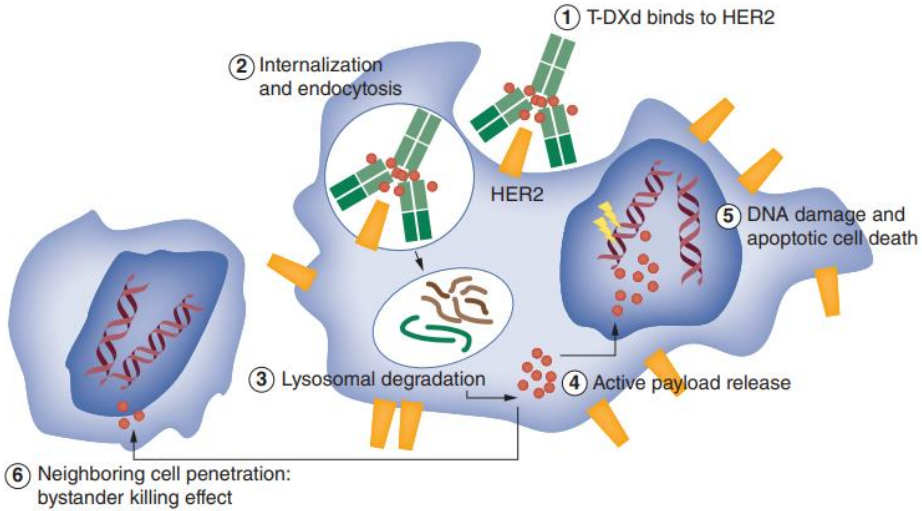
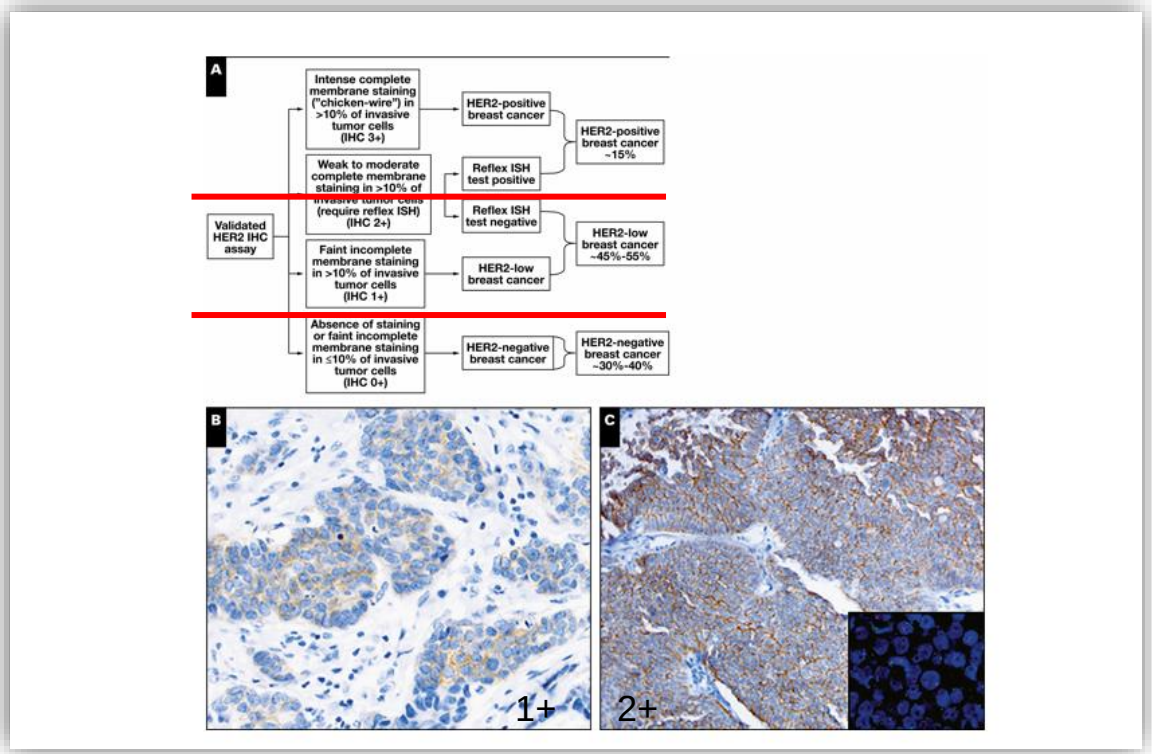


Figure 1. Mechanism of action of trastuzumab deruxtecan.

Lee, Future Oncol, 2022

HER2 low positive tumors: 3-tier scoring system



Zhang, AJCP, 2022

Problems with Diagnosis of HER2-Low Breast Cancer

- HER2 IHC assays developed to detect HER2+ breast cancers (i.e., HER2 3+)
- Insufficient dynamic range to reliably distinguish between HER2 0 and HER2 1+ cases and were not meant to be used for this purpose
- Very poor interobserver reproducibility in distinguishing HER2 0 from HER2 1+

Problems with Diagnosis of HER2-Low Breast Cancer

- Many labs do not use Ventana platform and 4B5 antibody used in DB-04 trial
- Sensitivity for identifying HER2 1+ cases varies among different assays (HercepTest > 4B5)
- Pre-analytic factors likely of critical importance
- Evolution of HER2-low expression over time
- For ~24 years (September 1998-June 2022), no need for us to distinguish HER2 0 from 1+ (both considered negative)

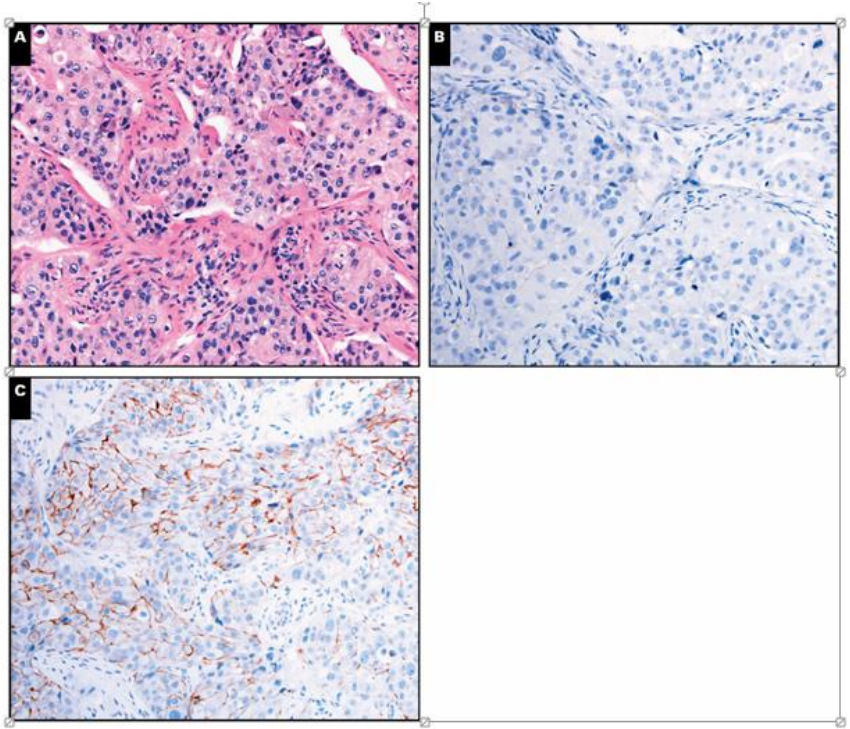
HER2-Low Breast Cancers

What are we as pathologists really being asked to do?

Identify breast cancer patients with HER2-low tumors who may benefit from T-DXd using a test that was never developed to identify those patients and cannot be done reproducibly

HER2 Low Positive Tumors-Variability in Staining

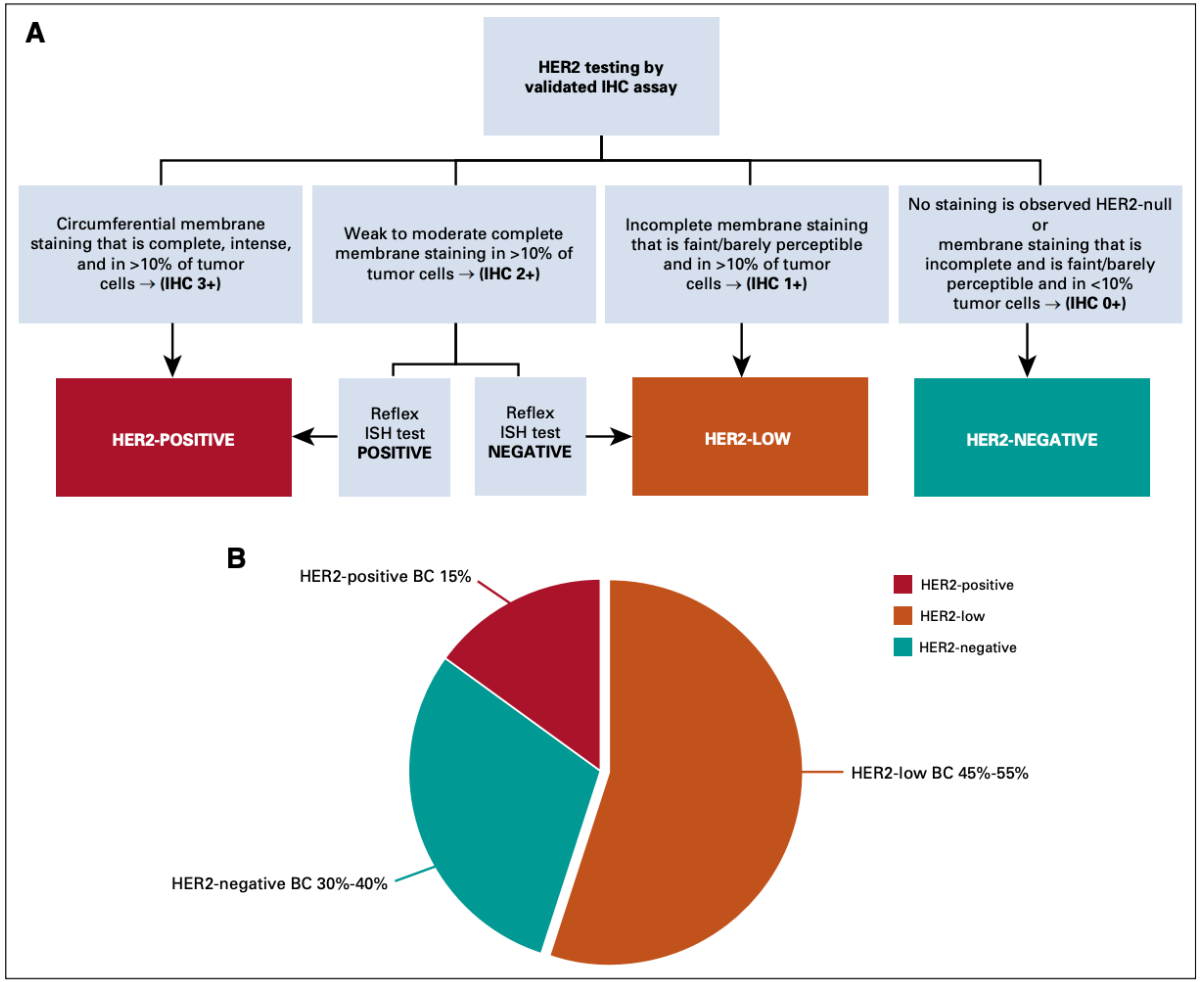
- Different staining intensity using different FDA approved-HER2 testing kits
- B. DAKO HercepTest showing essentially no staining (score 0)
- C. Ventana antibody 4B5 clone showing weak to moderate, incomplete staining in more than 10% of tumor cells (score 1+)



