Biological Markers and Molecular Signatures

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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
- 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

AJCC 8th Edition

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

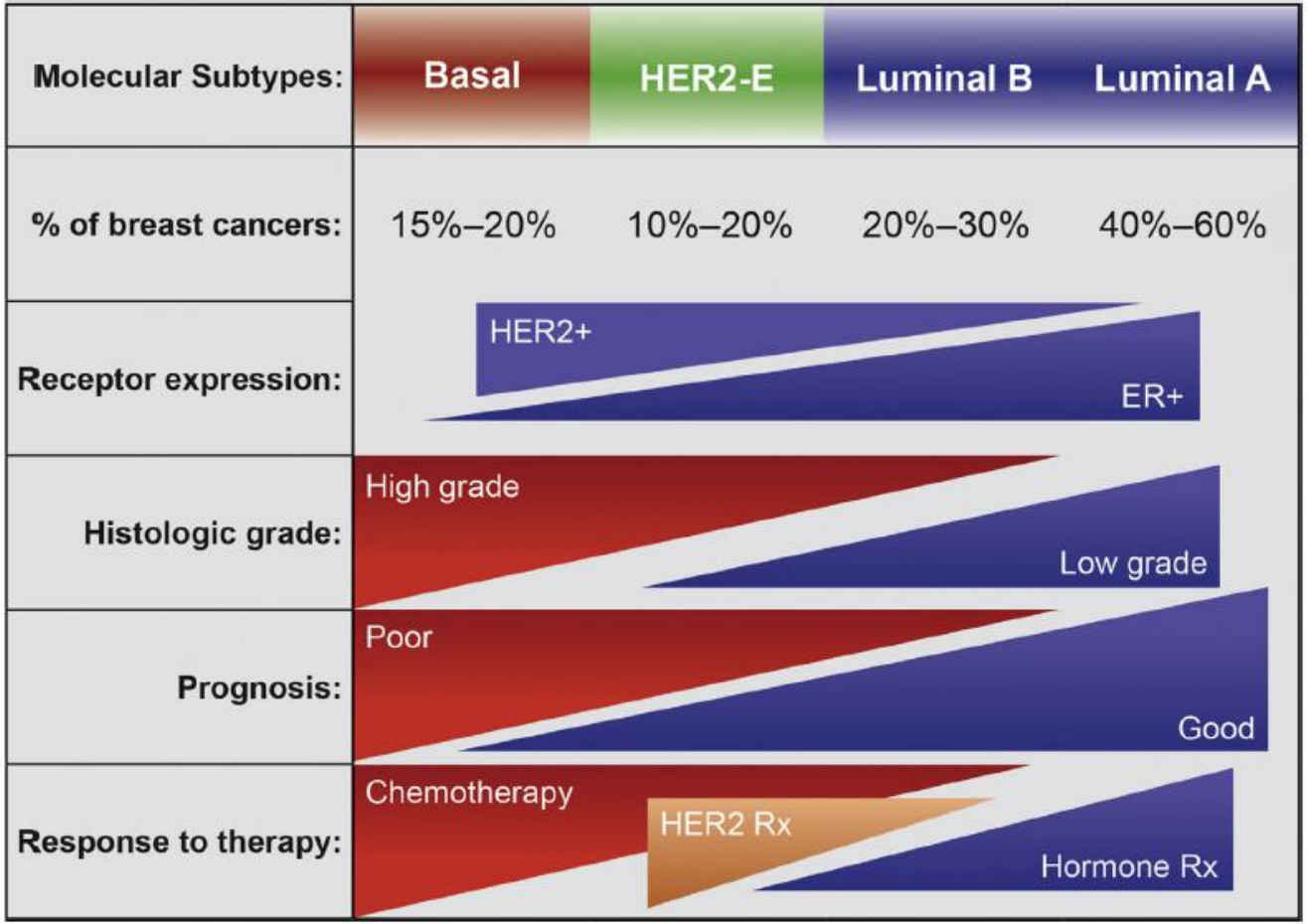
1. Obtaining genomic profiles is NOT required for assigning Pathological Prognostic Stage. However genomic profiles may be performed for use in determining appropriate treatment. If the OncotypeDx® test is performed in cases with a T1N0M0 or T2N0M0 cancer that is HER2-negative and ER-positive, and the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



Allison, Surg Pathol Clin, 2018

Ancillary Testing: Further Refinements

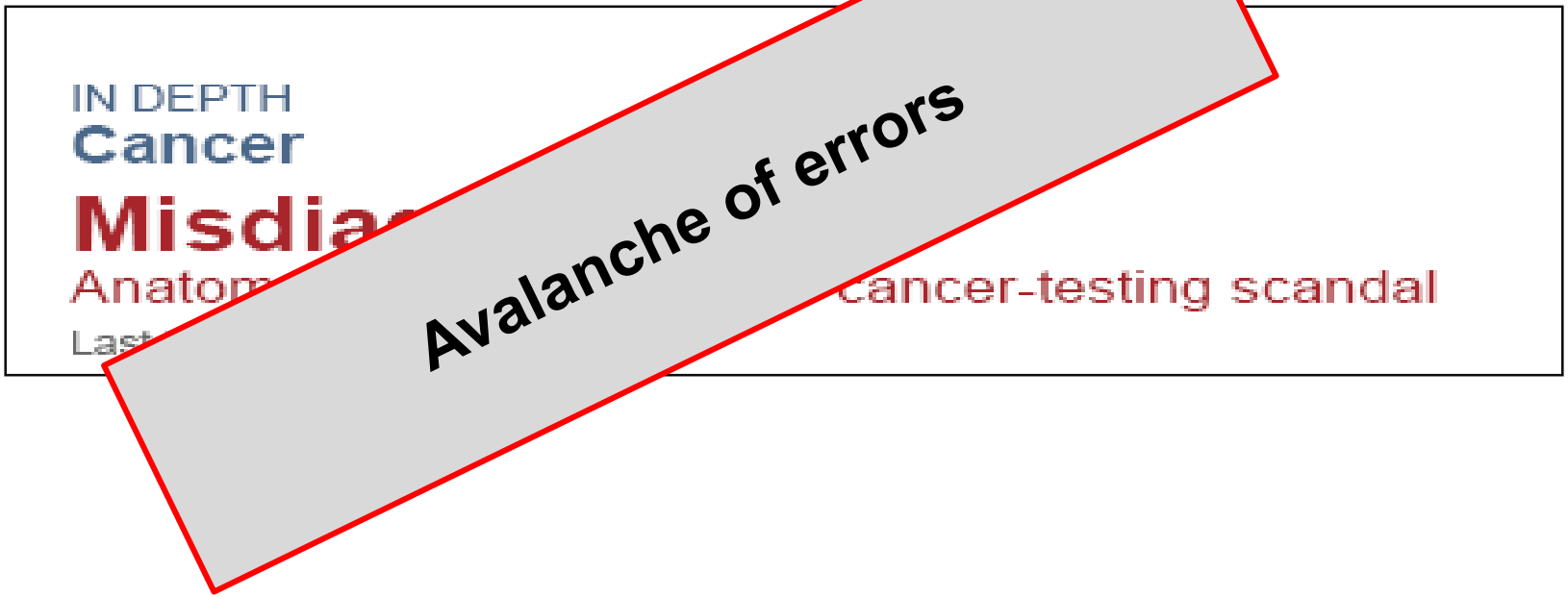
- ER, PR and HER2
- ER low positive tumors
- ER positive, node positive tumors, Ki-67 high
- HER2 low positive tumors
- Molecular assays to guide need for chemotherapy in ER+ tumors with low burden of nodal disease (and ?tumors with Ki-67 index between 5-30%)

ER, PR and HER2

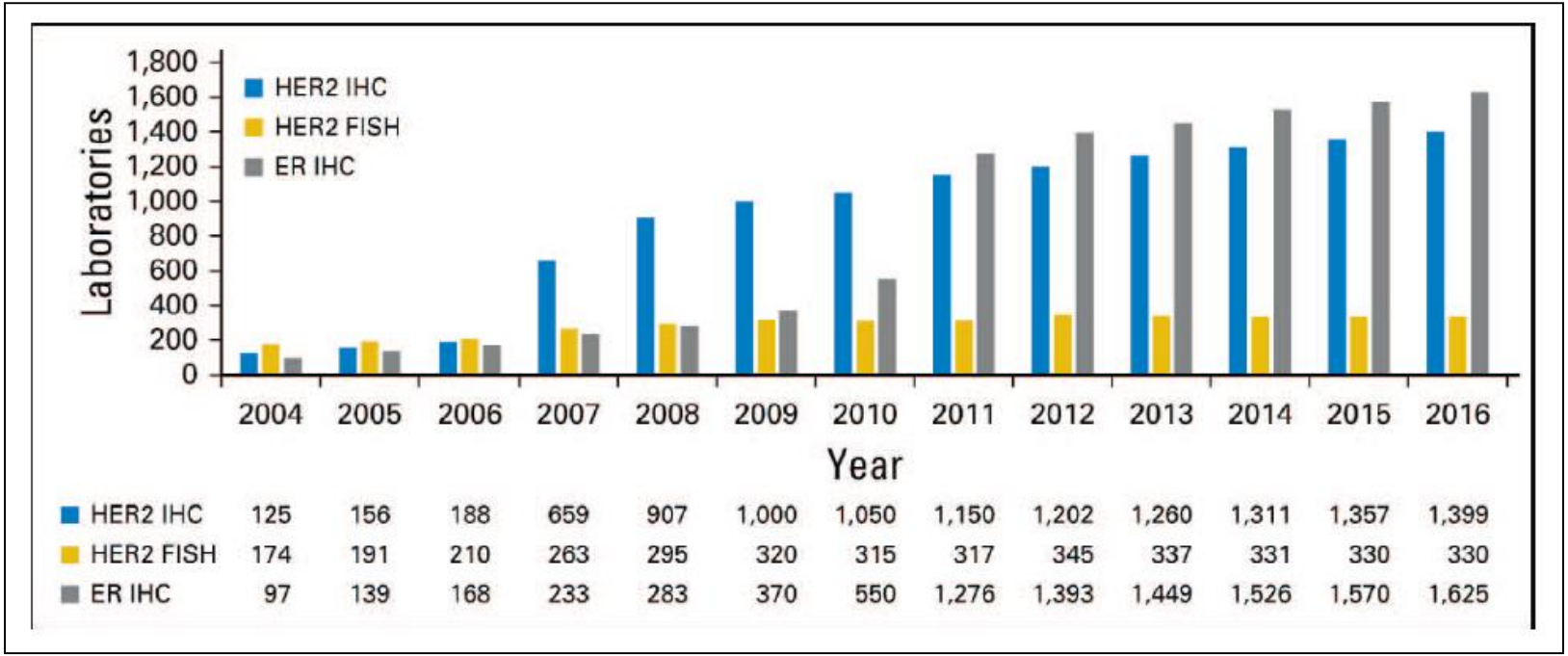
- High stakes tests
- Not only provide overall treatment and prognostic groupings, also determine specific “targeted” therapies
- Consequences of errors are significant
  - Deprive potentially responsive patients of treatment
  - Treat potentially unresponsive patients with possibility of treatment related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

Estrogen Receptor Testing

Biggest concern is over false negatives



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

Optimal Algorithm for ER/PR Testing

These definitions depend on laboratory documentation of the following:

1. Proof of initial validation in which positive ER or PgR categories are 90% concordant and negative ER or PgR categories are 95% concordant with a clinically validated ER or PgR assay.[3]

2. Ongoing internal QA procedures, including use of external controls of variable ER and PgR activity with each run of assay, regular assay reassessment, and competency assessment of technicians and pathologists.
3. Participation in external proficiency testing according to the proficiency testing program guidelines.
4. Biennial accreditation by valid accrediting agency.

Hammond, Arch Pathol Lab Med, 2010

Estrogen Receptor Testing

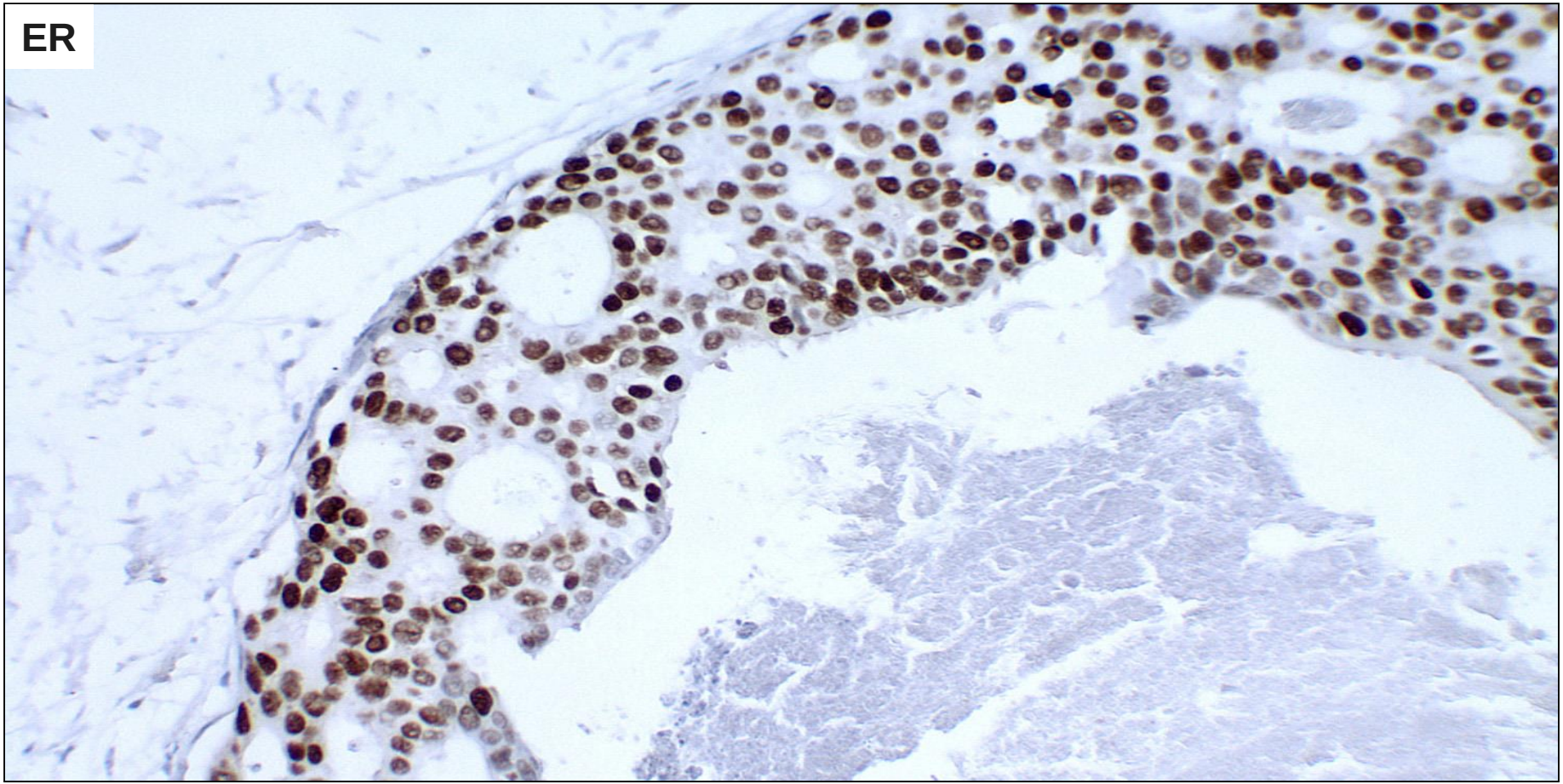
Estrogen Receptor

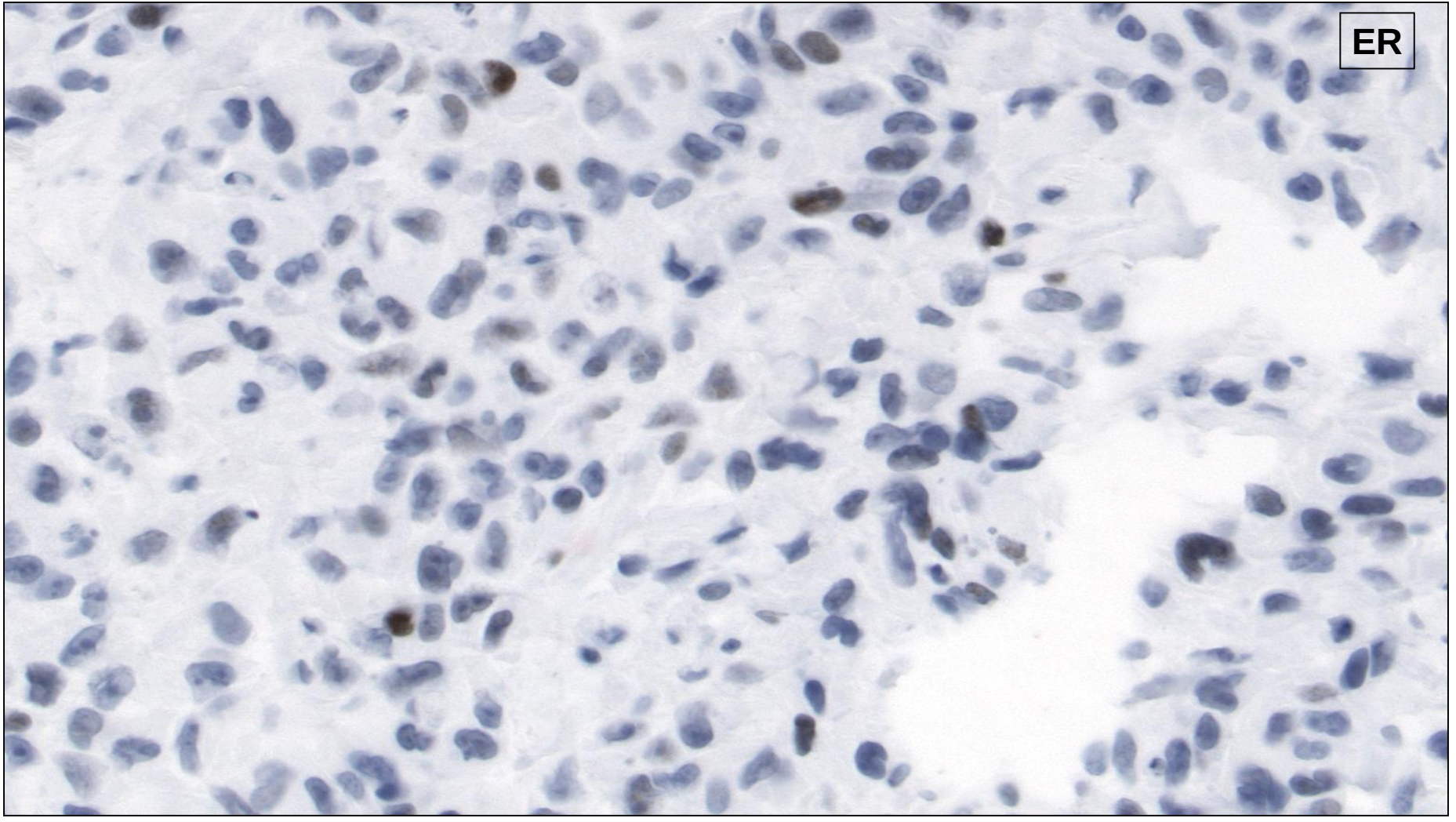
- Nuclear receptor, activated upon binding to estrogen (17-beta-estradiol)
- Role in normal breast development, differentiation and lactation
- ERα encoded by *ESR1* on chromosome 6
- ERβ encoded by *ESR2* on chromosome 14
- ER IHC antibodies recognize ERα

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


ER

Influence of Fixation Time

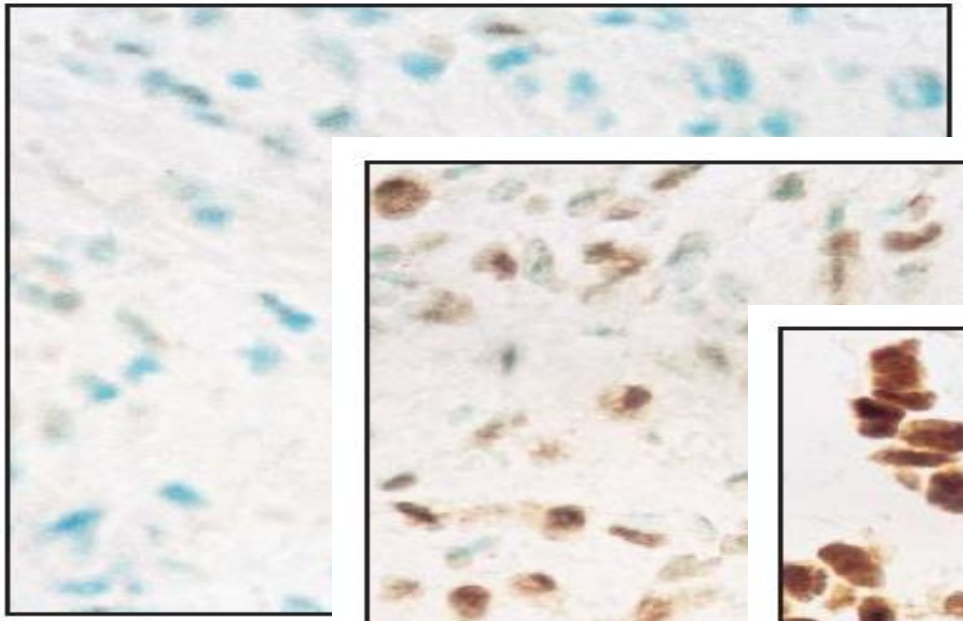


Image 1 Fixation, 3

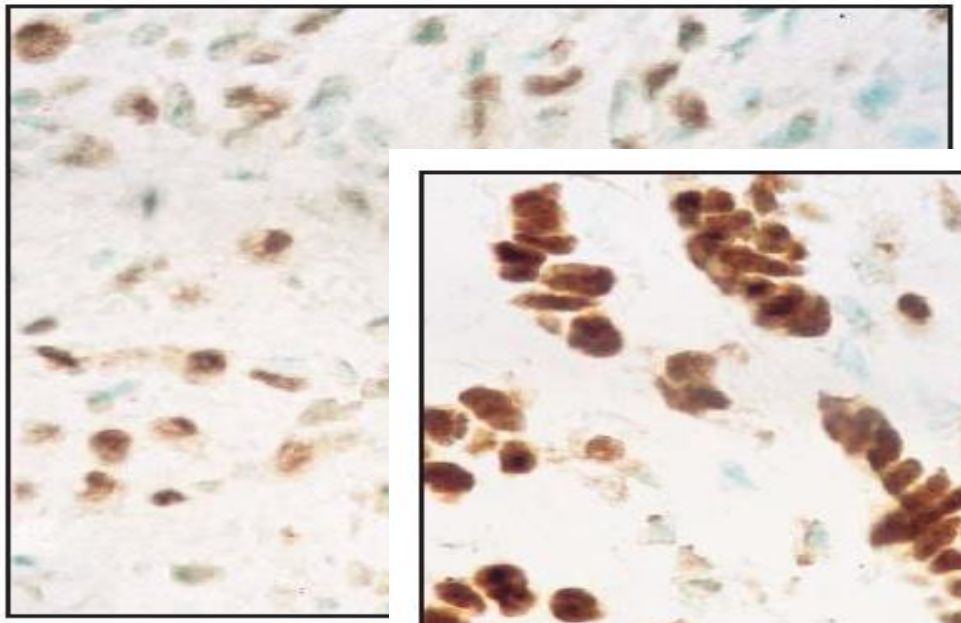


Image 2 Fixation, 6 h; a

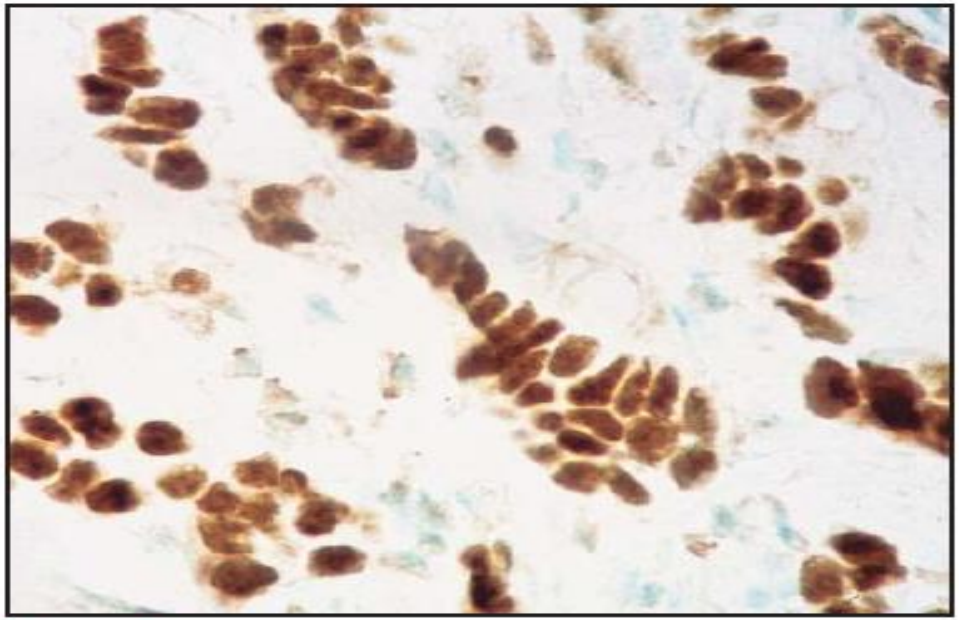
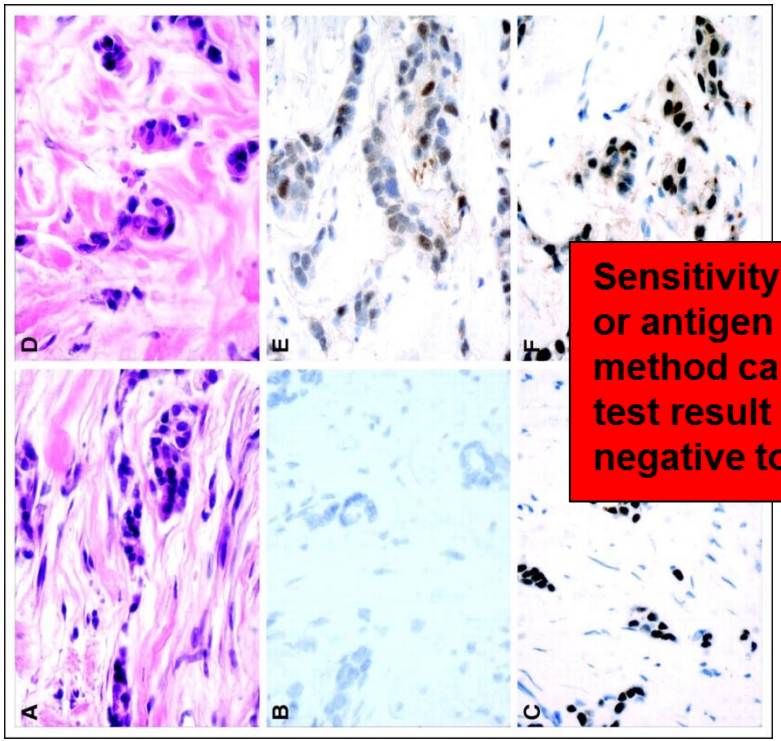
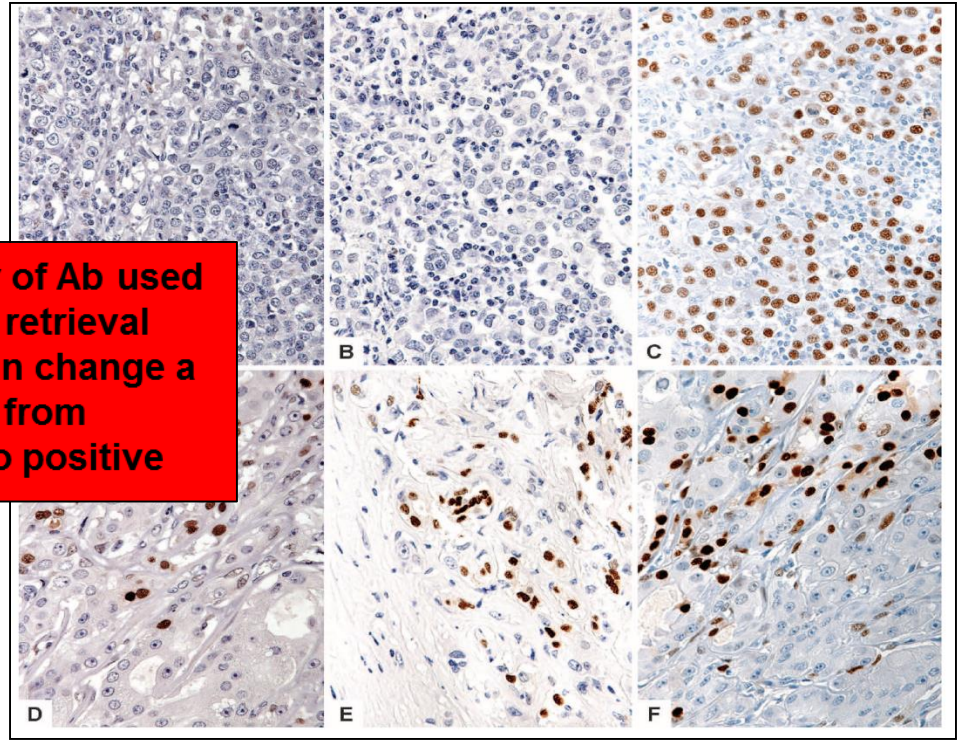


Image 3 Fixation, 8 h; antigen retrieval, 40 min.