Zhang, AJCP, 2022

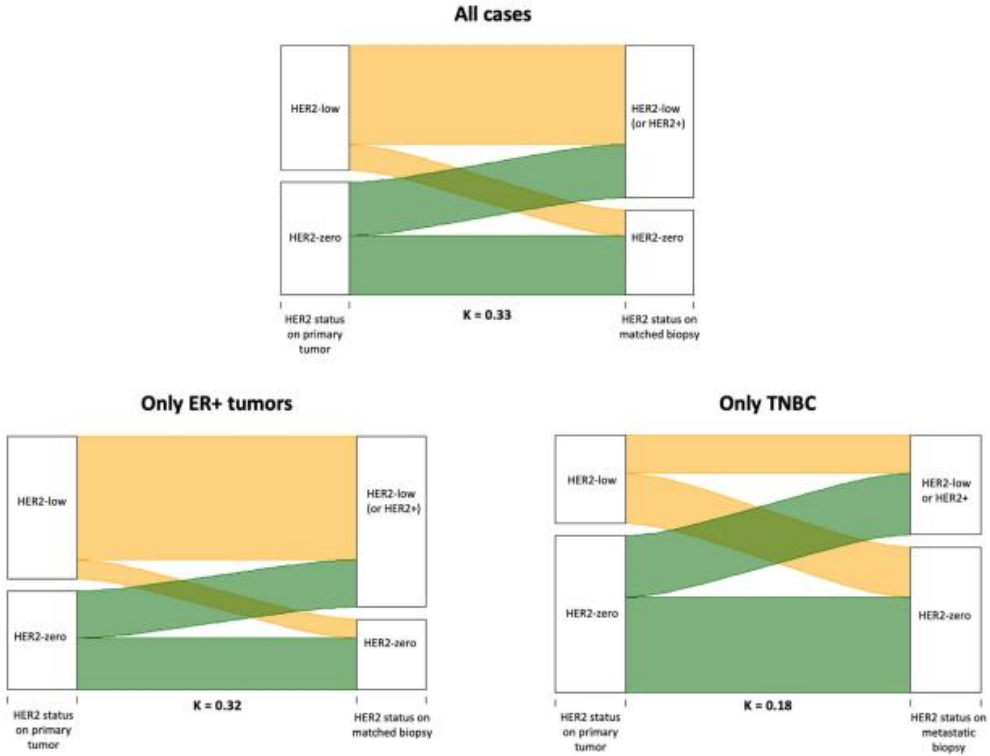
Rationale for Not Using HER2-Low Terminology in Reports

- "HER2-low" is a clinical interpretation of the IHC results (analogous to "triple negative")
- Only some cases considered "HER2-low" can be categorized as such by IHC alone (i.e., those that are HER2 1+)
- Would result in inconsistency in reporting cases in "HER2-low" based on IHC findings



Tarantino, JCO, 2020

Additional Challenge:
Evolution of HER2 low expression over time



Tarantino, Eur J Can, 2022

Lessons Learned from monarchE and Ki67 Testing

October 12, 2021

FDA approves abemaciclib with endocrine therapy for early breast cancer

(Companion diagnostic assay for Ki67 approved at the same time to distinguish Ki67 ≥20% from <20%)

Pathologists feverishly scramble to determine how best to distinguish Ki67 <20% vs ≥20% in their laboratories

March 3, 2023

Lilly

U.S. FDA Broadens Indication for Verzenio® (abemaciclib) in HR+, HER2-, Node-Positive, High Risk Early Breast Cancer

Ki67 testing no longer required

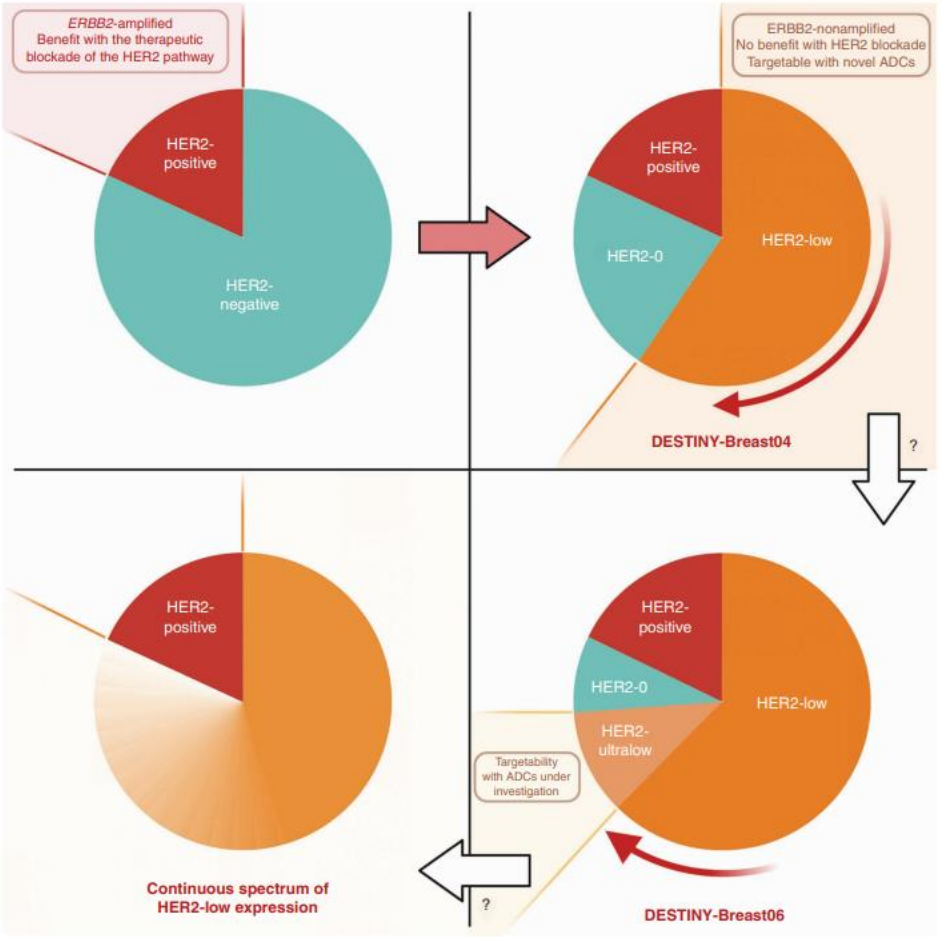
What about HER2 0?

- Unknown whether patients with HER2 0 tumors will respond to ADCs
- Limited data from the single-arm phase II “DAISY” study suggest that T-DXd may harbor relevant antitumor activity even in patients with HER2 0 tumors, with an objective response rate of 30%.
- DESTINY-Breast-06 phase 3 trial includes a subset of patients with HER2 0 tumors (those with HER2 IHC >0 and <1+, considered by some as HER2 “ultralow”); does not include patients with tumors with no HER2 protein expression at all (considered by some as HER2 “null”)

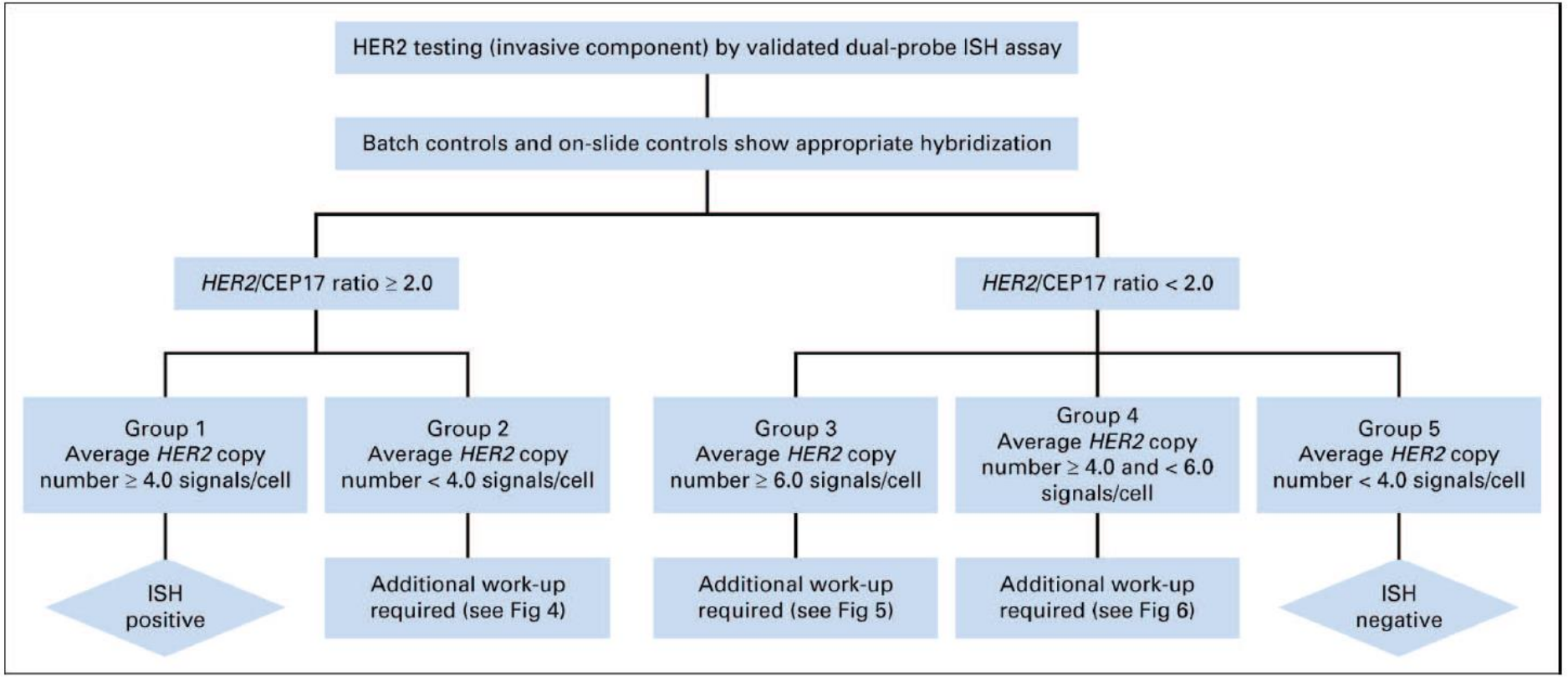
Schnitt, AJCP, 2023

Could all be unnecessary angst…

If patients with HER2 0 tumors are demonstrated to have a response rate to ADCs similar to that seen in HER2-low tumors, the attempt to distinguish HER2 1+ from HER2 0 (ultralow and null) cases may become clinically irrelevant



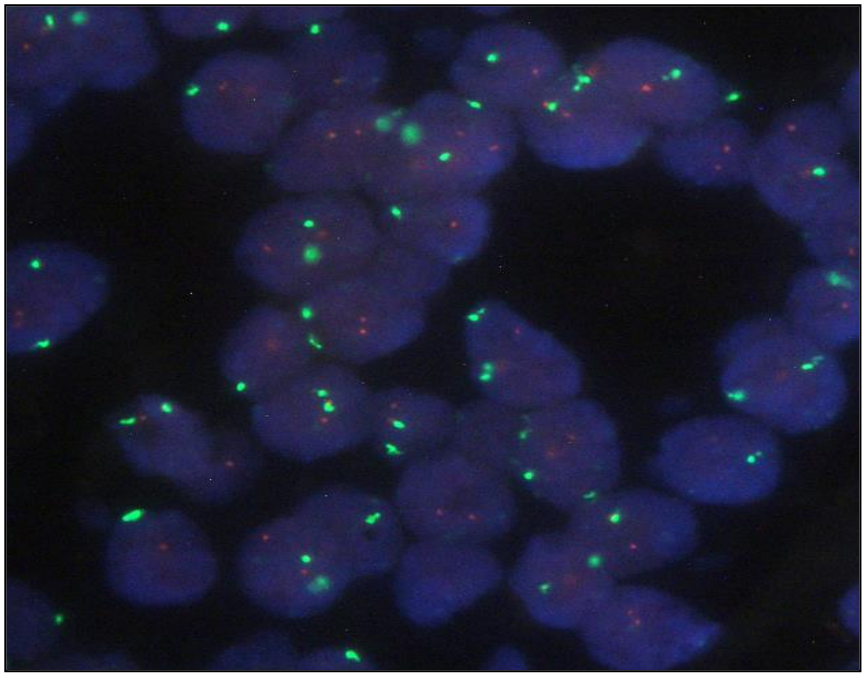
Tarantino, Cancer Discovery, 2022



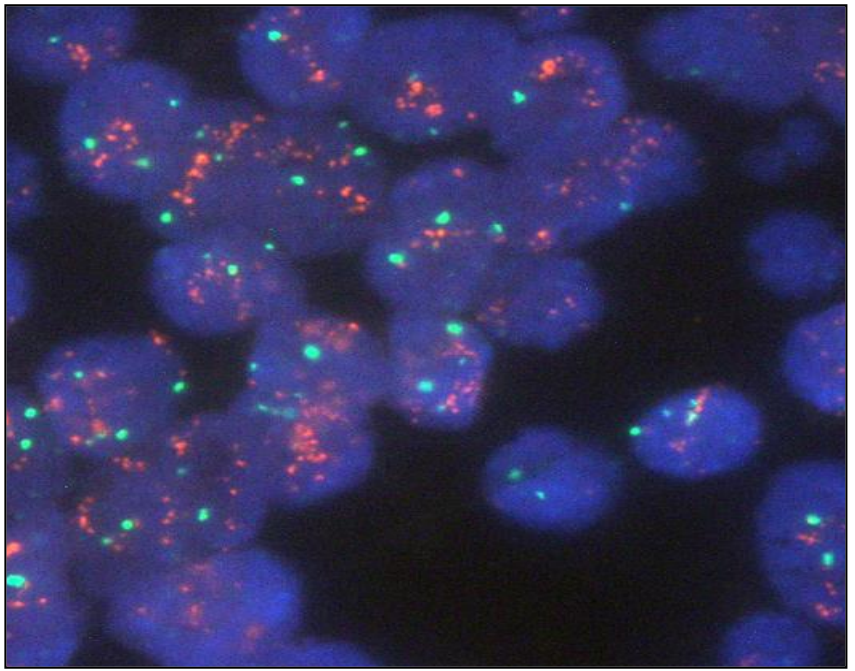
Wolff, Arch Pathol Lab Med, 2018

FISH for HER2, Dual Probe (Vysis PathVysion)

Not Amplified



Amplified

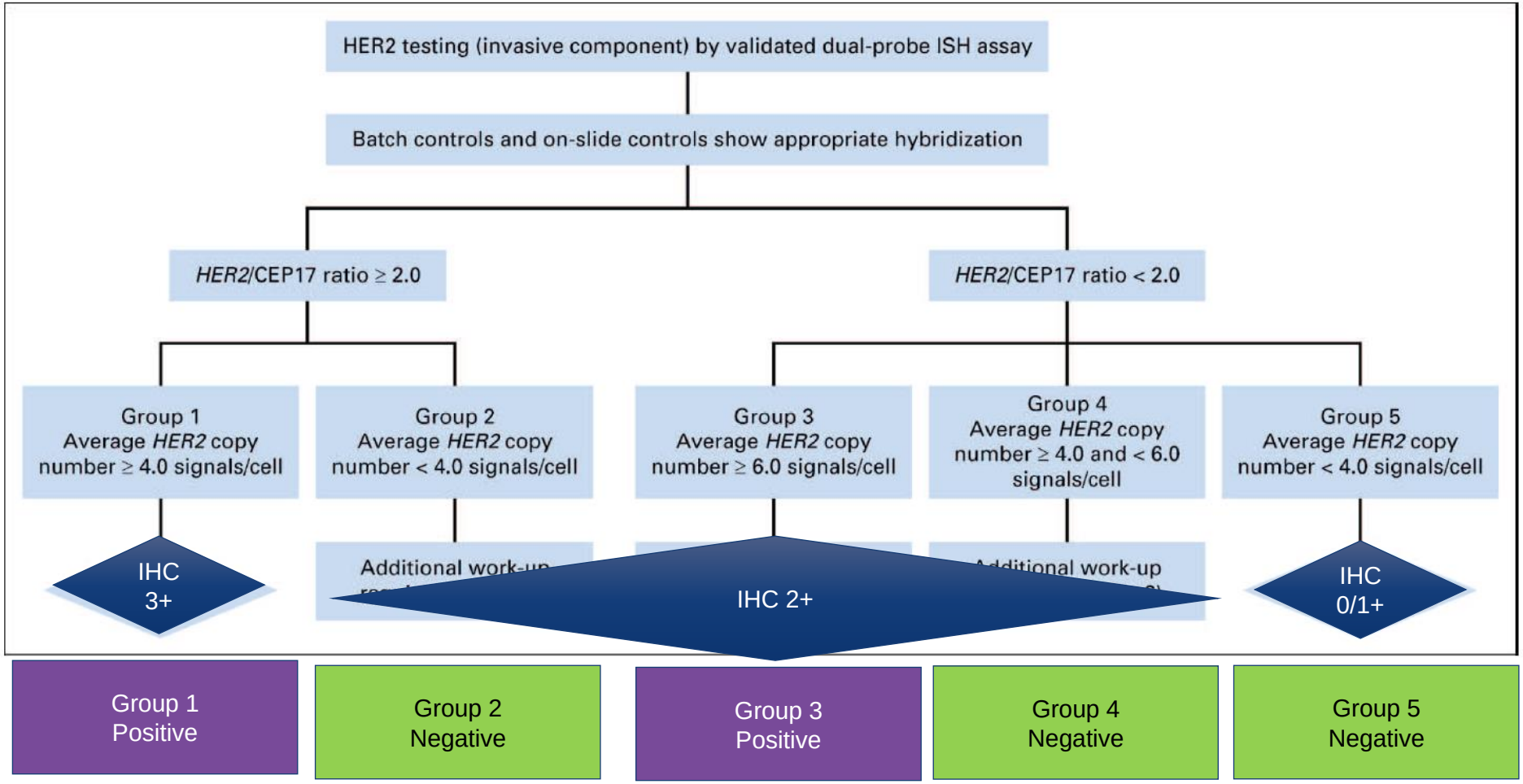


Our Practice

Beth Israel Deaconess Medical Center

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

- At BIDMC all cases have IHC and FISH performed
- For ~5% of cases in groups 2-4, IHC slide is reviewed before FISH interpretation is rendered
- Refer to guidelines for comments associated with HER2 interpretations for groups 2-4



IHC vs. FISH, Comparative Studies

- Concordance rates: 80-95%
- Very high concordance for cases scored as either negative (0-1+) or strongly positive (3+) by IHC
- Only a minority of cases with weak (2+) staining by IHC show amplification by FISH
- Current guidelines mandate additional testing with ISH for all equivocal (2+) cases
- Patients treated based on positive result (IHC 3+, or IHC 2+/FISH+)

HER2 Targeted Therapy

- Patients with breast cancers demonstrating HER2 overexpression or amplification have significantly reduced risk of recurrence and mortality
- But false positive interpretations of HER2 (IHC) has significant consequences

Modi, JCO, 2020
Denkert, Lancet Oncol, 2021

HER2 IHC False Positives

- Inappropriate patient treatments
- Incorrect tumor classification for clinical trials
- Economic ramifications to society
  - Treatment costs 10s of $1000/year
  - Cost of confirmatory test ~$90-$400
- Overstaining-normal epithelium should be negative
- Edge artifact, particularly noticeable in lobular carcinomas
- Cytoplasmic positivity-only membranous expression counts
- Overinterpretation of granular or incomplete membranous expression

HER2 Heterogeneity

Beth Israel Deaconess Medical Center

HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

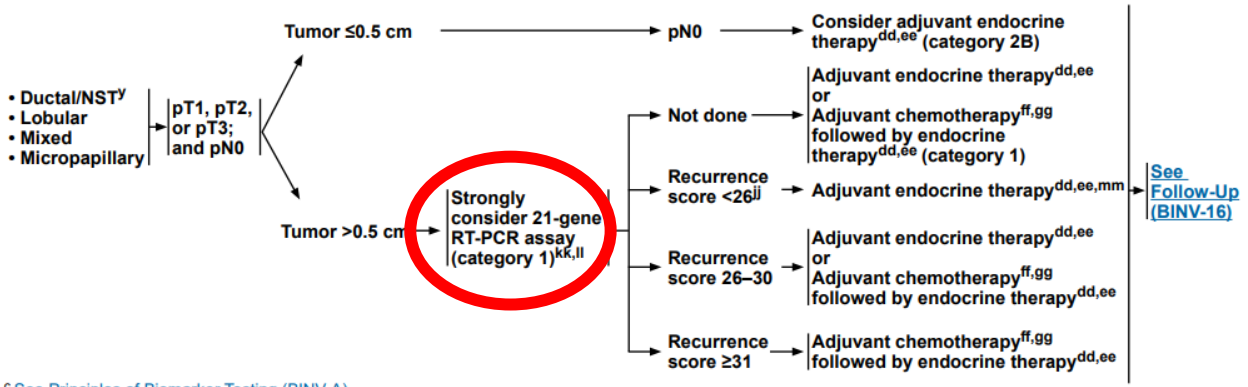
- May be seen when tumor is composed of different morphologic types or when there is subclonal diversity
- Subclonal diversity is rare, but important as there are treatment implications
- Interpretations must be on a *contiguous* area of tumor
- Report proportion of HER2+ tumor in heterogeneous cases

Address Discordant Results

- HER2+ cancers are typically:
  - High grade
  - Often have abundant eosinophilic cytoplasm or apocrine differentiation
  - High proliferative rate
- But tumors with the above features may be HER2 negative
- Good prognosis tumors are usually HER2 negative
- Review morphology and consider additional testing when result does not make sense
- Consider additional testing if tumor is HER2 negative on CNB and high grade on excision

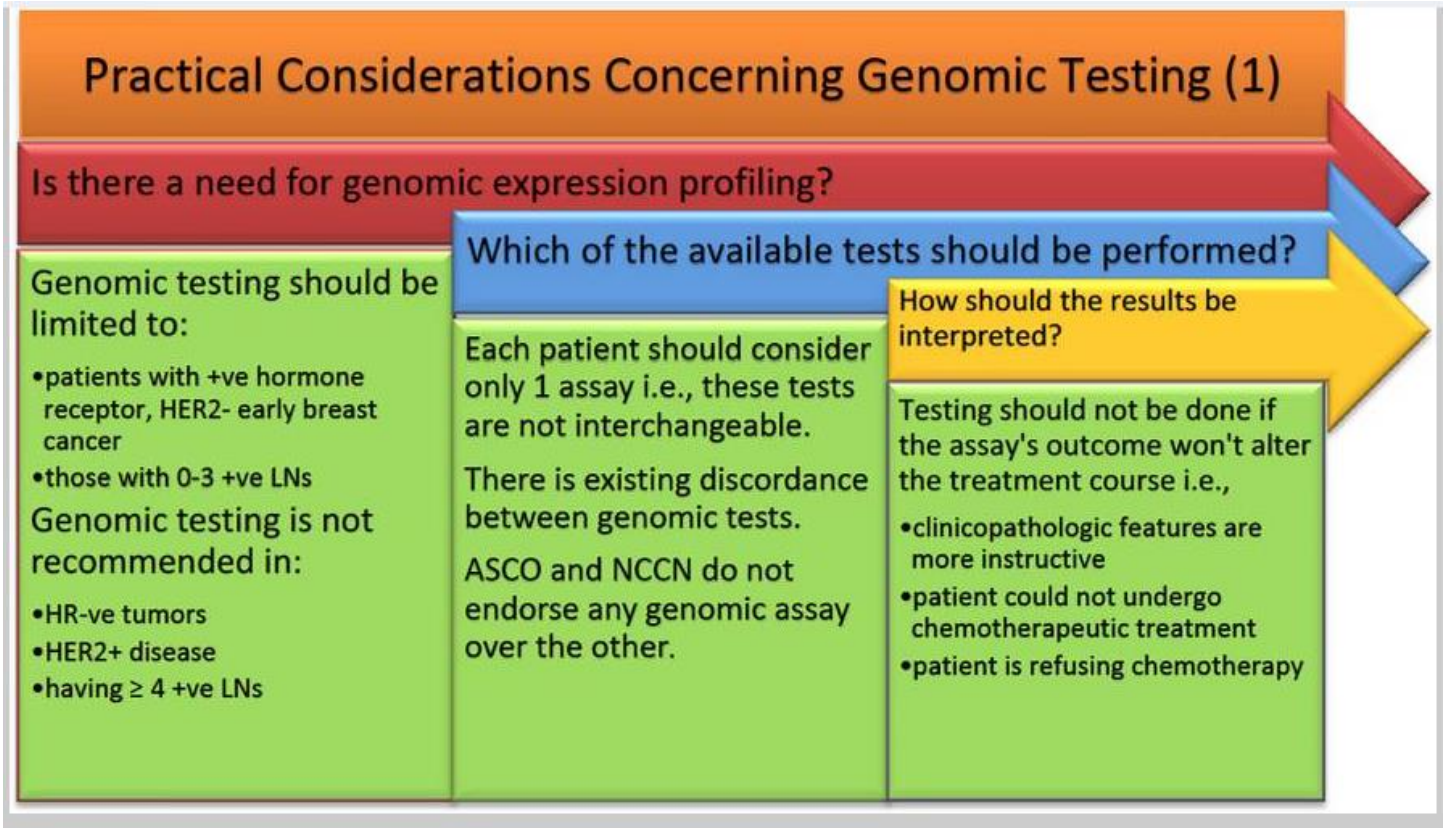
Multigene assays

SYSTEMIC ADJUVANT TREATMENT: NODE-NEGATIVE - HORMONE RECEPTOR-POSITIVE - HER2-NEGATIVE DISEASE[c,v,cc]