Goldstein, Am J Clin Pathol, 2003

Comparison of ER/PR Antibody Reagents



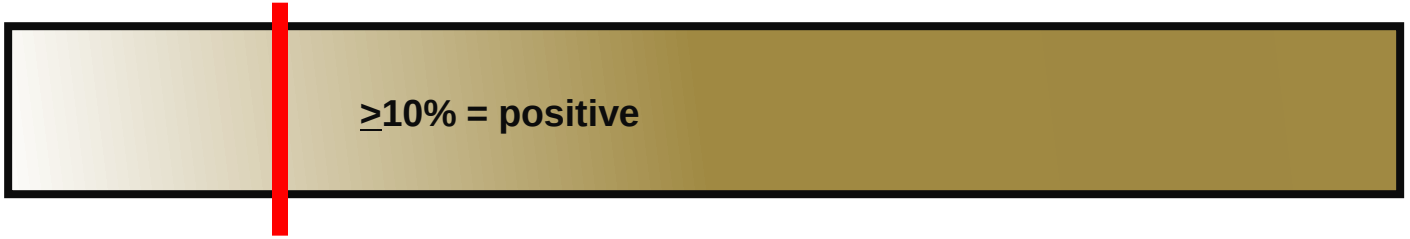


Sensitivity of Ab used or antigen retrieval method can change a test result from negative to positive

1D5 **SP1** **1D5** **6F11** **SP1**

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

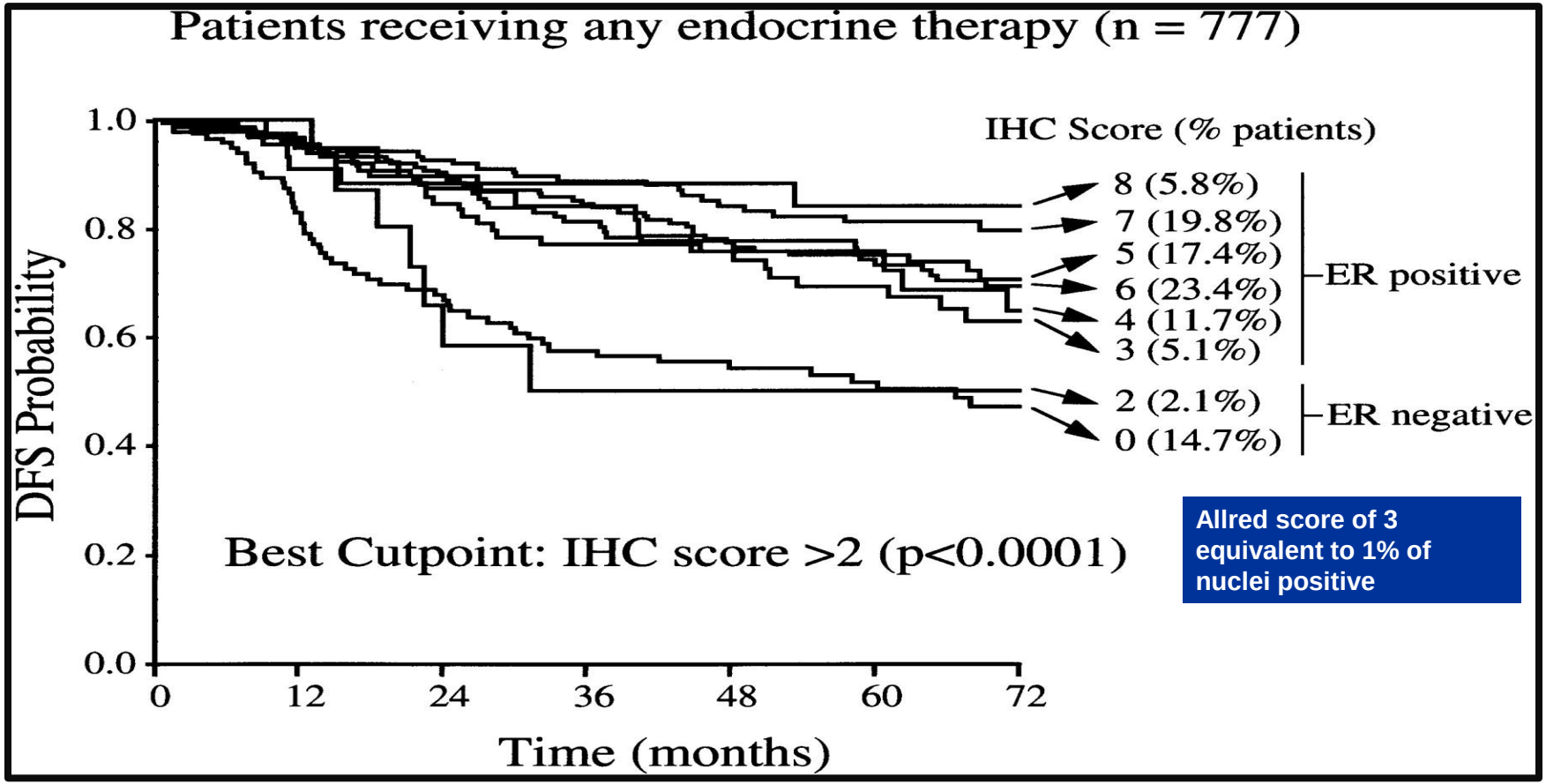
Quantification of ER

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

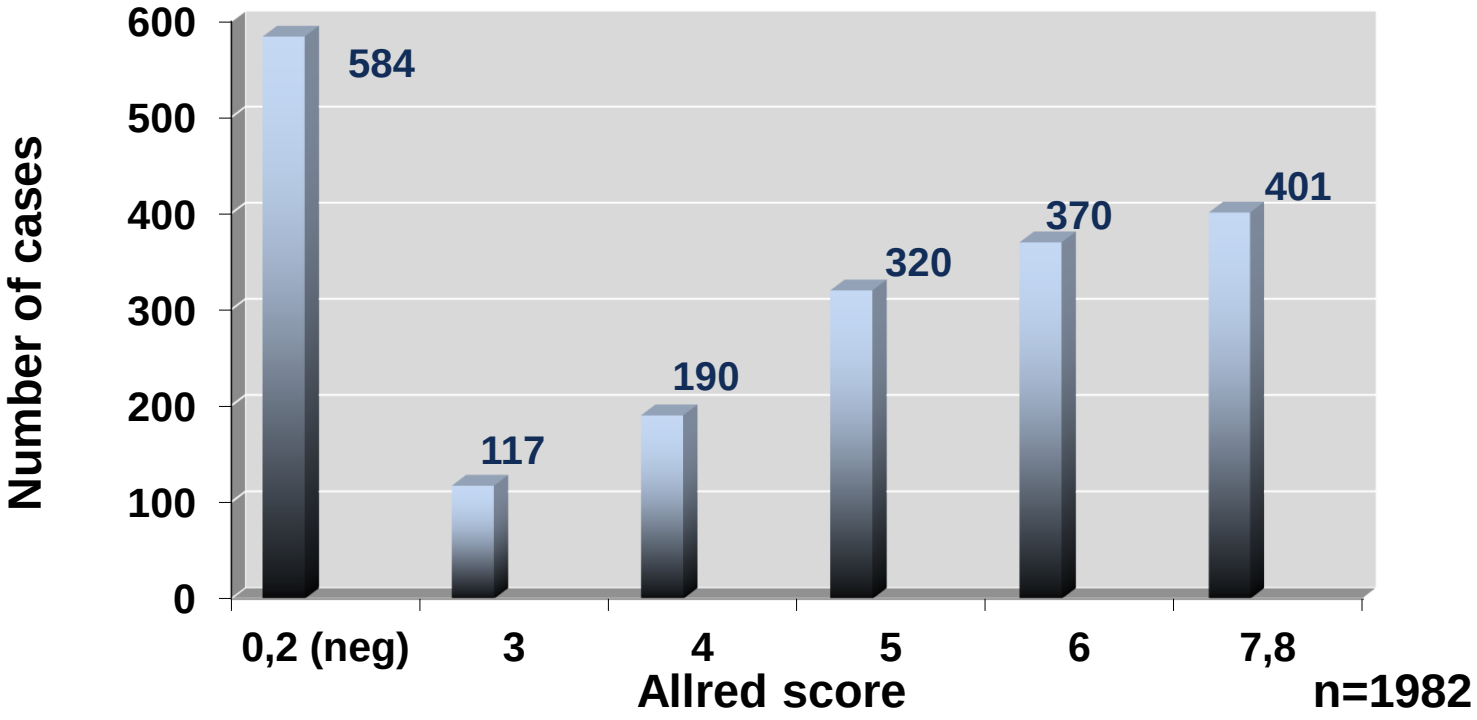


Allred score of 3 equivalent to 1% of nuclei positive

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

JOURNAL OF CLINICAL ONCOLOGY

Allred Score Distribution



Harvey, 1999

Categories of Endocrine Responsiveness

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

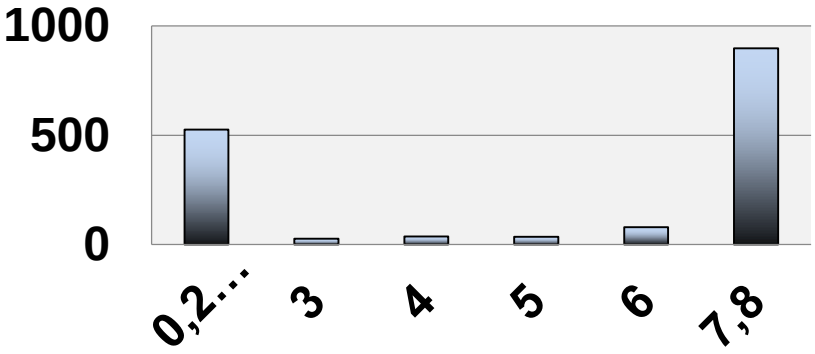
All positively associated with ER levels

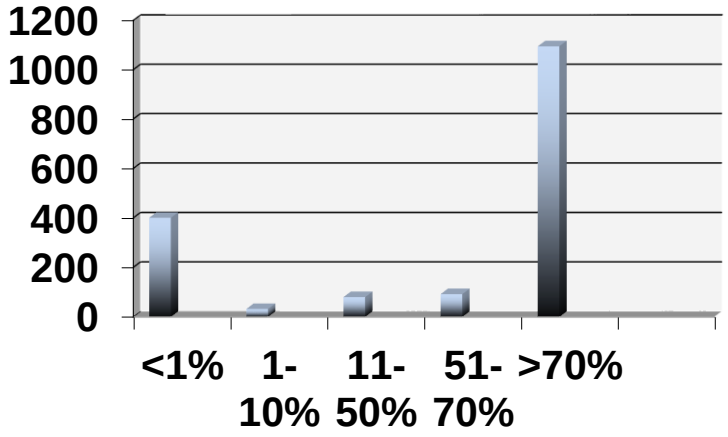
Cowen PN, 1990, Histopathology
Esteban JM, 1994, J Cell Biochem Suppl
Elledge RM, 2000 In J Cancer
Stendahl M, 2006, Clin Cancer Res
Yamashita H, 2006, Breast Cancer
Dowsett M, 2008, JCO

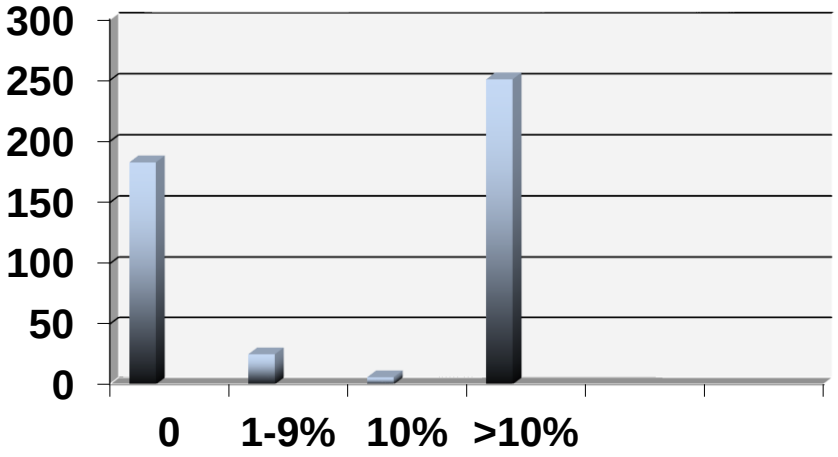
Does IHC Permit Reliable Quantification of ER?

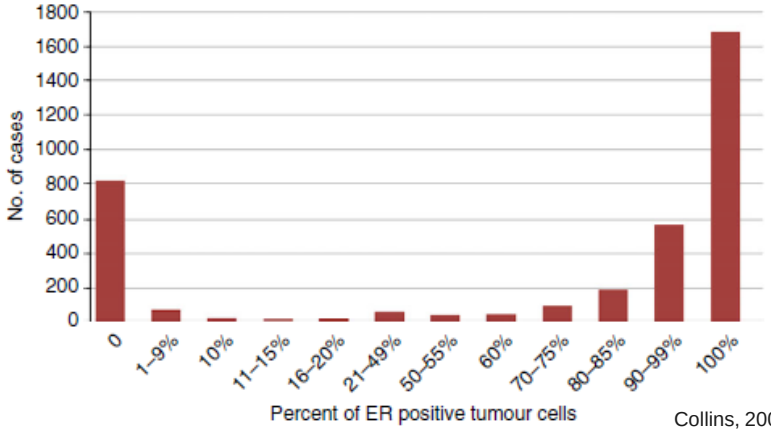
Current IHC methods utilize highly sensitive antibodies and detection systems and often employ signal enhancement