[c] See Principles of Biomarker Testing (BINV-A).
[v] See Special Considerations for Breast Cancer in Men (BINV-J).
[y] According to WHO, carcinoma of NST encompasses multiple patterns including medullary pattern, cancers with neuroendocrine expression, and other rare patterns.
[cc] Although patients with cancers with 1%–100% ER IHC staining are considered ER-positive and eligible for endocrine therapies, there are more limited data in the subgroup of cancers with ER-low–positive (1%–10%) results. The ER-low–positive group is heterogeneous with reported biologic behavior often similar to ER-negative cancers. This should be considered in decision-making for other adjuvant therapy and overall treatment pathway. See Principles of Biomarker Testing (BINV-A).
[dd] Consider adjuvant bisphosphonate therapy in postmenopausal (natural or induced) patients receiving adjuvant therapy.
[ee] Evidence supports that the magnitude of benefit from surgical or radiation ovarian ablation in premenopausal women with hormone receptor-positive breast cancer is similar to that achieved with CMF alone. See Adjuvant Endocrine Therapy (BINV-K).
[ff] Chemotherapy and endocrine therapy used as adjuvant therapy should be given sequentially with endocrine therapy following chemotherapy. Available data suggest that sequential or concurrent endocrine therapy with RT is acceptable. See Adjuvant Endocrine Therapy (BINV-K) and Preoperative/Adjuvant Therapy Regimens (BINV-L).
[gg] There are limited data to make chemotherapy recommendations for those >70 y of age. See NCCN Clinical Practice Guidelines for Older Adult Oncology.
[kk] Other prognostic gene expression assays may be considered to help assess risk of recurrence but have not been validated to predict response to chemotherapy. See Gene Expression Assays for Consideration of Addition of Adjuvant Systemic Chemotherapy to Adjuvant Endocrine Therapy (BINV-N).
[ll] Patients with T1b tumors with low-grade histology and no lymphovascular invasion should be treated with endocrine monotherapy as the TAILORx trial did not include patients with such tumors.
[mm] In women 50 years of age or younger with a recurrence score of 16–25, an exploratory analysis from the TAILORx study demonstrated a potential benefit to chemotherapy in younger patients. See Discussion.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.



Zerdan, Front Oncol, 2020

Commercially Available Multigene Prognostic Tests

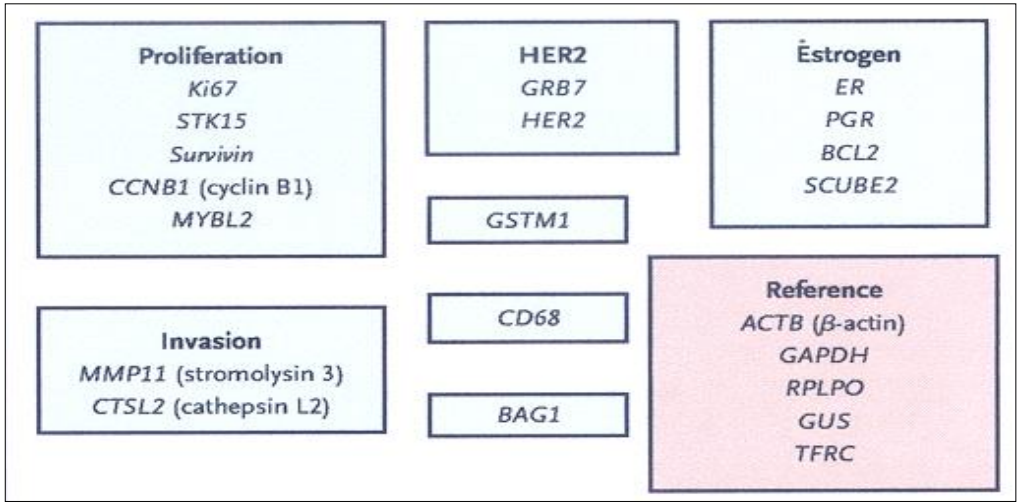
Assay	# of genes assayed	Traditional prognostic factors included	Sendout test	Cost (2018)	Score reporting
OncotypeDx (RS)	21	No	Yes	~$4000	0-100 Low/Int/High Risk
Mammaprint	70	No	Yes	~$4000	-1 to +1 Low/High Risk
Breast Cancer Index	2 + Molecular Grade Index	No	Yes	~$4000	0-10 Low/High Risk
EndoPredict Clinical (EPClin)	12	Tumor size Node status	Yes	~$2000	0-6 Low/High Risk
Prosigna (ROR)	50 + Proliferation signature	Tumor size	No	~$2080	0-100 N0 Low/Int/High N1a Low/High Risk

Adapted from Jane Brock MD PhD, Current Concepts and Controversies in Breast Pathology, 2018

A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer

Soonmyung Paik, M.D., Steven Shak, M.D., Gong Tang, Ph.D., Chungyeul Kim, M.D., Joffre Baker, Ph.D., Maureen Cronin, Ph.D., Frederick L. Baehner, M.D., Michael G. Walker, Ph.D., Drew Watson, Ph.D., Taesung Park, Ph.D., William Hiller, H.T., Edwin R. Fisher, M.D., D. Lawrence Wickerham, M.D., John Bryant, Ph.D., and Norman Wolmark, M.D.

Oncotype Dx (Genomic Health, Inc.)



NEJM 2004;351:2817

RS = +0.47 x HER2 group score
-0.34 x ER group score
+1.04 x proliferation group score
+0.10 x invasion group score
+0.05 x CD68
-0.08 x GSMT1
-0.07 x BAG1

<18	Low
18-31	Intermediate
>31	High

Recurrence Score and Prognosis in ER+, N- Breast Cancer

Table 1. Kaplan–Meier Estimates of the Rate of Distant Recurrence at 10 Years, According to Recurrence-Score Risk Categories.*

Risk Category	Percentage of Patients	Rate of Distant Recurrence at 10 Yr (95% CI)†
		percent
Low	51	6.8 (4.0–9.6)
Intermediate	22	14.3 (8.3–20.3)
High	27	30.5 (23.6–37.4)‡

* A low risk was defined as a recurrence score of less than 18, an intermediate risk as a score of 18 or higher but less than 31, and a high risk as a score of 31 or higher.
† CI denotes confidence interval.
‡ P<0.001 for the comparison with the low-risk category.

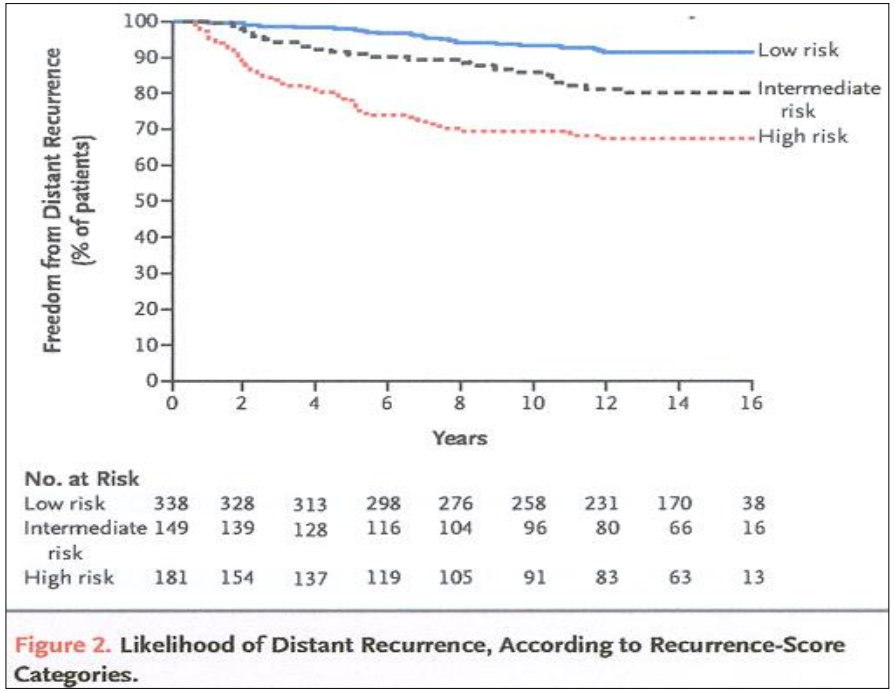


Figure 2. Likelihood of Distant Recurrence, According to Recurrence-Score Categories.

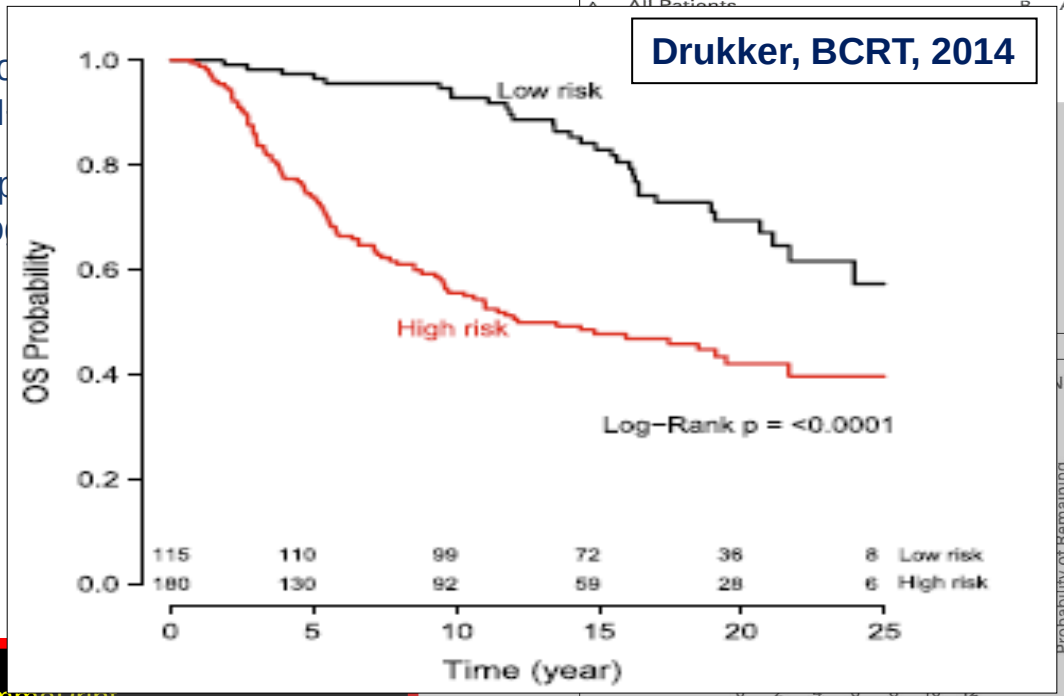
Paik, 2004

A GENE-EXPRESSION SIGNATURE AS A PREDICTOR OF SURVIVAL IN BREAST CANCER

Marc J. van de Vijver, M.D., Ph.D., Yudong D. He, Ph.D., Laura J. van 't Veer, Ph.D., Hongyue Dai, Ph.D., Augustinus A.M. Hart, M.Sc., Dorien W. Voskuil, Ph.D., George J. Schreiber, M.Sc., Johannes L. Peterse, M.D., Chris Roberts, Ph.D., Matthew J. Marton, Ph.D., Mark Parrish, Douwe Atsma, Anke Witteveen, Annuska Glas, Ph.D., Leonie Delahaye, Tony van der Velde, Harry Bartelink, M.D., Ph.D., Sjoerd Rodenhuis, M.D., Ph.D., Emiel T. Rutgers, M.D., Ph.D., Stephen H. Friend, M.D., Ph.D., and René Bernards, Ph.D.