ER Distribution







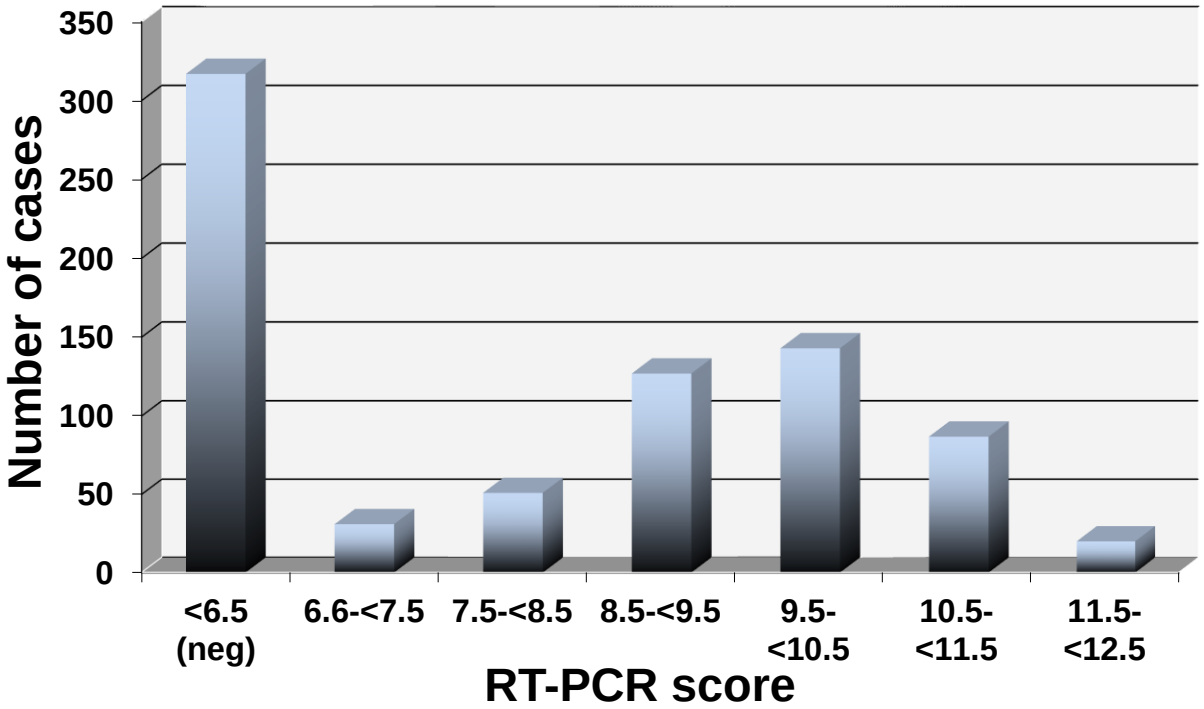


Collins, 2005
Badve, 2008
Iwamoto, 2012
Zhang, 2014
Muftah, 2017

Quantification of ER

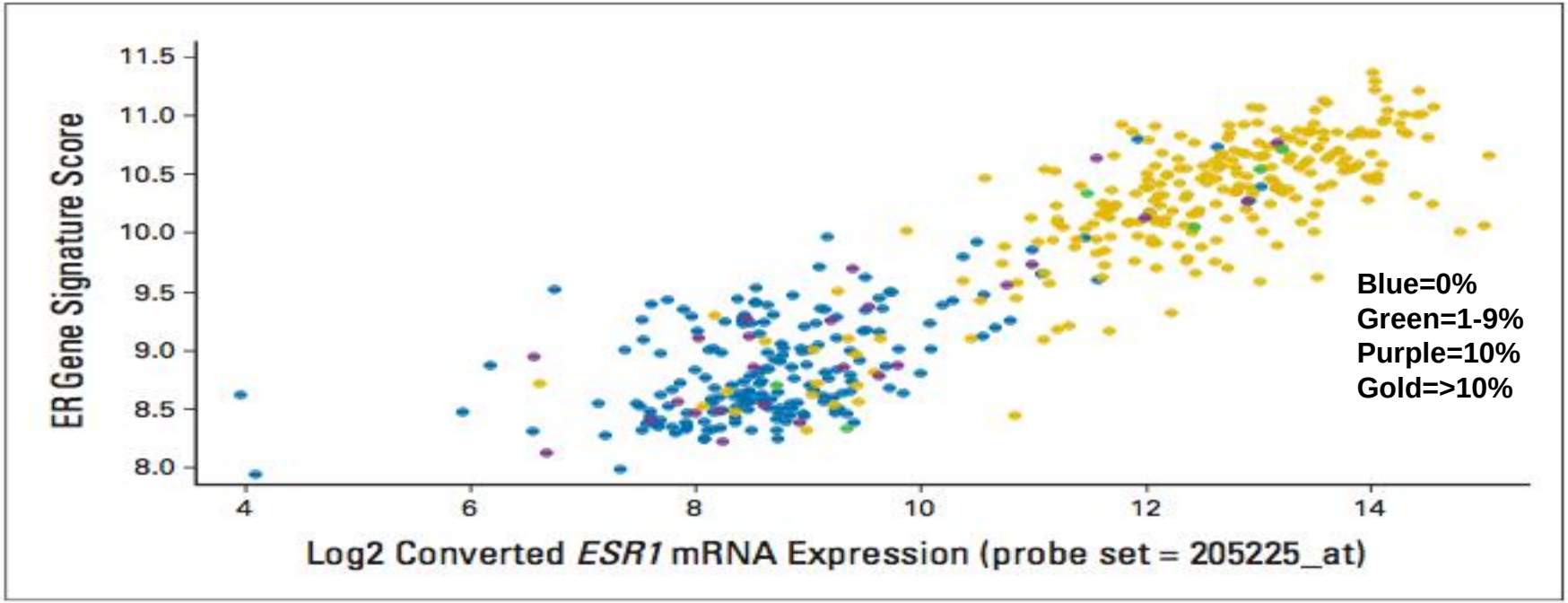
- We know from ligand binding assay days that ER in breast cancer is a continuous variable
- ER is not biologically bimodal
- ?Need for alternative methodologies

ER by RT-PCR



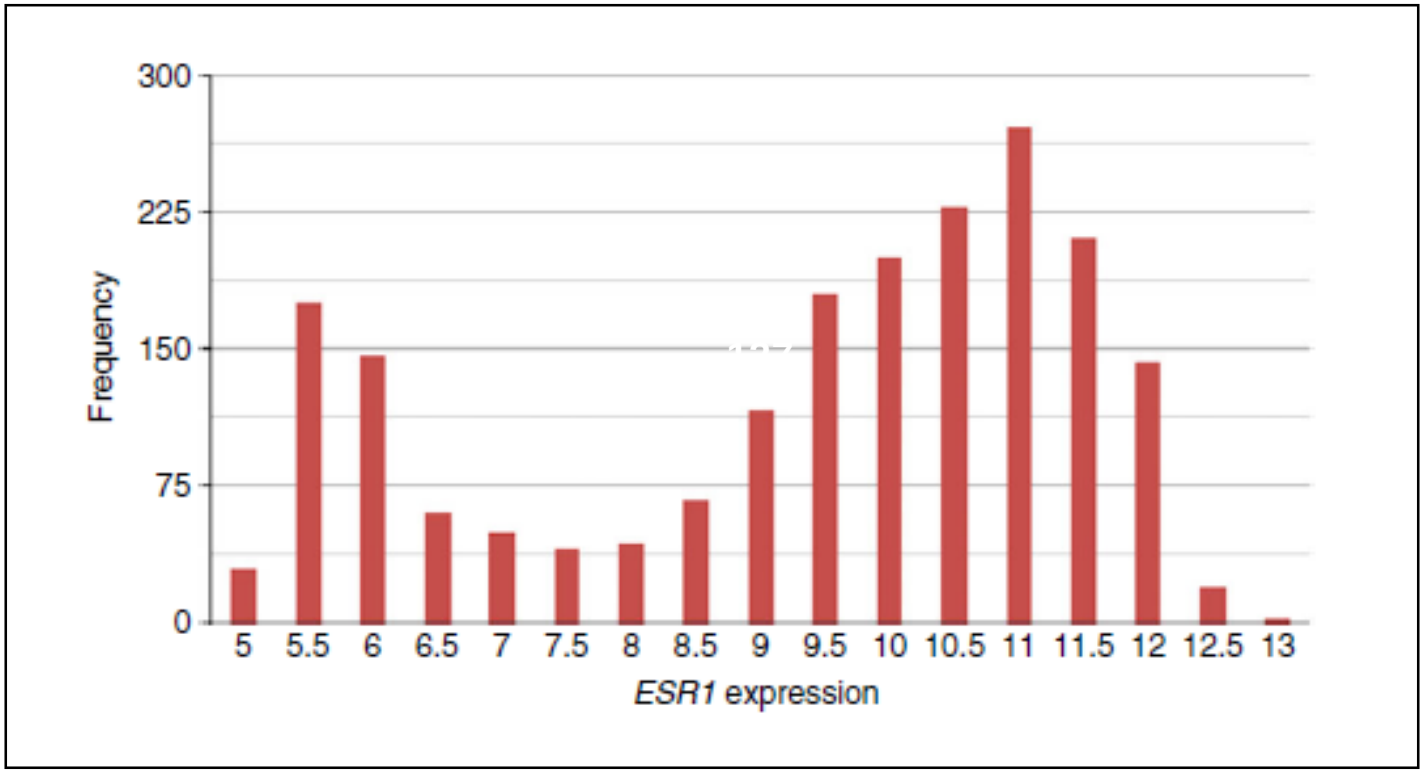
Badve, et al, 2008

Comparison of ER IHC, Gene Signature Score and mRNA Expression



Iwamoto, JCO, 2012

ER by mRNA Expression



Muftah, 2017 [38]

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
- Newer data support bimodal distribution for ER, suggesting dichotomization of results by IHC is appropriate
- 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
- Some triple negative trials now including patients with low ER+

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

Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update

New reporting of low positive group (1-10%)

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

Evaluate concordance of result

Additional requirements for ensuring testing conditions and laboratory proficiency

IHC is the gold standard; ER negativity by mRNA testing does not negate an IHC ER+ result

ER testing in DCIS now recommended

Allison, JCO, 2020

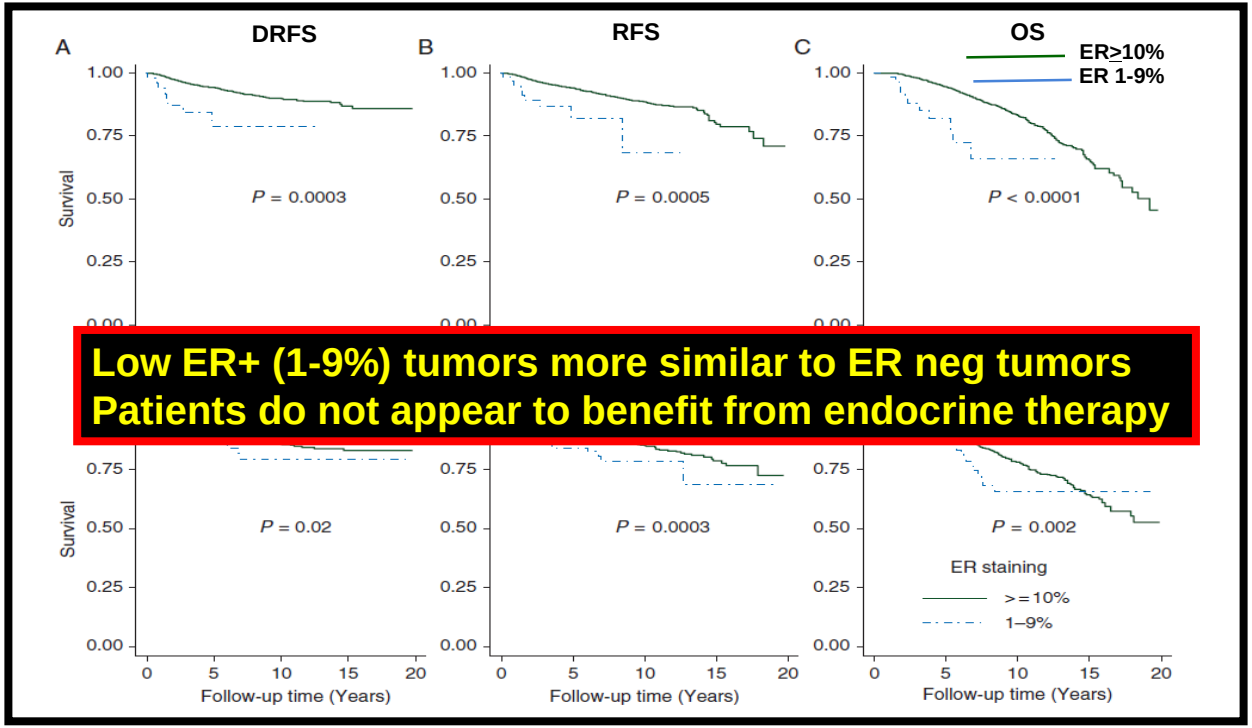
What about low ER group?

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

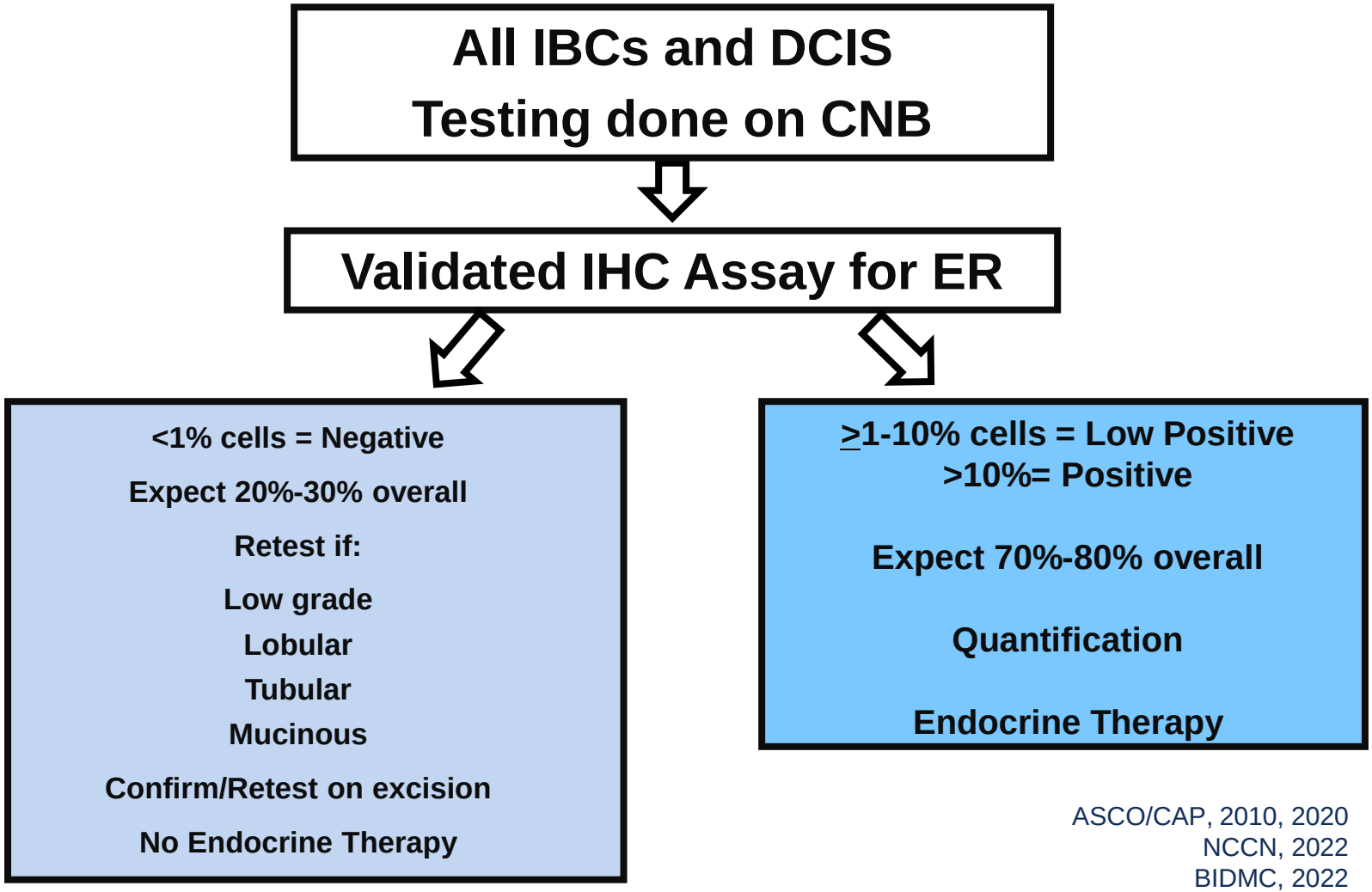
2.6% of tumors ER borderline (1-9%)