Expression signature
identified good and po
among both N- and N

Better than standard p
on clinical and histolo
Gallen, NIH)

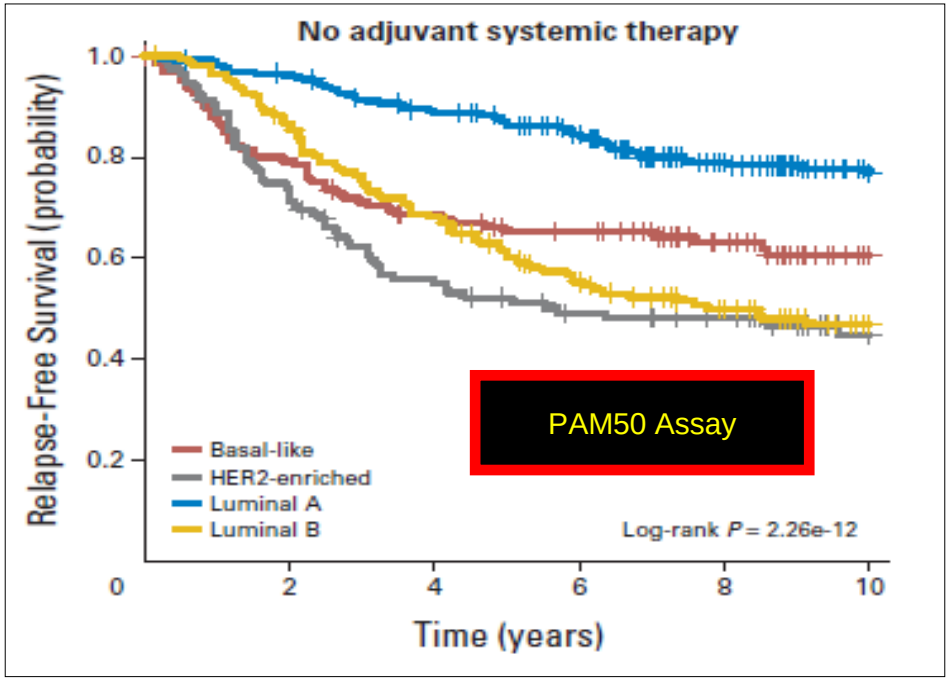


MammaPrint
(Agendia)

NEJM, 2002

Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes

Joel S. Parker, Michael Mullins, Maggie C.U. Cheang, Samuel Leung, David Voduc, Tammi Vickery, Sherri Davies, Christiane Fauron, Xiaping He, Zhiyuan Hu, John F. Quackenbush, Inge J. Stijleman, Juan Palazzo, J.S. Marron, Andrew B. Nobel, Elaine Mardis, Torsten O. Nielsen, Matthew J. Ellis, Charles M. Perou, and Philip S. Bernard



Prognostic value independent of:

- **Nodal status**
- **Size**
- **Grade**
- **ER status**

Predicted benefit from neoadjuvant chemotherapy

J Clin Oncol 2009

Comparison of the Performance of 6 Prognostic Signatures for Estrogen Receptor–Positive Breast Cancer

A Secondary Analysis of a Randomized Clinical Trial

Table 3. Univariate HRs and C Indexes for All Prognostic Signatures According to Nodal Status During Years 5 to 10

Gene Signature	Patient Group			
	Node-Negative Disease (n = 535)		Node-Positive Disease (n = 154)	
	HR (95% CI)[a]	C Index (95% CI)	HR (95% CI)[a]	C Index (95% CI)
CTS	1.95 (1.43-2.65)	0.721 (0.654-0.788)	1.61 (1.05-2.47)	0.644 (0.534-0.753)
IHC4	1.59 (1.16-2.16)	0.660 (0.576-0.745)	1.20 (0.79-1.81)	0.579 (0.460-0.697)
RS	1.46 (1.09-1.96)	0.585 (0.467-0.702)	1.24 (0.81-1.90)	0.555 (0.418-0.693)
BCI	2.30 (1.61-3.30)	0.749 (0.668-0.830)	1.60 (1.04-2.47)	0.633 (0.514-0.751)
ROR	2.77 (1.93-3.96)	0.789 (0.724-0.854)	1.65 (1.08-2.51)	0.643 (0.528-0.758)
EPclin	2.19 (1.62-2.97)	0.768 (0.701-0.835)	1.87 (1.27-2.76)	0.697 (0.594-0.799)

Sestak, JAMA Oncol, 2018

Ki-67

- Ki67 most widely used proliferation marker
- Tumor grade is a surrogate for proliferation
- Use of Ki67 shifts some luminal A-like tumors to luminal B-like
- International Ki-67 working group (IKWG) has developed guidelines
- Ki-67 (MIB-1 pharmDx (Dako Omnis) assay) approved as a companion diagnostic for the CDK 4/6 inhibitor, abemaciclib, in patients with ER+, HER2- tumors and LN+ and Ki-67 index ≥20% (though benefit independent of Ki-67 index)

Cirqueria, Breast J, 2015
Harbeck, Ann Oncol, 2021

St Gallen 2015 subtyping of luminal breast cancers: impact of different Ki67-based proliferation assessment methods

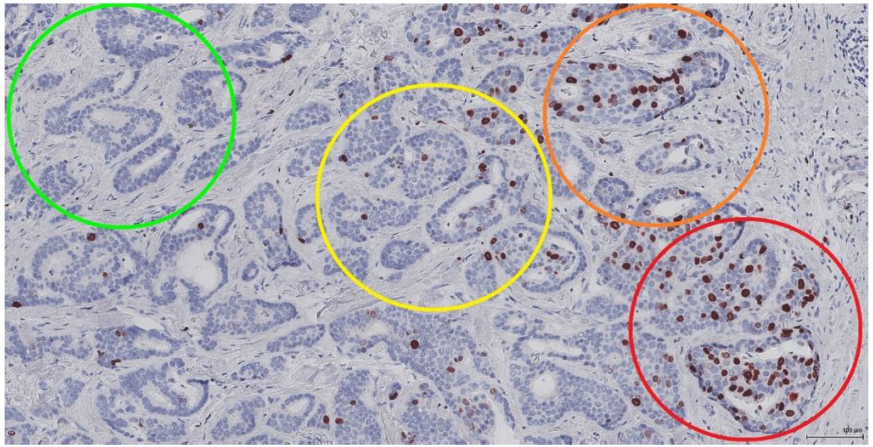
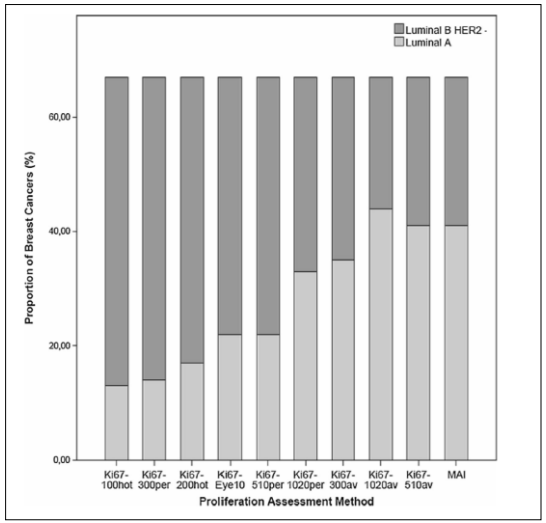


Fig. 1 Simplified example of a Ki67-labeled breast cancer showing hot spot (*red circle*), cold spot (*green circle*), periphery area (*orange circle*), and area of intermediate proliferation (*yellow circle*)

Using <20% cut point to define luminal A tumors



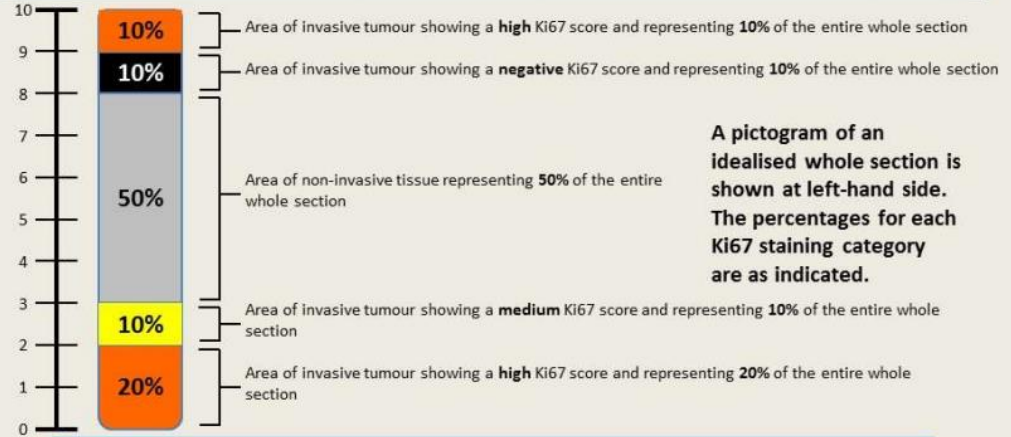
Focke, BCRT, 2016

Ki-67

- Ki-67 useful in determining prognosis in ER+, HER2 negative breast cancer to identify those who do not need adjuvant chemotherapy (IKWG)
- Analytical validity for <5% or >30% tumors
- Tremendous observer variability in the clinically relevant 10-20% range
- Preanalytic variables, such as delay in fixation, can lead to decrease in labeling index
- In the 5-30% range, multigene expression assays recommended by ASCO
- Requirement for ki-67 index >20% (companion diagnostic assay) has been removed as the indication for abemecliclib; now any ER+ high risk patient (≥4+ LNs or 1-3 positive nodes, and either histologic grade 3 or tumor size >50 mm
- A new tool for technical standardization of the Ki67 immunohistochemical assay; cell line with Ki-67 + and – cells present in incremental standardized ratios

Nielsen, JNCI, 2021
Royce, JCO, 2022
Harbeck, Ann Oncol, 2021
Aung, Mod Pathol, 2021

ESTIMATING THE PERCENTAGE OF KI67 STAINED INVASIVE TUMOUR NUCLEI: EXAMPLE 1



Area of invasive tumour showing a **high** Ki67 score and representing **10%** of the entire whole section

Area of invasive tumour showing a **negative** Ki67 score and representing **10%** of the entire whole section

Area of non-invasive tissue representing **50%** of the entire whole section

A pictogram of an idealised whole section is shown at left-hand side. The percentages for each Ki67 staining category are as indicated.

Area of invasive tumour showing a **medium** Ki67 score and representing **10%** of the entire whole section

Area of invasive tumour showing a **high** Ki67 score and representing **20%** of the entire whole section

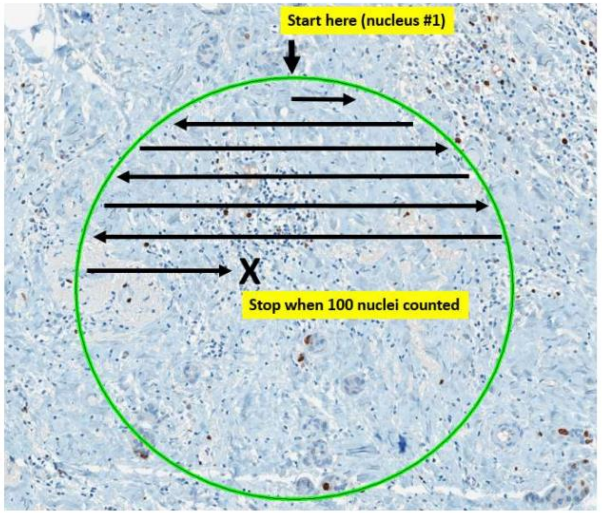
$$\text{Relative \% of invasive tumour nuclei in a particular Ki67 staining category} = \frac{\text{Total \% of invasive tumour nuclei in that category}}{\text{Total \% of all invasive tumour nuclei present}} \times 100$$

In this whole section the invasive tumour represents 50% of the total nuclei present (the other 50% is non-invasive tumour or non-tumoural). Therefore, when estimating the percentages of invasive tumour nuclei exhibiting various categories of staining the calculation is as shown in the table:

Category	Absolute % of total nuclei	Relative % of invasive tumour nuclei
Negative	**10%**	10/50 x 100 = **20%**
Low	**0%**	**0%**
Medium	**10%**	10/50 x 100 = **20%**
High	**10% + 20% = 30%**	30/50 x 100 = **60%**

Appendix A. Typewriter pattern

The following image shows a typewriter nuclei counting pattern. The green circle indicates the selected scoring field.



$$unweighted\ Ki67\ score = \frac{total\ \#\ of\ +ve\ tumor\ nuclei\ counted\ in\ all\ fields}{total\ \#\ of\ tumor\ nuclei\ counted\ in\ all\ fields} \times 100$$

$$weighted\ Ki67\ score = \sum_{i\ in\ \{neg,low,med,high\}} \%\ of\ slide\ with\ i^{th}\ staining\ category \times \frac{total\ \#\ of\ +ve\ tumor\ nuclei\ counted\ in\ fields\ with\ i^{th}\ staining\ category}{total\ \#\ of\ tumor\ nuclei\ in\ fields\ with\ it\ \ staining\ category} \times 100$$