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, 2022

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

IHC for HER2

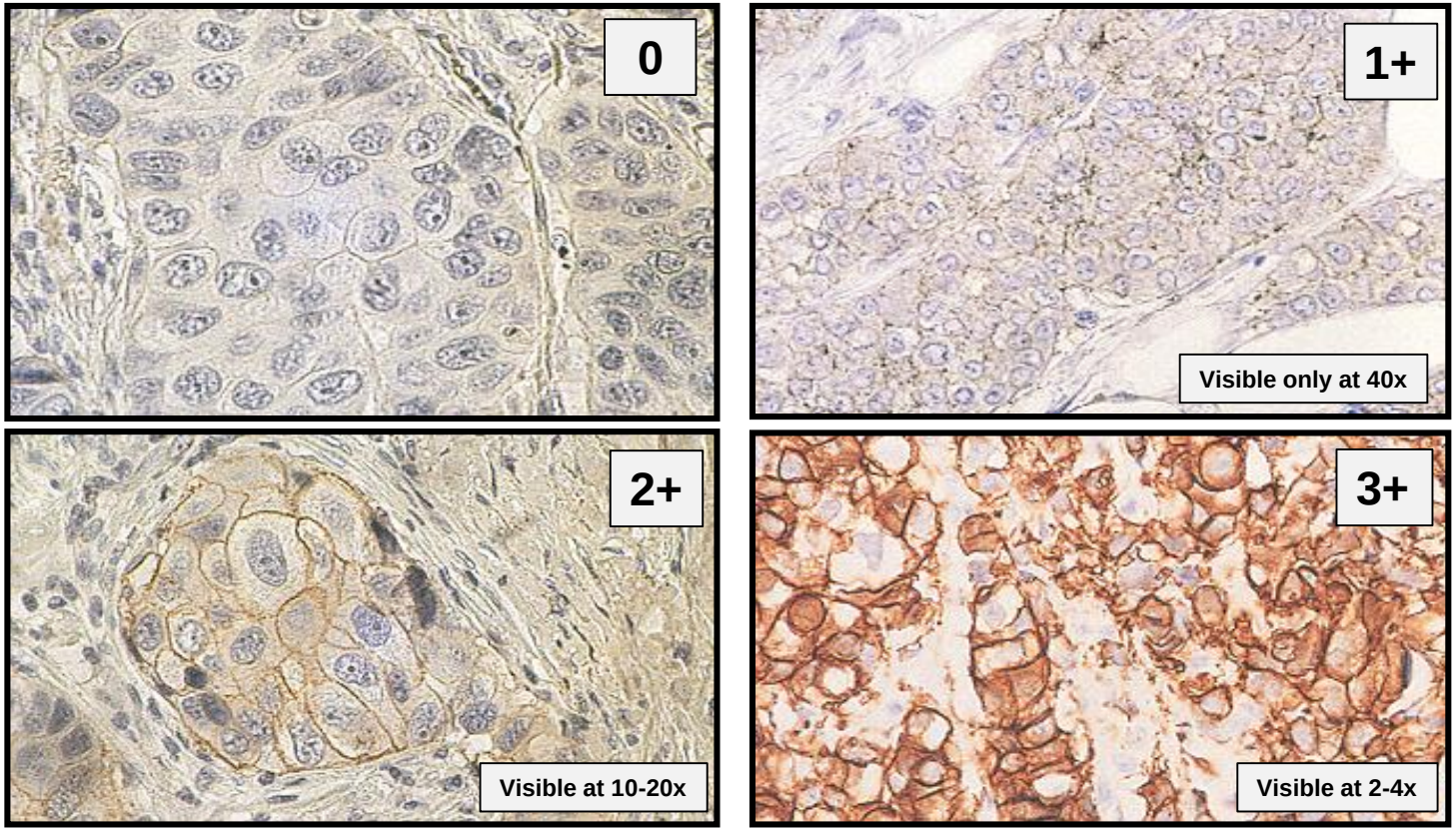
Pros

- Can be performed in any laboratory performing IHC
- Short procedure time
- Rapid, light microscope-based interpretation
- Morphology preserved
- Inexpensive
- Linked to clinical outcome and therapeutic response

Cons

- Numerous antibodies; vary in sensitivity and specificity
- Results may be highly affected by preanalytic factors

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)

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

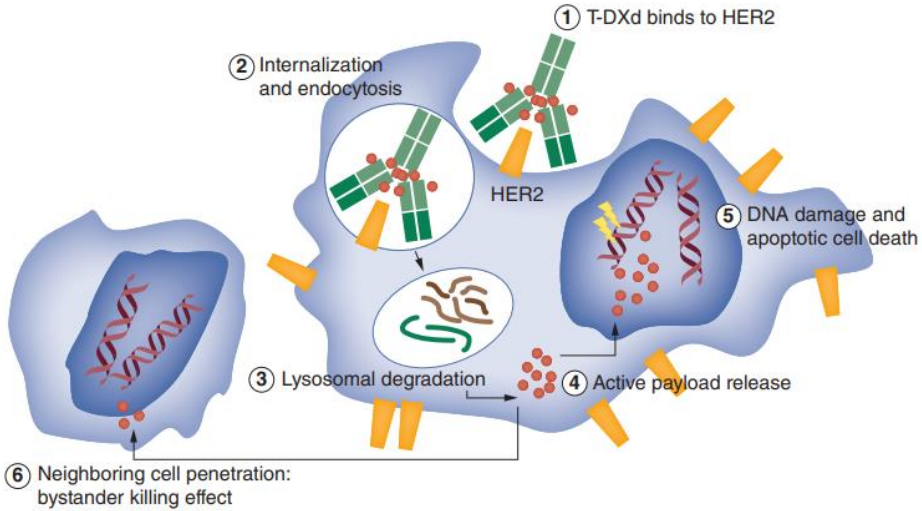
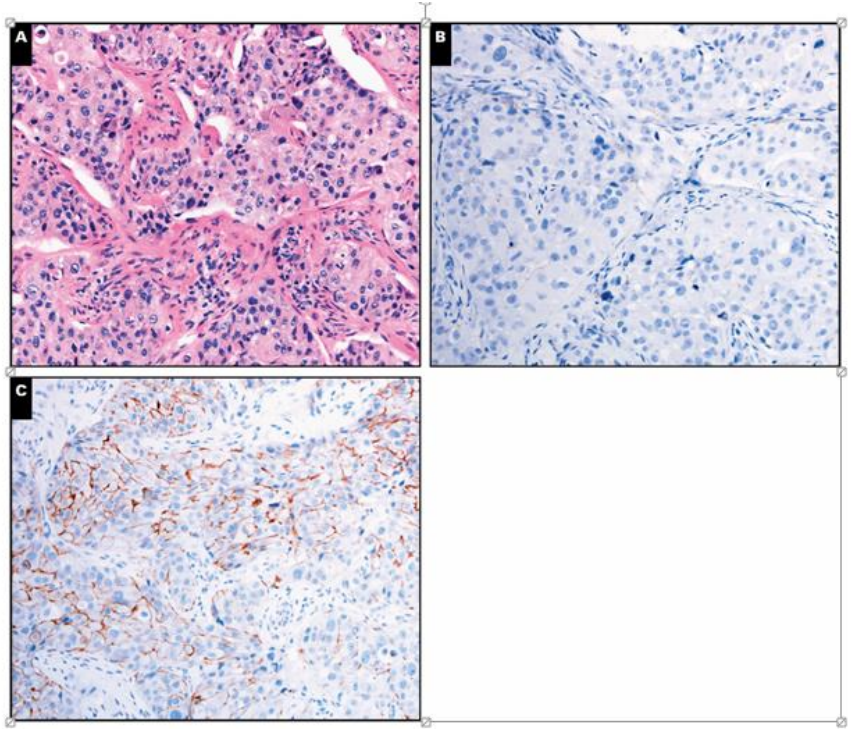


Figure 1. Mechanism of action of trastuzumab deruxtecan.

Lee, Future Oncol, 2022

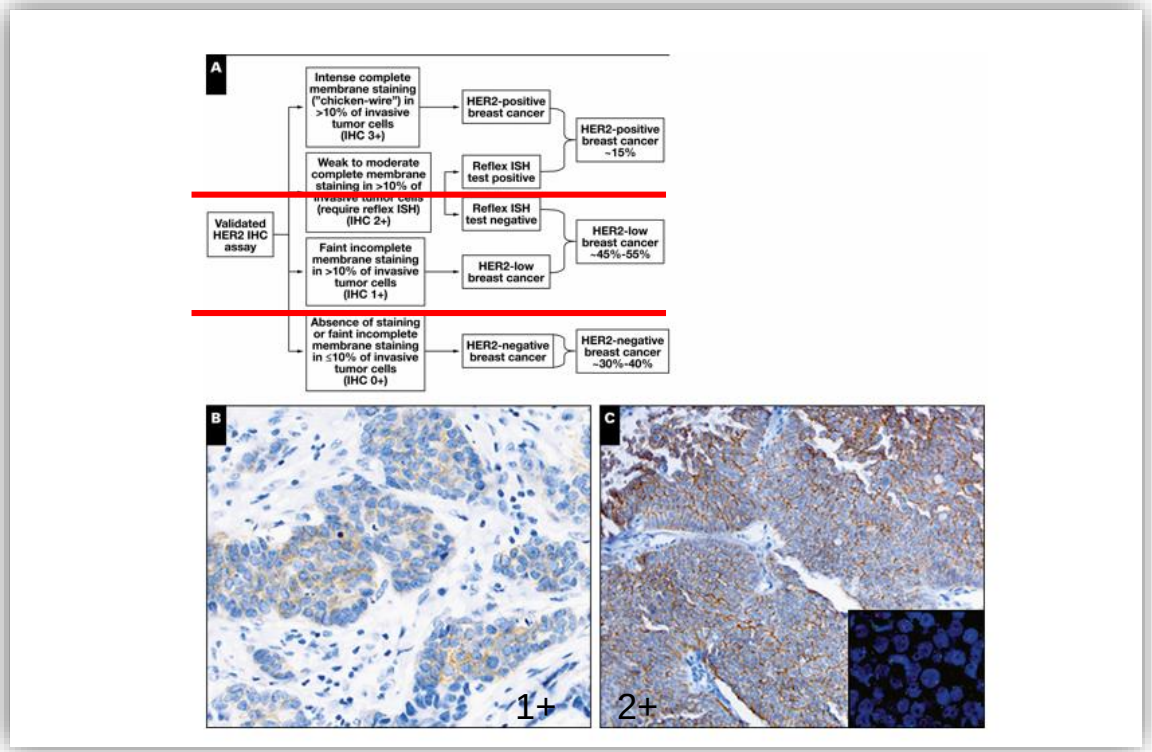
HER2 Low Positive Tumors-Variability in Staining

- 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+)