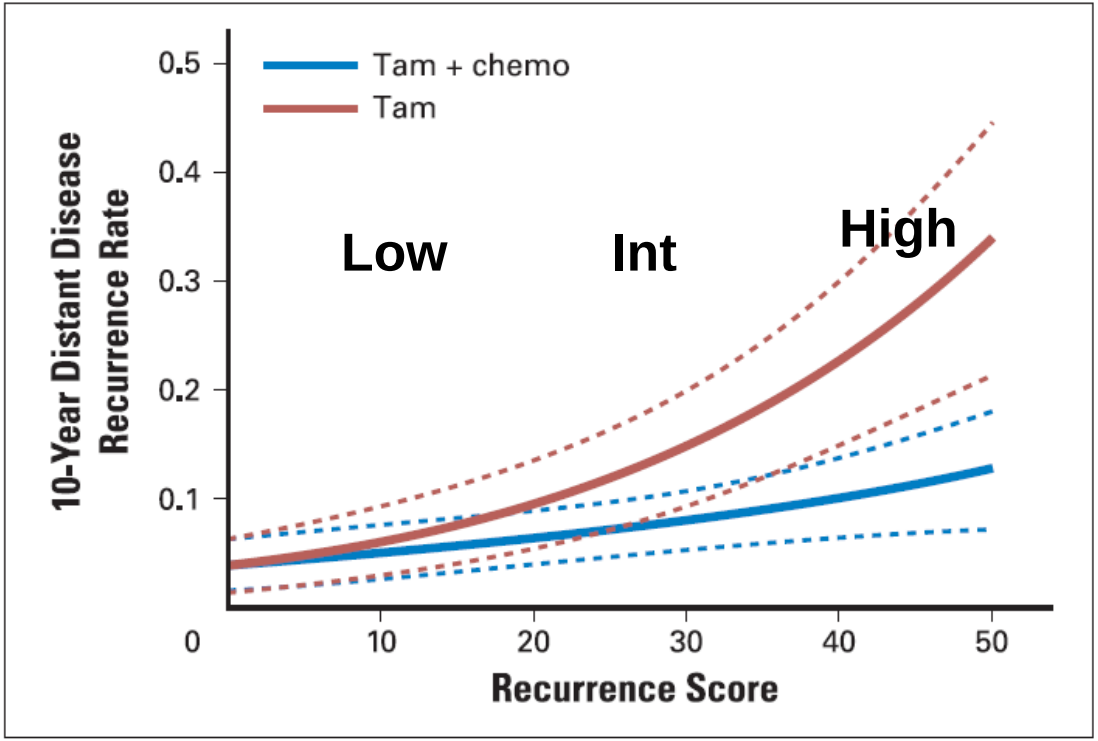
IKWG, website

Multigene Signatures
and
Predictive Factors

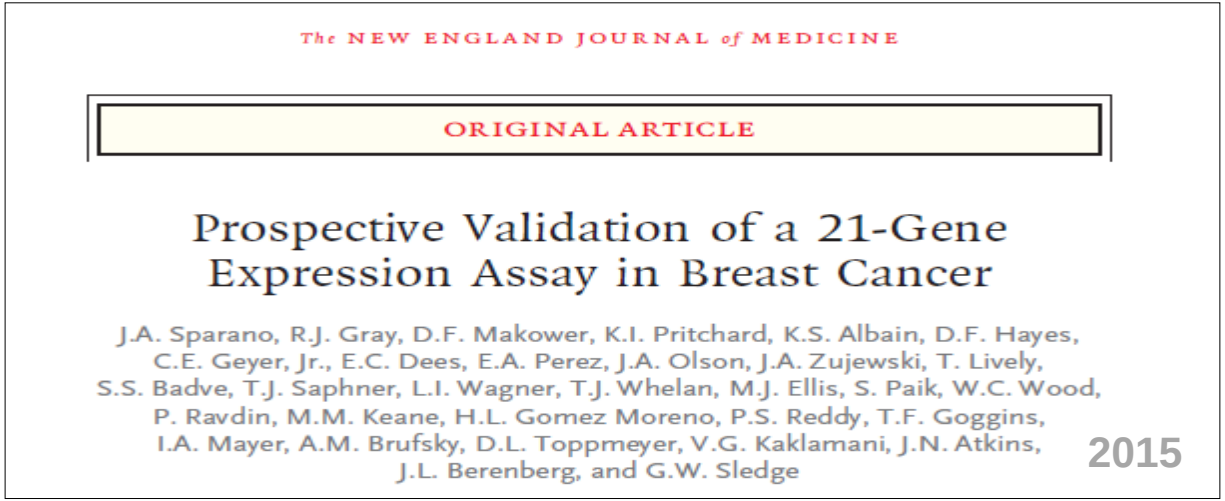
Multigene Assays for Consideration of Adjuvant Systemic Therapy in addition to Endocrine Therapy					
Test	**Predictive**	**Prognostic**	**NCCN category of preference**	**NCCN category of evidence**	**Recurrence Risk**
21 gene assay (OncotypeDX) Node negative	YES	Yes	Preferred	1	Low Intermediate High
21 gene assay (OncotypeDX) Node positive	N/A, awaiting results of RxPonder Study	Yes	Other	2A	Low Intermediate High
70 gene assay (Mammaprint) pN0 and 1-3 positive nodes	Not determined	Yes	Other	1	Low High
50 gene assay (PAM50) pN0 and 1-3 positive nodes	Not determined	Yes	Other	2A	Low Intermediate High
12 gene assay (EndoPredict) pN0 and 1-3 positive nodes	Not determined	Yes	Other	2A	Low High
Breast Cancer Index (BCI)	Not determined	Yes	Other	2A	Low High

Adapted from Goetz, JNComp Can Netw, 2019

Recurrence Score and Chemotherapy Benefit in ER+, N- Breast Cancer



Paik, 2006



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Prospective Validation of a 21-Gene Expression Assay in Breast Cancer

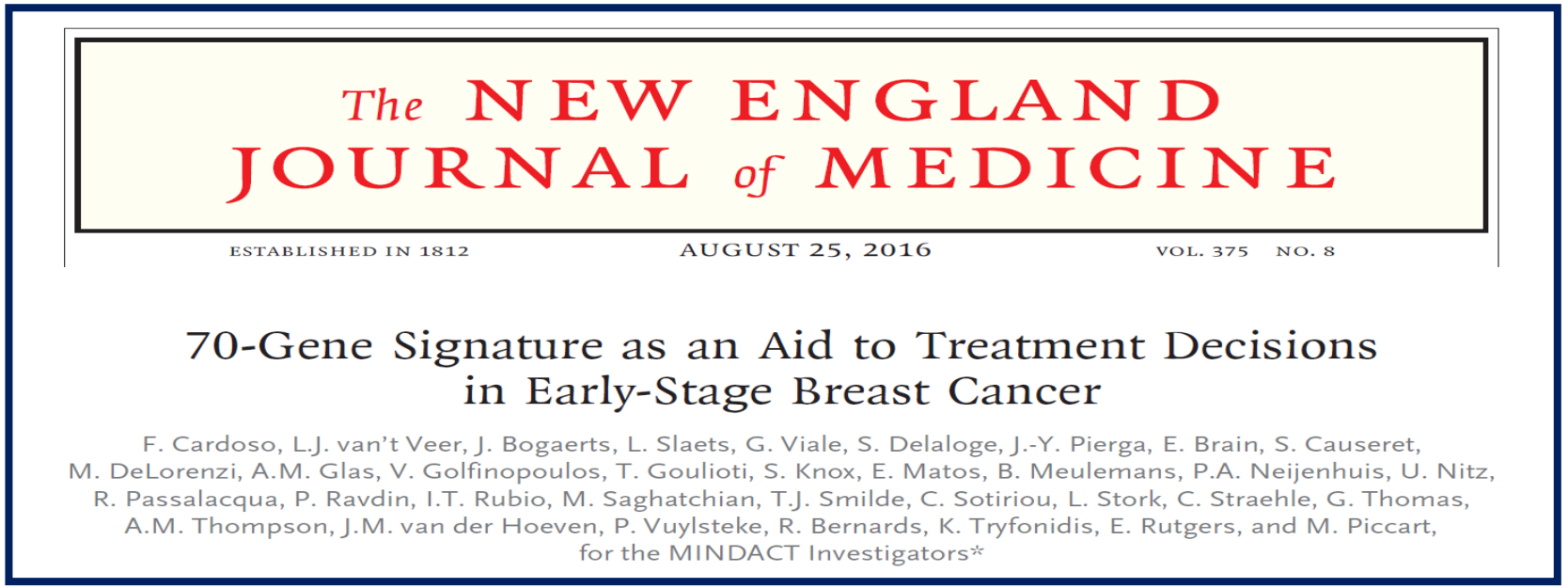
J.A. Sparano, R.J. Gray, D.F. Makower, K.I. Pritchard, K.S. Albain, D.F. Hayes, C.E. Geyer, Jr., E.C. Dees, E.A. Perez, J.A. Olson, J.A. Zujewski, T. Lively, S.S. Badve, T.J. Saphner, L.I. Wagner, T.J. Whelan, M.J. Ellis, S. Paik, W.C. Wood, P. Ravdin, M.M. Keane, H.L. Gomez Moreno, P.S. Reddy, T.F. Goggins, I.A. Mayer, A.M. Brufsky, D.L. Toppmeyer, V.G. Kaklamani, J.N. Atkins, J.L. Berenberg, and G.W. Sledge

2015

Very low rates of recurrence reported among patients with low RS in whom chemotherapy was omitted

Therefore, we are seeing 21-gene RS being used clinically with increasing frequency to identify patients with ER+ breast cancer *who may safely be spared cytotoxic therapy*

Overall survival *98%* at 5 years in TAILORx



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 25, 2016 VOL. 375 NO. 8

70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer

F. Cardoso, L.J. van't Veer, J. Bogaerts, L. Slaets, G. Viale, S. Delaloge, J.-Y. Pierga, E. Brain, S. Causeret, M. DeLorenzi, A.M. Glas, V. Golfinopoulos, T. Goulioti, S. Knox, E. Matos, B. Meulemans, P.A. Neijenhuis, U. Nitz, R. Passalacqua, P. Ravdin, I.T. Rubio, M. Saghatchian, T.J. Smilde, C. Sotiriou, L. Stork, C. Straehle, G. Thomas, A.M. Thompson, J.M. van der Hoeven, P. Vuylsteke, R. Bernards, K. Tryfonidis, E. Rutgers, and M. Piccart, for the MINDACT Investigators*

Clinical-Path High/Mammaprint-Low group:

- Distant metastasis-free survival *94.8%* at 5 years
- Overall survival only 1.5% less than those receiving chemotherapy
- *14%* absolute reduction in use of CT when risk assessed with Mammaprint

Impact of Expression Signatures For Selecting Treatment

- For patients with ER+ early breast cancer the benefits of OncotypeDX outweigh the acquisition costs
- Arguments have been made for use of alternate algorithms, such as Magee Equation (or variations thereof) which demonstrate significant cost savings to the health care economy
- In a recent study of 1,396 pts with low RS (<18) treated at MSKCC, LRR was 0.9%; 0.7% in women treated with endocrine therapy alone

Rouzier, BCRT, 2013
Turner, Cancer Med, 2019
Turashvili, BMC Cancer, 2018

Use of Biomarker to Guide Decision on Adjuvant Systemic Therapy for Women with Early-Stage Invasive Breast Cancer ER+, HER2-, node negative breast cancer		
Age	**Recurrence Score**	**Recommendation**
≥50 years old	**<26**	**Endocrine Therapy**
	26-30	**Consider Chemotherapy**
	>30	**Chemotherapy**
<50 years old	**<16**	**Endocrine Therapy**
	16-30	**Consider Chemotherapy**
	>30	**Chemotherapy**

Andre, JCO, 2019
Poorvu, JCO, 2020

Chemotherapy Benefit?

- Three prospective randomized trials-MINDACT, TAILORx and RxPONDER-have demonstrated the usefulness of gene signatures in predicting benefit from adjuvant chemotherapy in patients with ER+ breast cancer in the intermediate risk groups
- No statistically significant benefit for the addition of chemotherapy in the intermediate risk groups; with the exception of some benefit demonstrated in women <50yrs of age

Tumor Infiltrating Lymphocytes

Tumor Infiltrating Lymphocytes (TILs)

- No current recommendation to report TILs
- High TILs (>30%) more frequently seen in HER2+ and TNBC; 15-20% of cases
- TILs predictive of response to NAST
- Linked to good prognosis in HER2+ and TNBC, but poor prognosis in ER+ disease
- 10% increase in TILs correlates with 15% improvement in survival

Denkert, J Clin Oncol, 2010
Stanton, JAMA Oncol, 2016
Curigliano, Ann Oncol, 2017
www.tilsinbreastcancer.org

The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014

- Guidelines to standardize assessment and reporting of TILs in breast cancer
- Method based on clinical validity and utility
- Inter-class correlation of 0.7
- With visual reference ranges provided ICC improved to 0.89

Salgado, Ann Oncol, 2015

The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014

- Only stromal TILs within the border of the invasive carcinoma counted
- Given as a percentage of stroma occupied by TILs (no high/low cutpoints defined)
- TILS=lymphocytes and plasma cells
- Overall assessment (not hotspots)

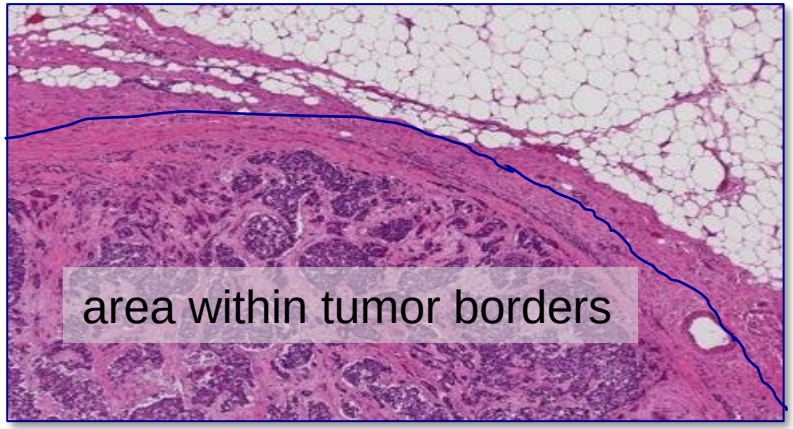
Salgado, Ann Oncol, 2015

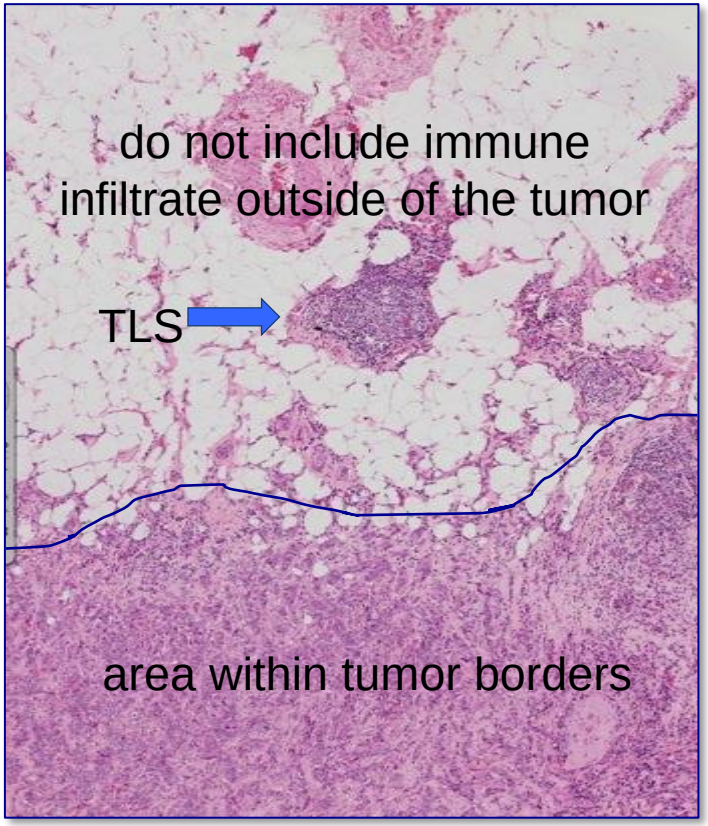
Step 1: Define area for TIL evaluation

Only TILs within the borders of the invasive tumors are evaluated

The invasive edge is included in the evaluation, but not reported separately

Immune infiltrates outside of the tumor borders, e.g. in adjacent normal tissue or DCIS are not included

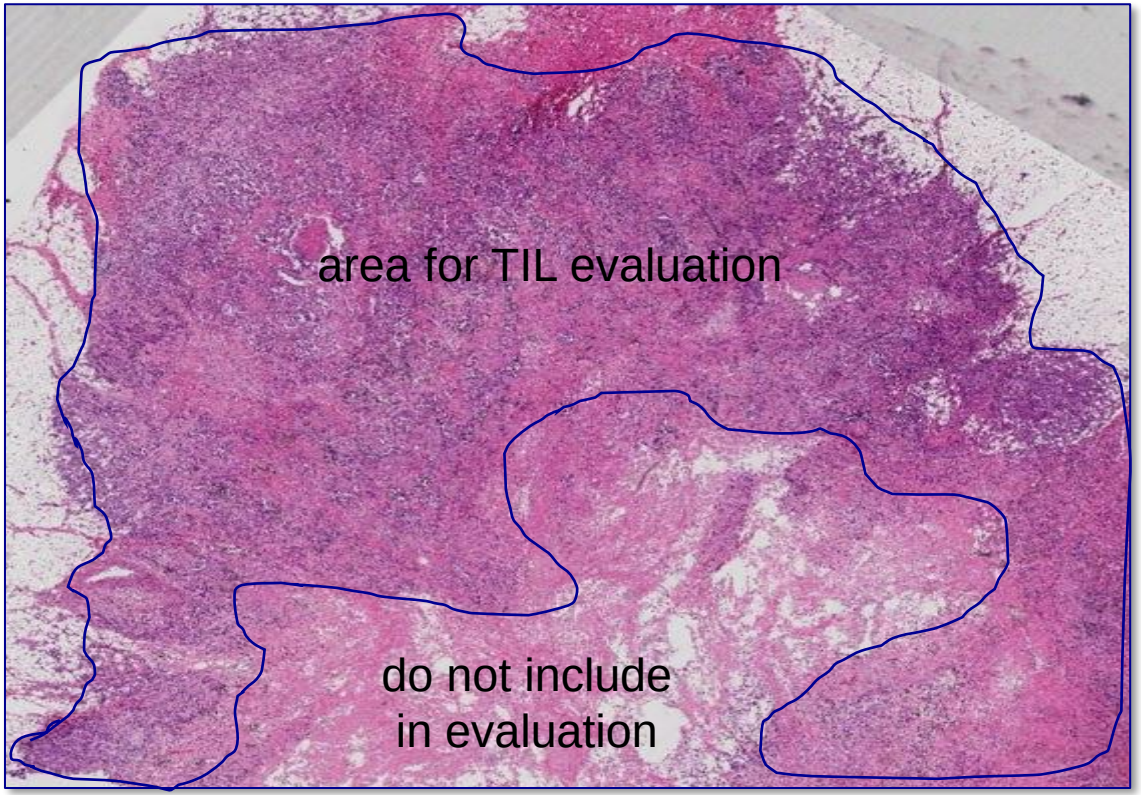




From TILs website www.tilsinbreastcancer.org

Step 1: Define area for TIL evaluation

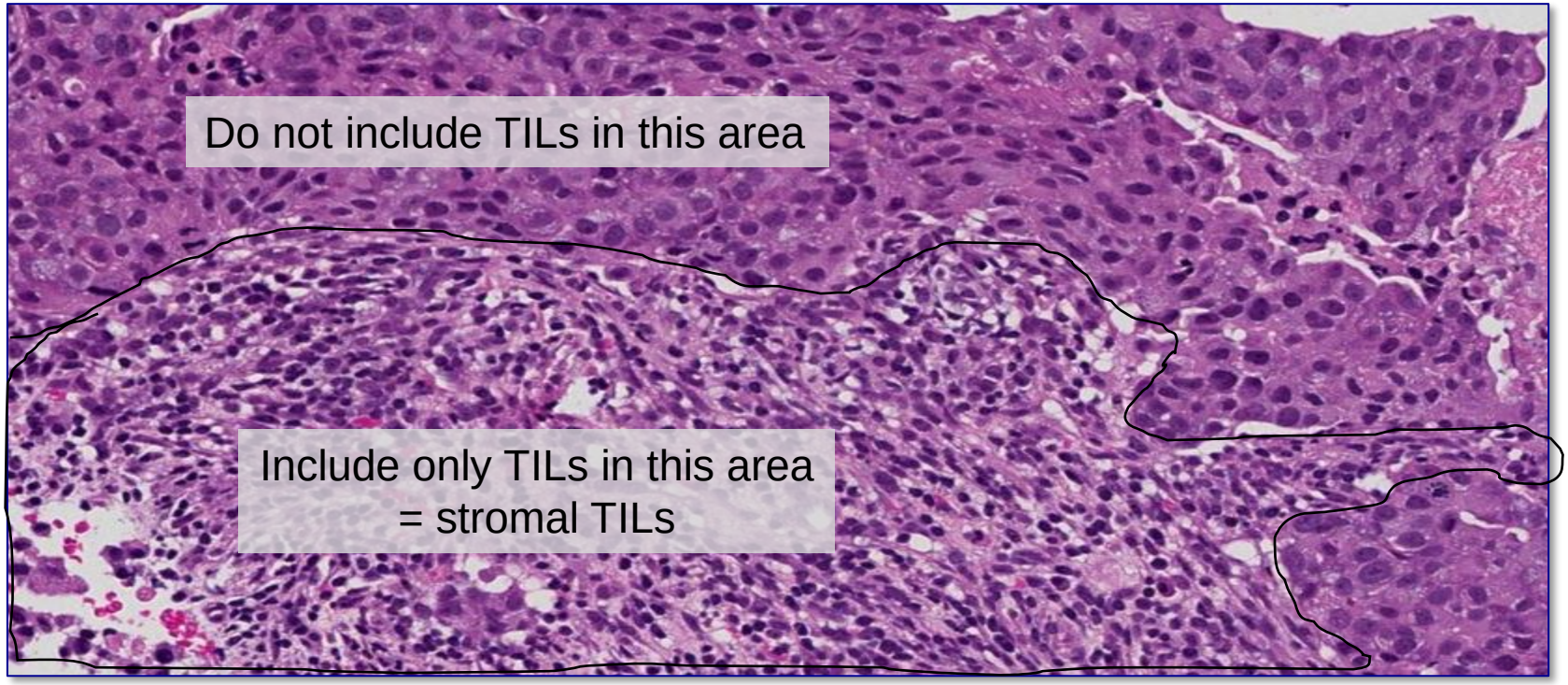
Large areas of central necrosis or fibrosis are not included in the evaluation



From TILs website www.tilsinbreastcancer.org

Step 2: Focus on stromal TIL

In the diagnostic setting, only stromal TILs are relevant



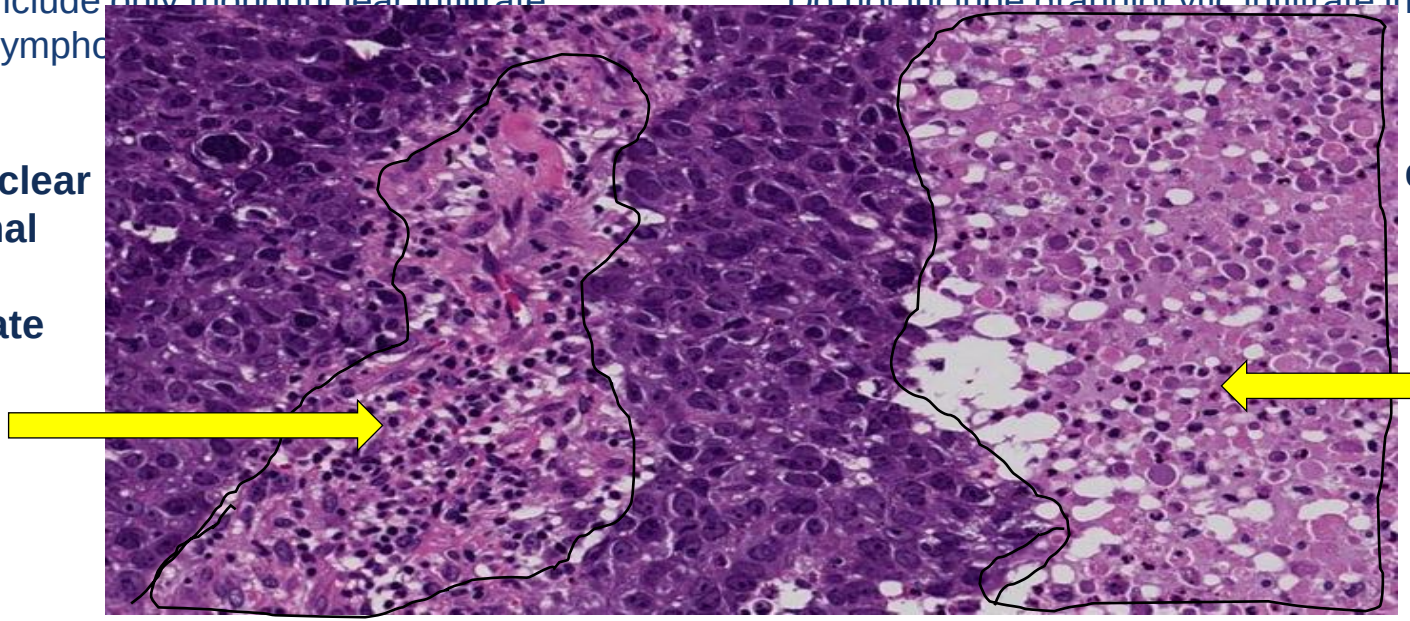
From TILs website www.tilsinbreastcancer.org