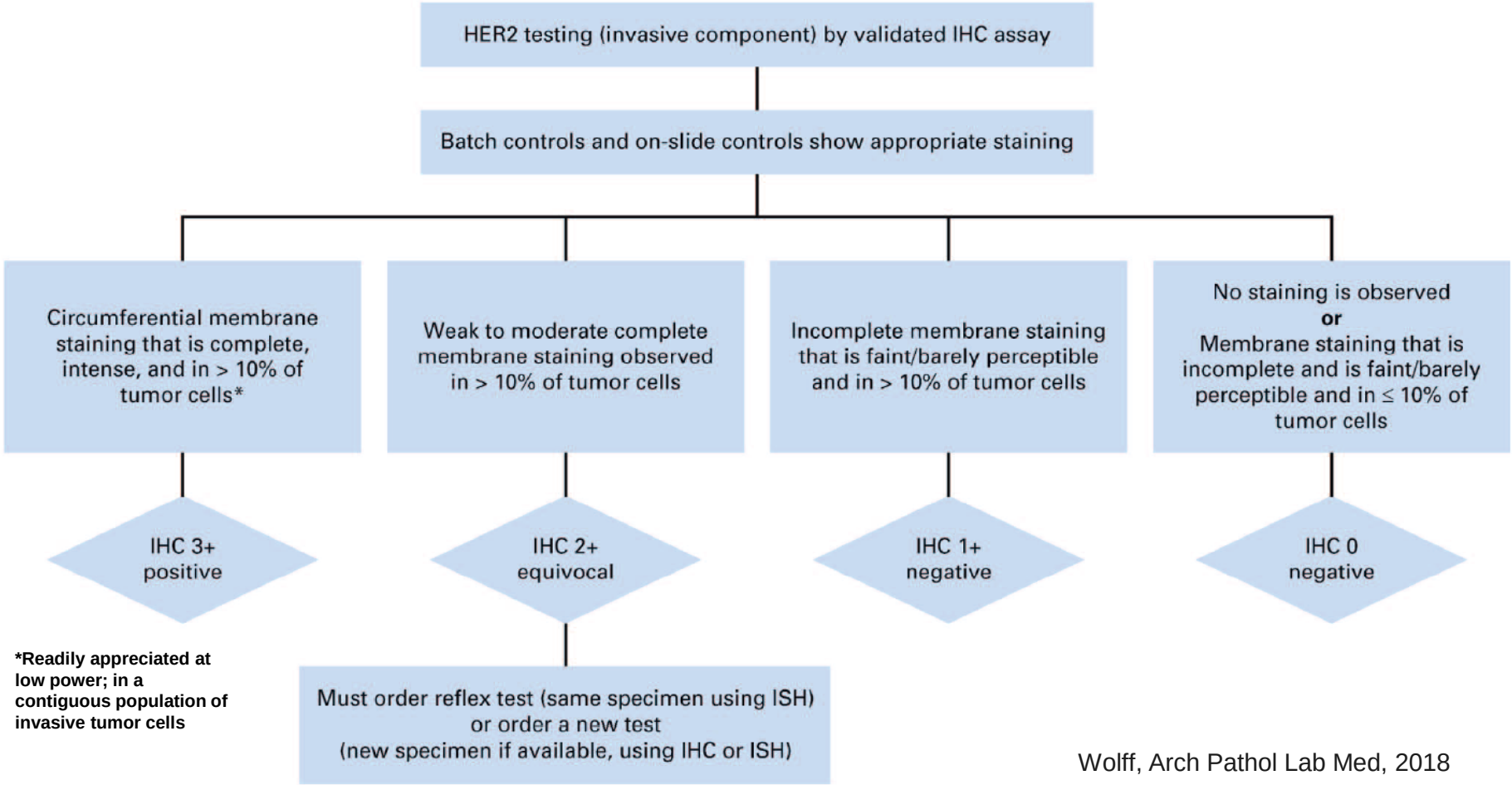


Zhang, AJCP, 2022

HER2 low positive tumors: 3-tier scoring system



Zhang, AJCP, 2022

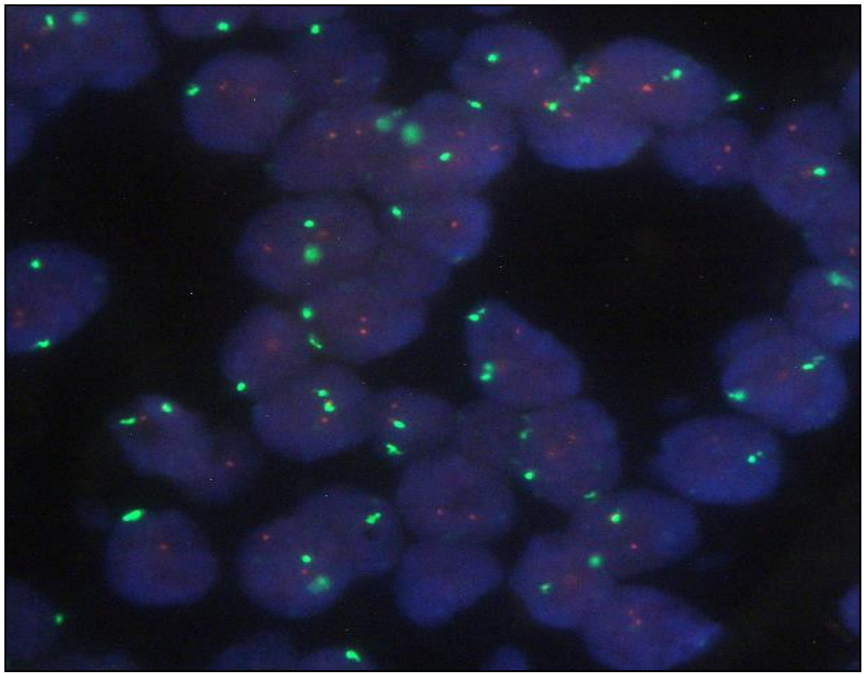


***Readily appreciated at low power; in a contiguous population of invasive tumor cells**

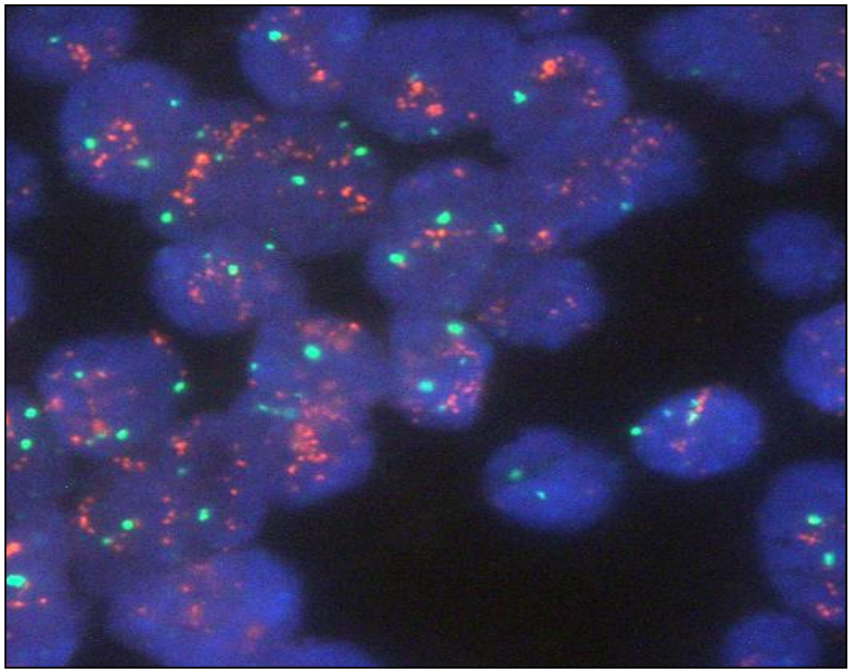
Wolff, Arch Pathol Lab Med, 2018

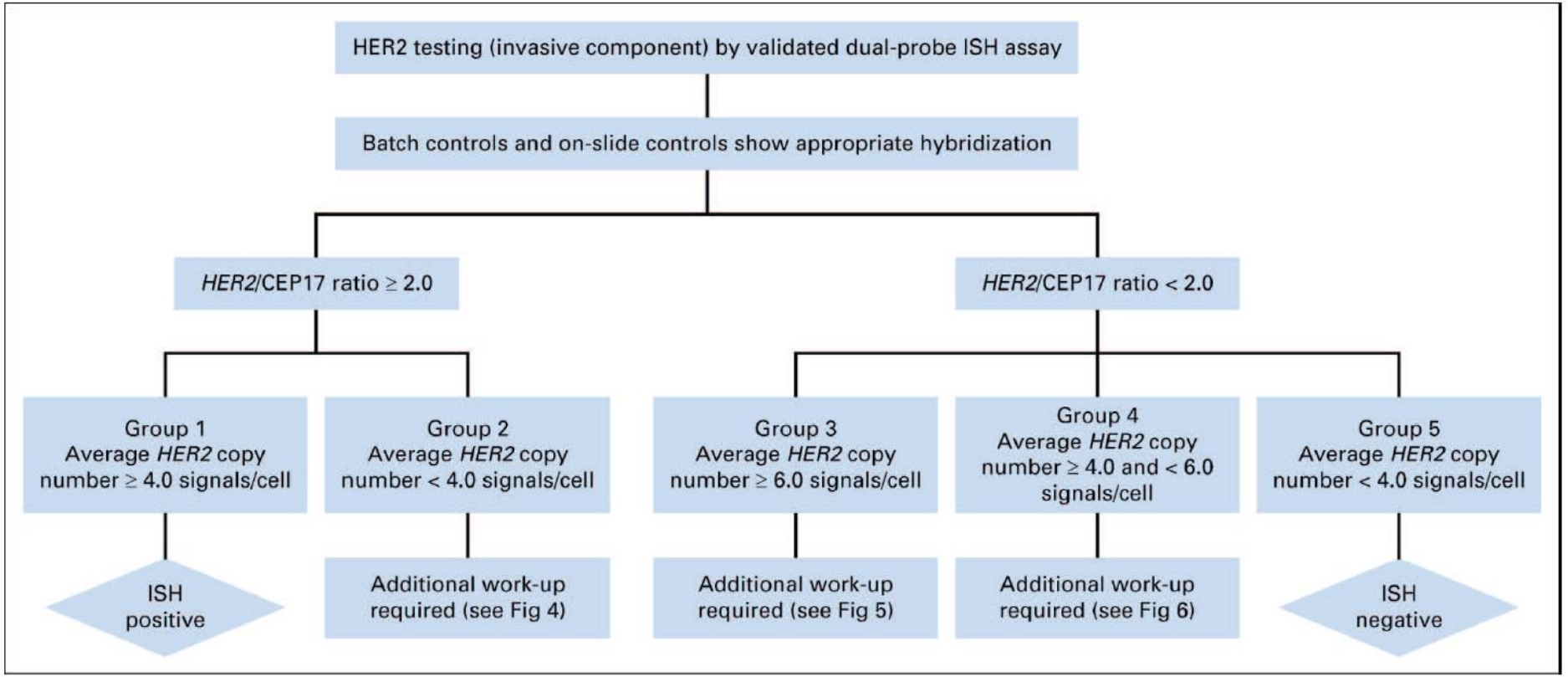
FISH for HER2, Dual Probe (Vysis PathVysion)

Not Amplified



Amplified





Wolff, Arch Pathol Lab Med, 2018

FISH for HER2

Pros

- Highly specific reagents commercially available
- Standardized threshold for positivity
- Results quantitative
- Internal controls
- Less affected by preanalytic factors
- Linked to clinical outcome and therapeutic response

Cons

- Not available in many labs
- Technically more difficult than IHC
- Longer procedure time than IHC
- Requires fluorescence microscope
- Poor morphology
- More expensive than IHC

Our Practice

- 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

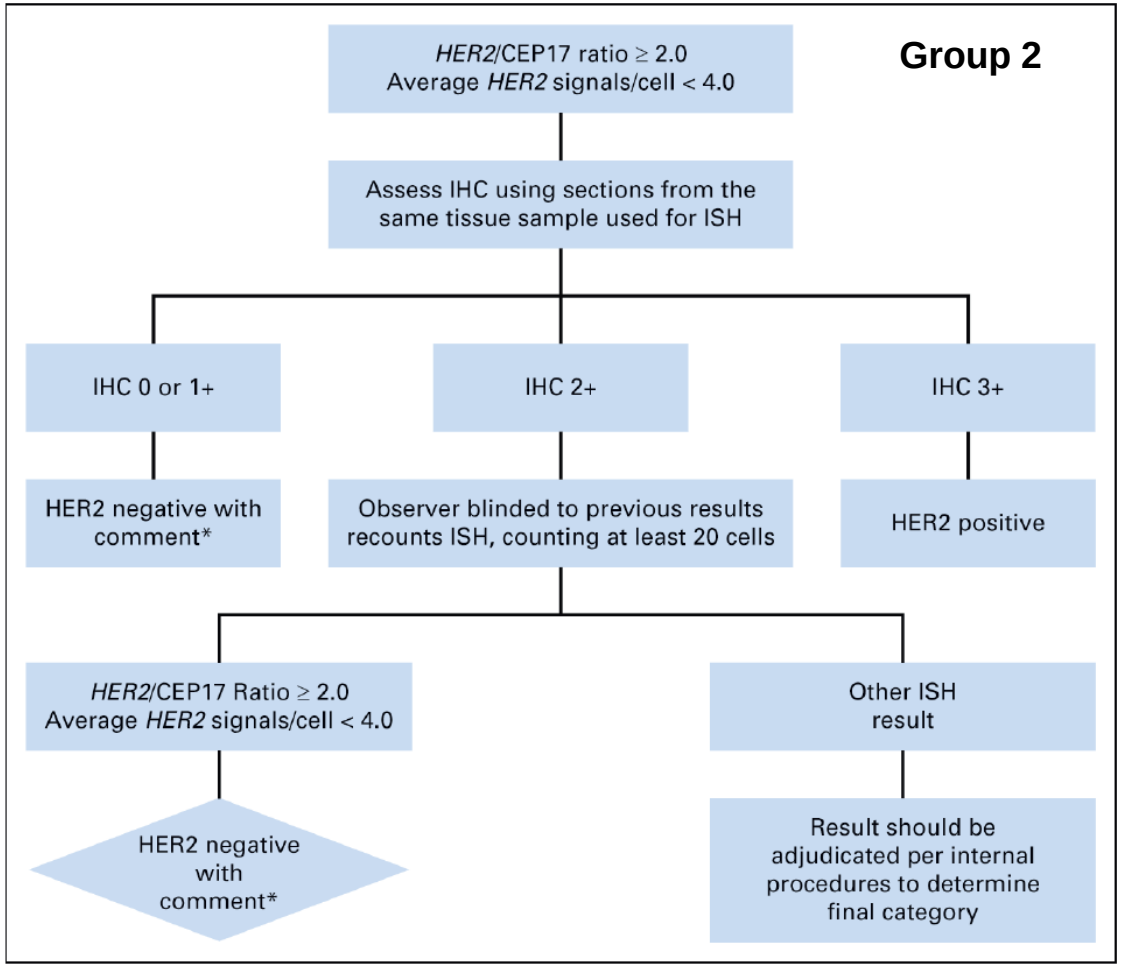


Figure 4. *Clinical Question 3, group 2. *Evidence is limited on the efficacy of human epidermal growth factor receptor 2 (HER2)–targeted therapy in the small subset of cases with an HER2/chromosome enumeration probe 17 (CEP17) ratio ≥2.0 and an average HER2 copy number of <4.0 per cell. In the first generation of adjuvant trastuzumab trials, patients in this subgroup who were randomly assigned to the trastuzumab arm did not seem to derive an improvement in disease-free or overall survival, but there were too few such cases to draw definitive conclusions. Immunohistochemistry (IHC) expression for HER2 should be used to complement in situ hybridization (ISH) and define HER2 status. If the IHC result is not 3+ positive, it is recommended that the specimen be considered HER2 negative because of the low HER2 copy number by ISH and the lack of protein overexpression.*

Wolff, Arch Pathol Lab Med, 2018

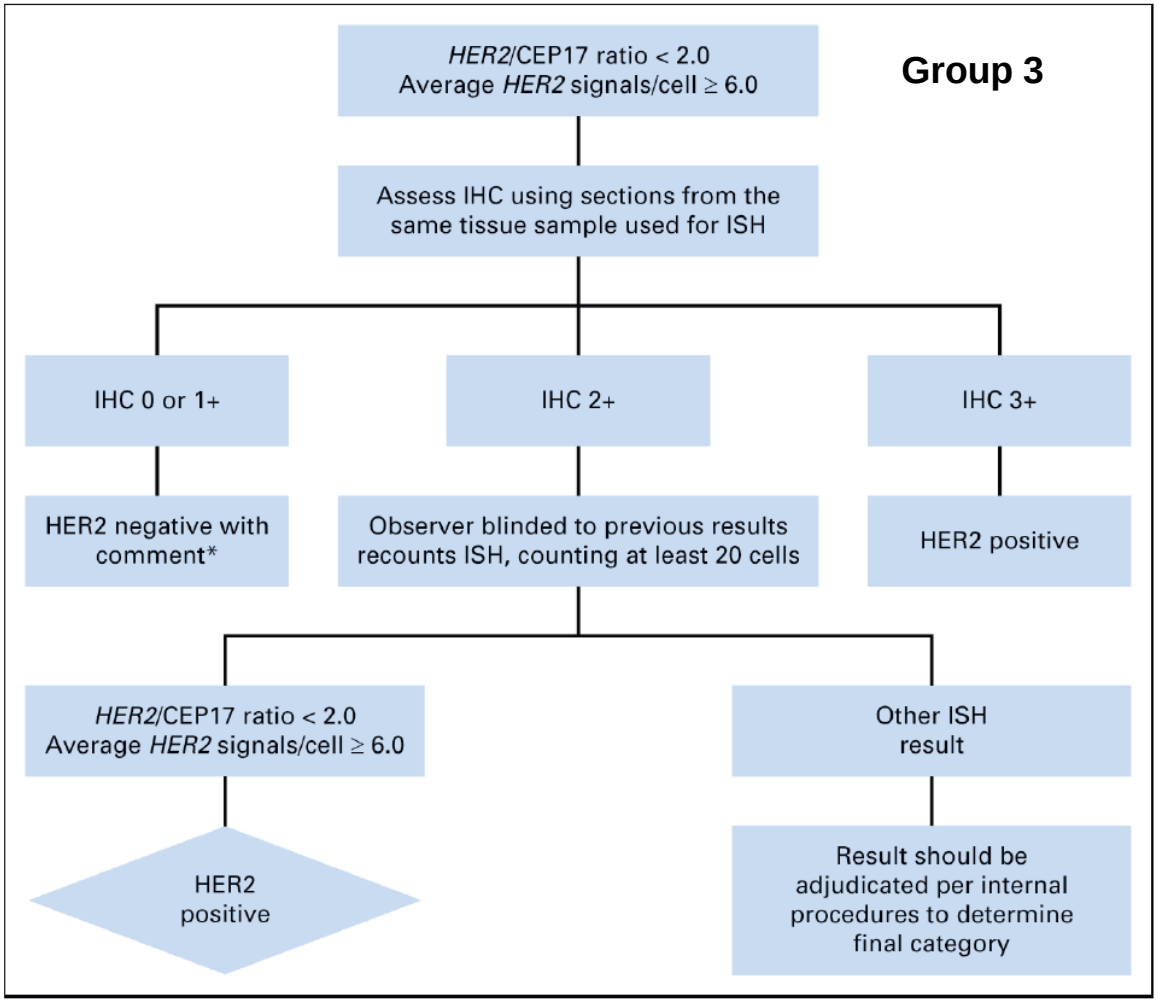
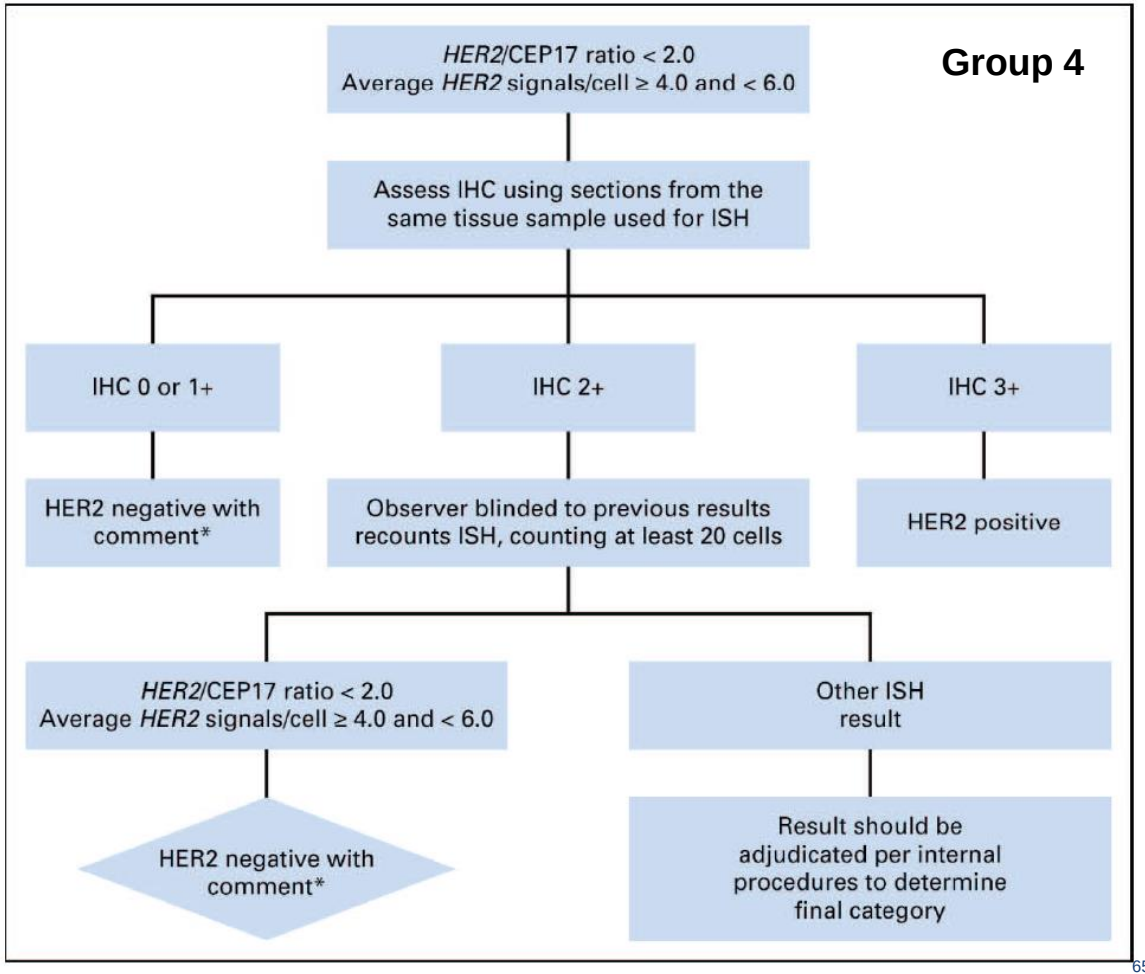