Step 3: Determine type of inflammatory infiltrate

Include only mononuclear infiltrate (lympho

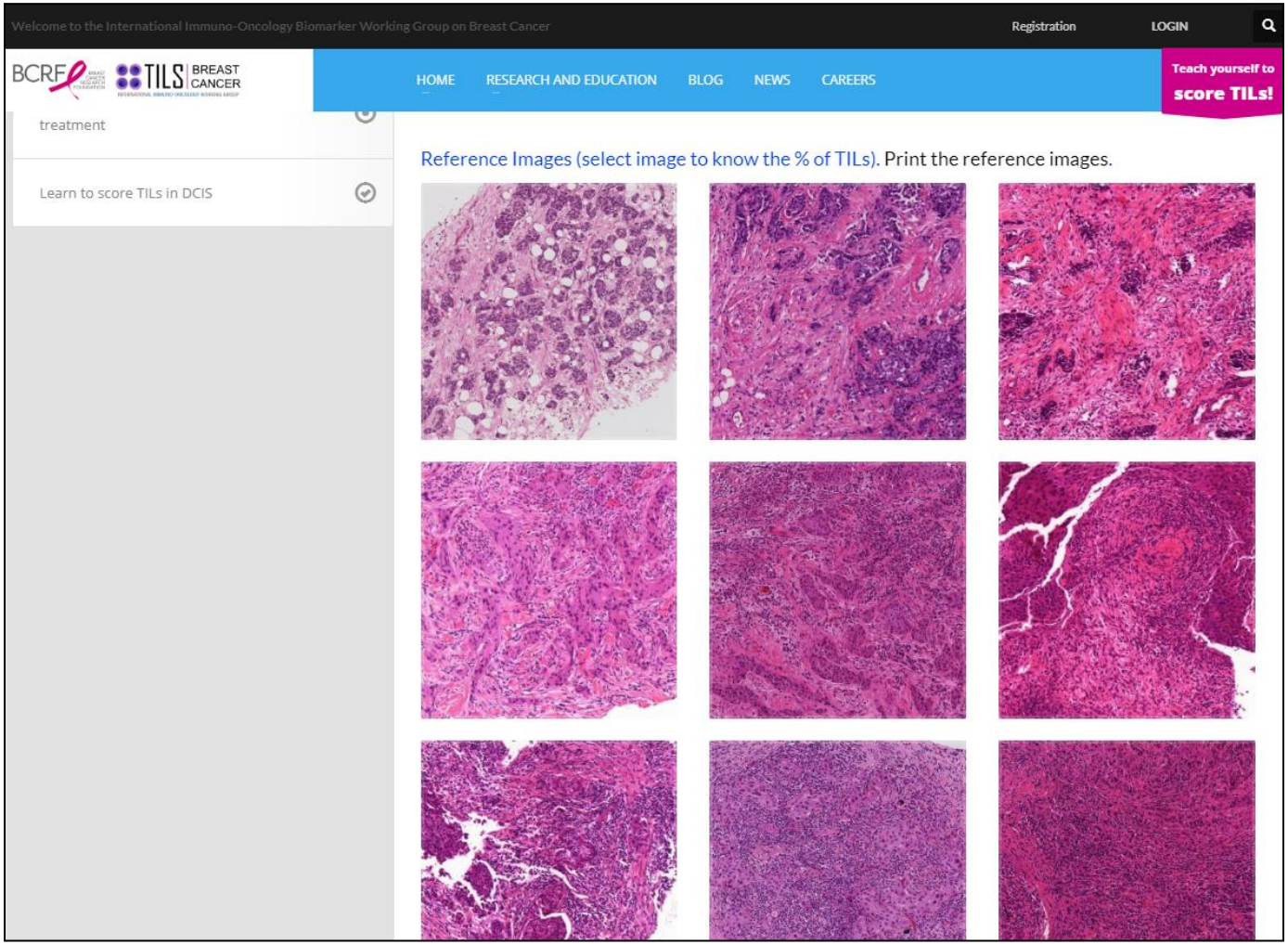
Do not include granulocytic infiltrate in areas of

mononuclear stromal TIL infiltrate



do not include granulocytes in necrotic areas

From TILs website www.tilsinbreastcancer.org



PD-L1

- Programmed death-ligand 1 (PD-L1) is a transmembrane protein that binds to the PD-1 receptor during immune system modulation
- The PD-1 receptor is typically expressed on cytotoxic T-cells and other immune cells, while the PD-L1 ligand is typically expressed on normal cells
- Normal cells use the PD-1/PD-L1 interaction as a mechanism of protection against immune recognition by inhibiting the action of T-cells
- Inactivation of cytotoxic T-cells downregulates the immune response such that the inactive T-cell is exhausted, ceases to divide, and might eventually die by programmed cell death, or apoptosis

PD-L1

- Tumor cells upregulate the expression of PD-L1 as a mechanism to evade immune response
- Activated T-cells recognize the PD-L1 marker on the tumor cell, and PD-L1 signaling renders the T-cell inactive
- The tumor cell escapes the immune cycle, continues to avoid detection for elimination, and is able to proliferate
- PD-1/PD-L1 interaction between tumor cells and activated T-cells is a mechanistic pathway used by immunotherapeutic agents
- When the tumor cell is unable to interact with the activated T-cell, the immune system remains active, thereby preventing immunosuppression

Companion Diagnostics

Beth Israel Deaconess Medical Center
HARVARD MEDICAL SCHOOL TEACHING HOSPITAL

4 FDA approved assays mTNBC (SP142, 22C3, 28-8, SP263)

- Different primary antibodies
- Different detection systems
- Different staining platforms
- Different scoring criteria (e infiltrating immue cells)
- Different definitio sitivity (>10%, ≥1% etc.)
- And, of course, di drugs

Atezolizumab Withdrawn

Decision becomes whether the choice of the drug drives the assay selection, or conversely, the result of the assays should inform the choice of the drug

Badve, JNCI, 2021
Gianni, Ann Oncol, 2022

PDL-1 testing

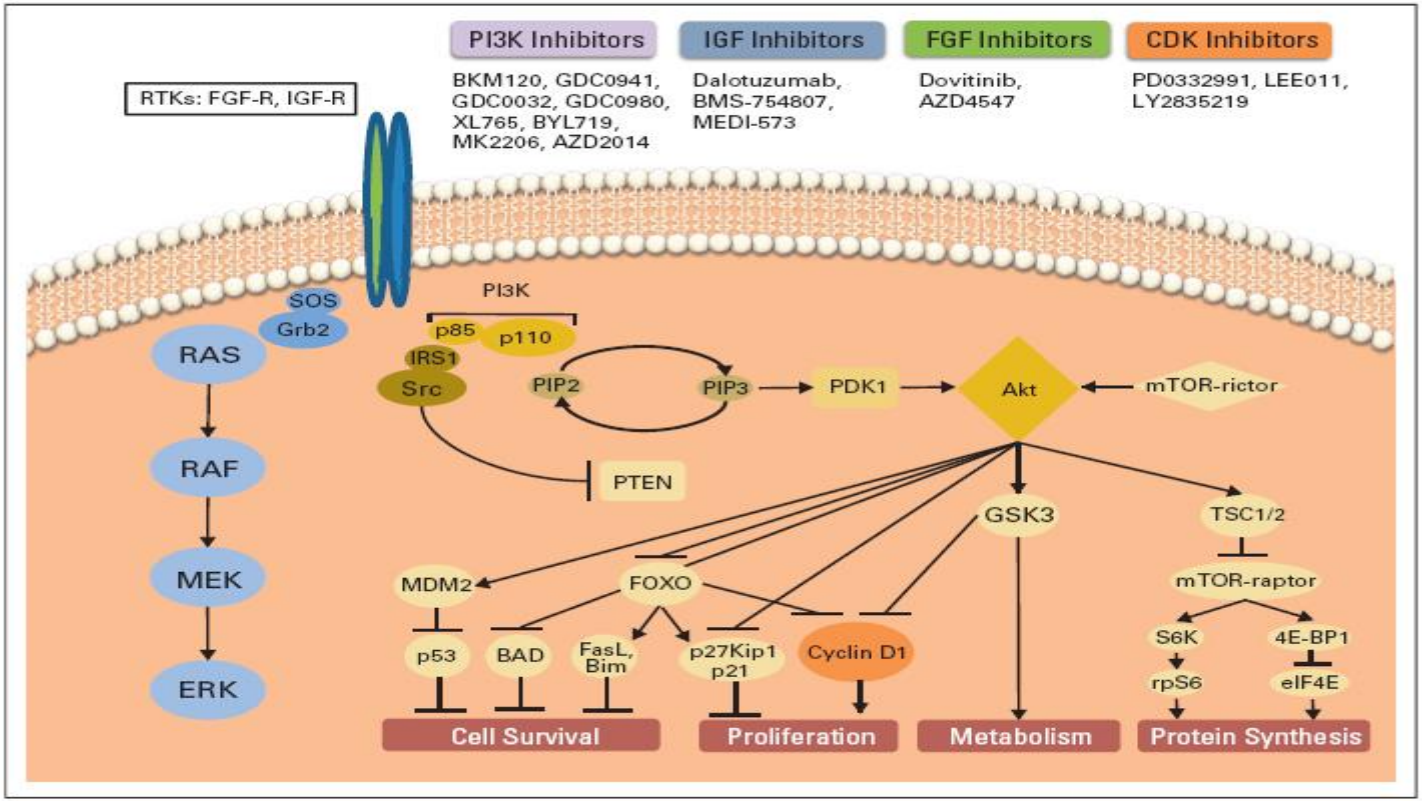
- PD-L1 testing in advanced TNBC used to predict benefit from pembrolizumab
- 22C3 antibody (companion diagnostic to pembrolizumab) is scored using the combined positive scoring system (CPS) [positive ≥ 10%]
- PDL-1 testing with SP142 no longer indicated [atezolizumab withdrawn for this indication]
- Rare patients with mismatch repair deficient (MSI-H/dMMR) TMB-H metastatic breast cancer may be candidates for pembrolizumab immunotherapy

Najjar, Virchows Arch, 2022

Where are we today?

- Targeted sequencing for genomic alterations/mutations in patients with metastatic disease to determine eligibility for clinical trials (e.g. for PI3 kinase inhibitors)

Signaling Pathways Under Blockade in Luminal Cancers



Ades, JCO, 2014

Discriminants of Benefits from Chemotherapy

- Histologic Type (eg, special TNC types)
- Histologic Grade
- Tumor Size
- LVI
- Biomarker status (ER, PR and HER2)
- Multigene assays in a subset of patients (ER+, >5mm, N0 or N1mi)
- (TILs)

Know your patient population

Be aware of overall ER+ vs. ER- rate in your lab; should be 60-80%, but will vary with patient population

Know your HER2 positive rate; should be 10-15%

Also useful to monitor your HER2 2+ IHC to HER2 amplified rate

Summary

- ER, PR and HER2 status are the major drivers of clinical decision making regarding the type of systemic therapy
- Performance of high-quality assays is critical to patient care
- Attention to common pitfalls, correlation with morphology and judicious additional testing can prevent errors
- Multigene assays are being utilized in patients with ER+, HER2, pN0 – pN1a to determine need for adjuvant chemotherapy