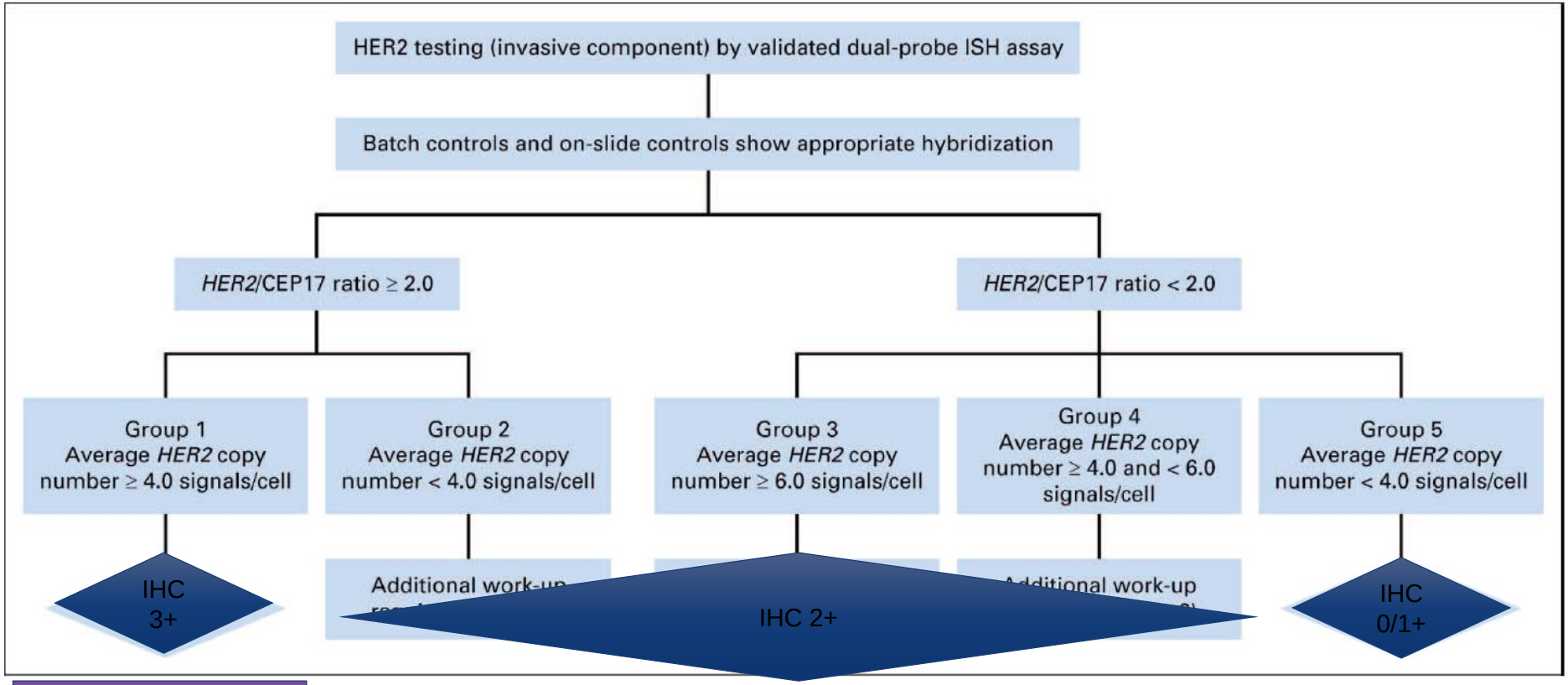
Figure 5. *Clinical Question 4, group 3. *There are insufficient data on the efficacy of human epidermal growth factor receptor 2 (HER2)–targeted therapy in cases with a HER2 ratio of <2.0 in the absence of protein overexpression because such patients were not eligible for the first generation of adjuvant trastuzumab clinical trials. When concurrent immunohistochemistry (IHC) results are negative (0 or 1+), it is recommended that the specimen be considered HER2 negative. CEP17, chromosome enumeration probe 17.*

Wolff, Arch Pathol Lab Med, 2018

Figure 6. *Clinical Question 5, group 4. *It is uncertain whether patients with an average of ≥4.0 and <6.0 human epidermal growth factor receptor 2 (HER2) signals per cell and a HER2/chromosome enumeration probe 17 (CEP17) ratio of <2.0 benefit from HER2-targeted therapy in the absence of protein overexpression (immunohistochemistry [IHC] 3+). If the specimen test result is close to the in situ hybridization (ISH) ratio threshold for positive, there is a higher likelihood that repeat testing will result in different results by chance alone. Therefore, when IHC results are not 3+ positive, it is recommended that the sample be considered HER2 negative without additional testing on the same specimen.*



Wolff, Arch Pathol Lab Med, 2018



Group 1 Positive	Group 2 Negative	Group 3 Positive	Group 4 Negative	Group 5 Negative

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
- Newer evidence of benefit in HER2-low positive tumors (IHC 1+ or 2+ and ISH negative) with antibody drug conjugates (ADC)
- e.g. Trastuzumab deruxtecan (T-DXd), a novel HER2-targeted ADC designed to deliver a topoisomerase I inhibitor payload to HER2-expressing cancer cells

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 ~$70,000/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

- 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

Alternative Probe Testing

- Following the ASCO/CAP 2013 Update, group 4 cases (i.e. ratio<2, HER2 copy number ≥4 and <6 signals/cell) were often tested with multiple chromosome 17 probes (alternate probes)
- Some of these assays were not analytically or clinically validated
- 2018 Expert Panel strongly recommends against this practice

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
- Consider additional testing or review of morphology when result does not make sense
- Consider additional testing if tumor is HER2 negative on CNB and high grade on excision

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

Multigene assays

Commercially Available Multigene Signatures

Gene expression test	Oncotype DX®	MammaPrint®	"Intrinsic gene molecular classification/PAM50/Prosigna™	MapQuant DX®	EndoPredict®	Breast Cancer Index℠ (HoxB13:IL17BR/MGI)
Provider	Genomic Health	Agendia BV	NanoString Technologies Inc.	Qiagen (formerly Ipsogen Inc.); still available?	Sividon Diagnostics	bioTheranostics
Assay	21-gene recurrence score	70-gene signature	"Intrinsic gene" list or 50-gene PCR	97-gene signature or 8-gene qRT-PCR	qRT-PCR 8 prognostic genes, 3 normalization gene	2-gene HOXB13:IL17R/ molecular-grade index
RNA isolated from	Formalin-fixed, paraffin-embedded tumor tissue	Frozen or formalin-fixed, paraffin-embedded tumor tissue	Frozen or formalin-fixed, paraffin-embedded tumor tissue	Frozen or formalin-fixed, paraffin-embedded tumor tissue	Formalin-fixed, paraffin-embedded tumor tissue	Formalin-fixed, paraffin-embedded tumor tissue
Outcome	Disease-free relapse at 10 years	Distant metastasis at 5 years	Disease-free, distant metastasis-free and overall survival	Good (GGI I) or poor (GGI III) prognosis	Distant metastasis at 10 years	Relapse-free and overall survival
Clinical Application	Prediction of recurrence risk in ER+ BC treated with tamoxifen	Prognosis of N0 BC, <5 cm diameter	Classification of invasive breast cancers	Molecular grading, for ER+, histological grade II BC	Prognosis of endocrine-treated BC	Prognostic in ER+ BC, prediction of response to tamoxifen
Risk groups identified	Three risk groups based on recurrence score	Dichotomous; good or poor prognosis	Classification of tumors into luminal A, luminal B, HER2, and basal-like subtypes	Dichotomous; GGI I or GGI III	Dichotomous; low risk or high risk	Continuous variable; risk of recurrence score

ER estrogen receptor, *BC* breast carcinoma, *GGI* Genomic Grade Index

Van de Vijver, 2014

National Comprehensive Cancer Network®

NCCN Guidelines Version 3.2020
Invasive Breast Cancer

NCCN Guidelines Index
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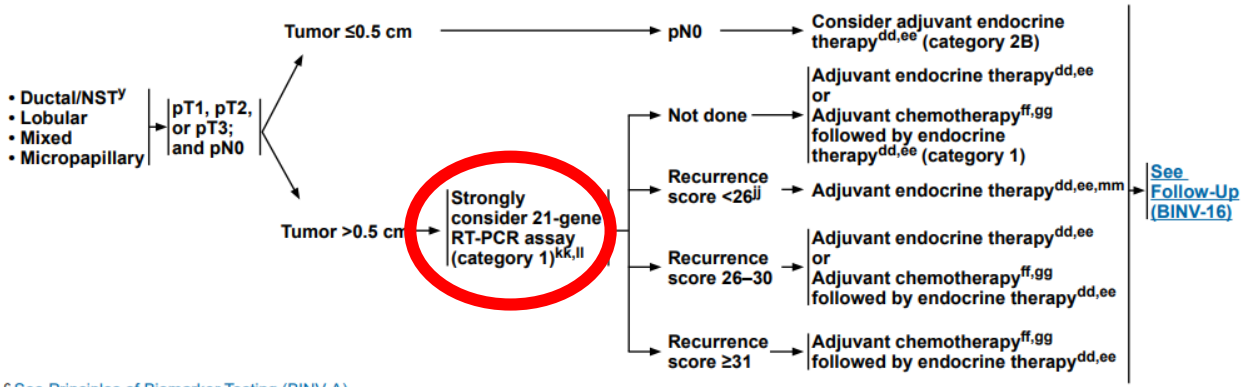
Discussion

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.

Comparing Breast Cancer Multiparameter Tests in the OPTIMA Prelim Trial: No Test Is More Equal Than the Others

Comparison of 5 different prognostic tests (including OncotypeDx, Prosigna, Mammaprint and IHC4)

Only modest agreement found when stratifying by low/intermediate vs. high risk of recurrence

All three subtype tests assigned between 59.5%-62.4% to luminal A category, but only 40% assigned to luminal A by all three tests

Only 19.2% uniformly assigned to non-luminal A subtypes

Implications for individual patient subtyping and risk stratification

Bartlett, JNCI, 2016

Multigene Prognostic Tests

Is this approach really better than using a combination of clinical and pathologic factors supplemented by appropriate biomarkers detected by IHC (eg, ER, PR, HER2 and Ki67)?

Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures

Pratyaksha Wirapati[1], Christos Sotiriou[2], Susanne Kunkel[1], Pierre Farmer[1,3], Sylvain Pradervand[4], Benjamin Haibe-Kains[2,5], Christine Desmedt[2], Michail Ignatiadis[2], Thierry Sengstag[1,3], Frédéric Schütz[1], Darlene R Goldstein[1,4,6], Martine Piccart[2] and Mauro Delorenzi[1,3]

- *Proliferation genes* are the common driving force in all prognostic signatures
- Factors associated with tumor burden (size, nodal status) remain independently associated with prognosis

Multigene Prognostic Tests

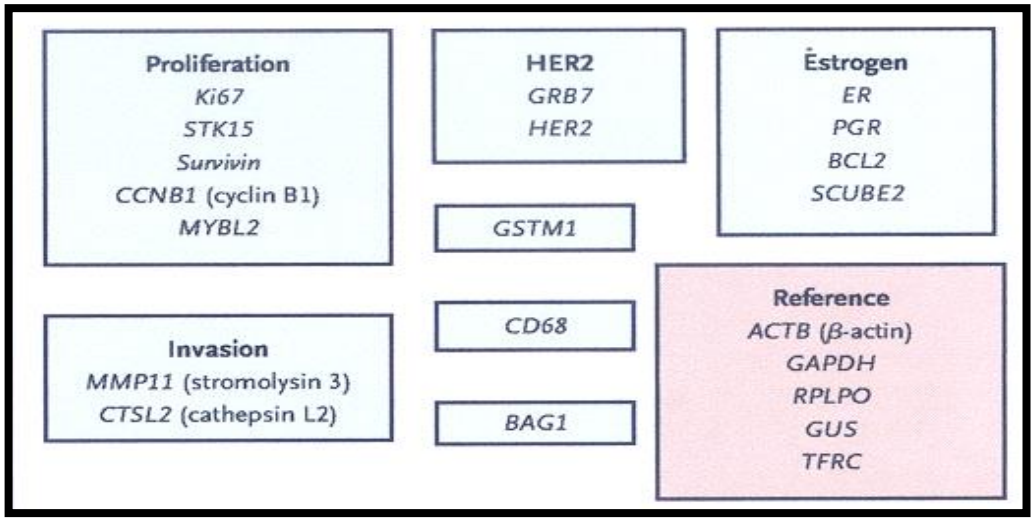
Assay	# of genes assayed	Traditional prognostic factors included	Sendout test	Current cost (2018)	Score reporting
OncotypeDx	21	No	Yes	~$4000	0-100 Low/Int/High Risk
Mammaprint	70	No	Yes	~$4000	-1 to +1 Low/High Risk
Breast Cancer Index	2 +Molecular Grade Index	No	Yes	~$4000	0-10 Low/High Risk
EndoPredict 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.)



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

NEJM 2004;351:2817

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.

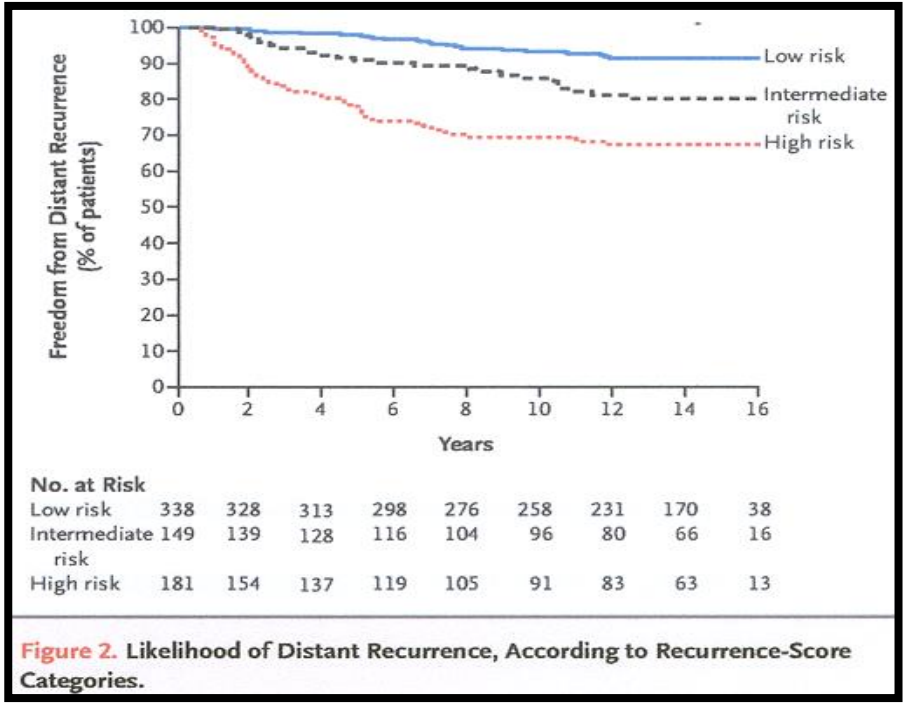


Figure 2. Likelihood of Distant Recurrence, According to Recurrence-Score Categories.

Paik, 2004

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

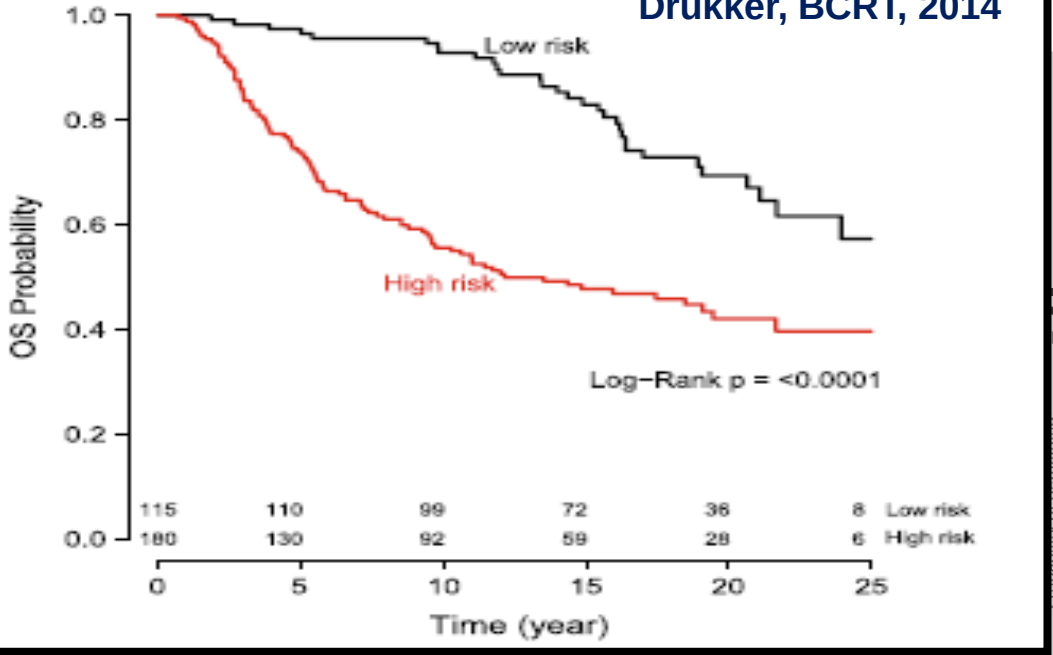
Marc J. van de Vijver, M.D., Ph.D., Yudong D. He, Ph.D., Laura J. van 't Veer, Ph.D., Hongyue Dai, Ph.D., Augustinus A.M. Hart, M.Sc., Dorien W. Voskuil, Ph.D., George J. Schreiber, M.Sc., Johannes L. Peterse, M.D., Chris Roberts, Ph.D., Matthew J. Marton, Ph.D., Mark Parrish, Douwe Atsma, Anke Witteveen, 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.

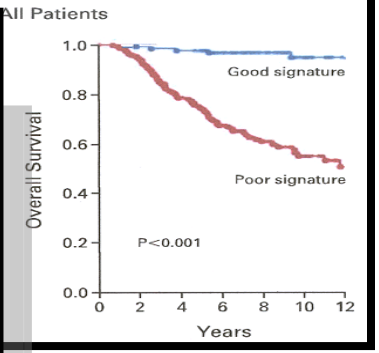
Expression signature
identified good and p
among both N- and N

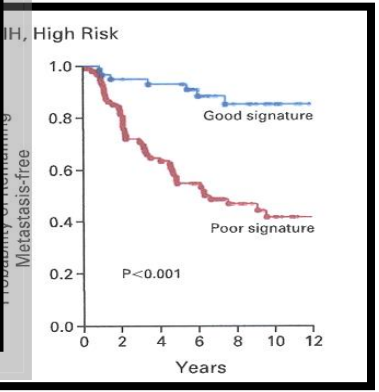
Better than standard
on clinical and histolo
Gallen, NIH)

Mar
(Agendia)

Drukker, BCRT, 2014



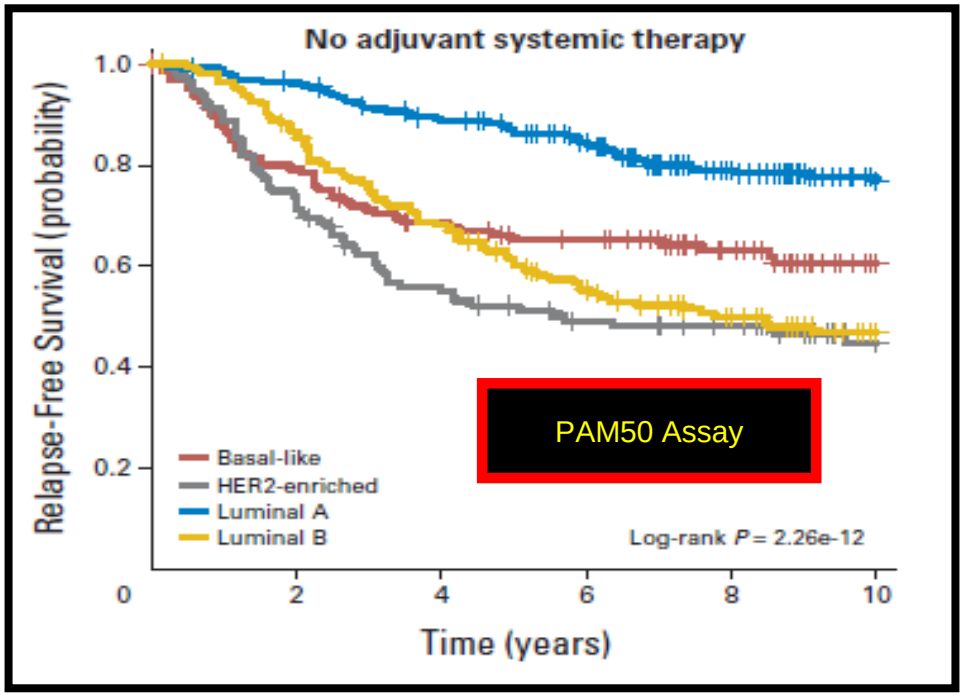




NEJM, 2002

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

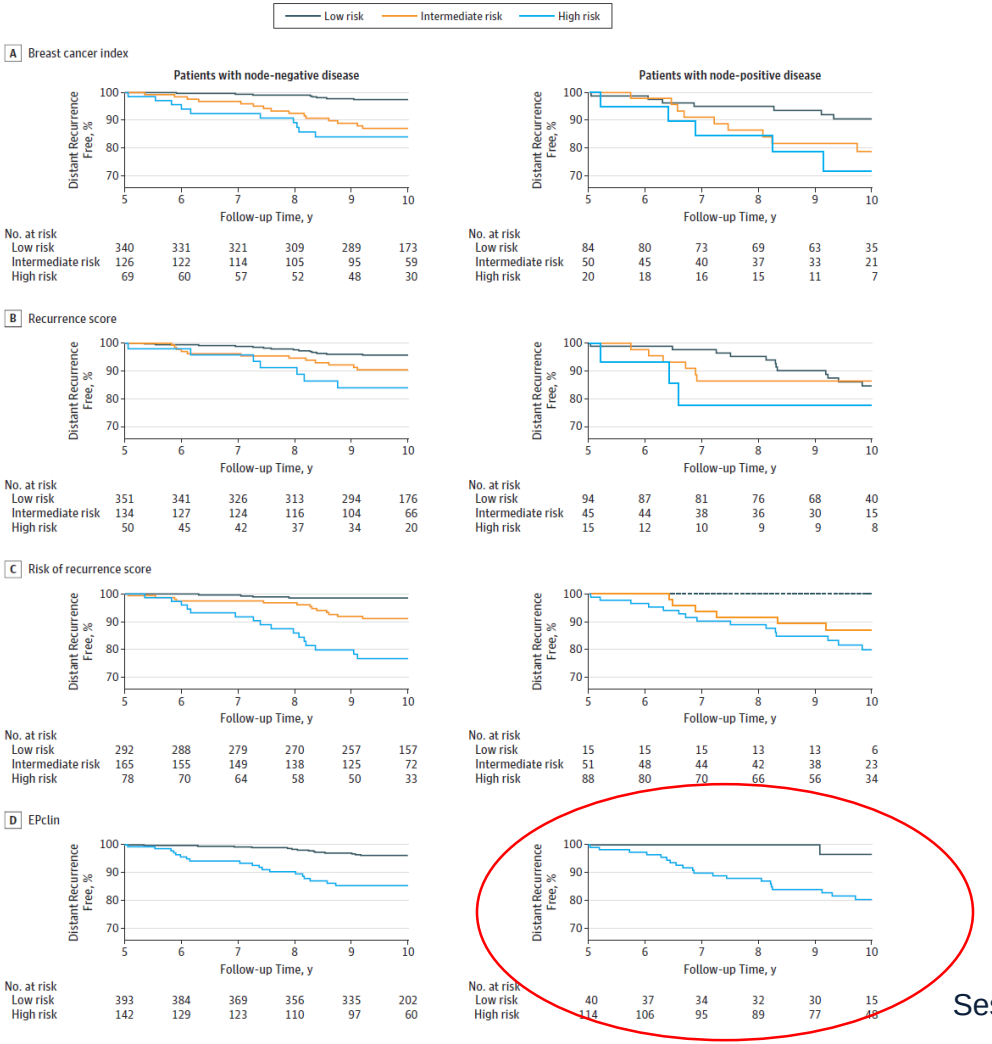
Comparison of the Performance of 6 Prognostic Signatures for Estrogen Receptor–Positive Breast Cancer

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



Sestak, JAMA Oncol, 2018

MULTIGENE ASSAYS FOR CONSIDERATION OF ADDITITON OF ADJUVANT SYSTEMIC CHEMOTHERAPY TO ADJUVANT ENDOCRINE THERAPY[a]

Assay	Predictive	Prognostic	NCCN Category of Preference	NCCN Category of Evidence and Consensus	Recurrence Risk	Treatment Implications (references on next page)
21-gene (Oncotype Dx) (for pN0 or node negative)	Yes	Yes	Preferred	1	<26	Patients with T1b/c and T2, hormone receptor-positive, HER2-negative and lymph node-negative tumors, with risk scores (RS) between 0-10 have a risk of distant recurrence of less than 4% and those with RS 11-25, derived no benefit from the addition of chemotherapy to endocrine therapy in the prospective TAILORx study.[1] In women 50 years of age or younger, with RS 16-25 addition of chemotherapy to endocrine therapy was associated with a lower rate of distance recurrence compared with endocrine monotherapy. Consideration should be given for the addition of chemotherapy to endocrine therapy in this group.[1]
					26-30	In patients with T1 and T2, hormone receptor-positive, HER2-negative and lymph node-negative tumors and a RS of 26-30, the omission of chemotherapy has not been studied prospectively. Clinicians should consider additional clinical and pathological factors with regard to the addition of chemotherapy to endocrine therapy in decision-making.[2]
					≥31	For patients with T1b/c and T2, hormone receptor-positive, HER2-negative and lymph node-negative tumor RS ≥31, the addition of chemotherapy to endocrine therapy is recommended.[2]
21-gene (Oncotype Dx) (for pN+ or node positive)	N/A* *awaiting results of Rxponder study	Yes	Other	2A	Low (<18)	The RS is prognostic in women with hormone receptor-positive, lymph node positive tumors receiving endocrine monotherapy.[3-10] A secondary analysis of a prospective registry of women with hormone receptor-positive, HER2-negative, lymph node positive tumors demonstrated a 5 year risk of distant recurrence of 2.7% in patients with a RS of <18 treated with endocrine monotherapy.[9] In the West German Plan B study, 110 women with hormone receptor-positive, HER2-negative, lymph node-positive tumors and a RS of <11, showed a 5 year disease free survival of 94.4% when treated with endocrine monotherapy.[6] For hormone receptor-positive, HER2-negative, lymph node-positive tumors, clinicians should be aware that the optimal RS cut-off (< 11 vs < 18) is still unknown both for prognosis (risk of recurrence) as well as prediction of chemotherapy benefit.
					Intermediate (18-30) or High (≥31)	In a secondary analysis of the SWOG 8814 trial of women with hormone receptor-positive, lymph node-positive tumors, high RS (≥31) was predictive of chemotherapy benefit. Because of a higher risk of distant recurrence, patients with hormone receptor-positive, 1-3 positive lymph nodes and RS of ≥18 should be considered for adjuvant chemotherapy in addition to endocrine therapy.[3]
70-gene (MammaPrint) (for node negative and 1-3 positive nodes)	Not determined	Yes	Other	1	Low	With a median follow-up of 5 years, among patients at high clinical risk and low genomic risk, the rate of survival without distant metastasis in this group was 94.7% (95% confidence interval, 92.5% to 96.2%) among those who did not receive adjuvant chemotherapy. Among patients with 1-3 positive nodes, the rates of survival without distant metastases were 96.3% (95% CI, 93.1 to 98.1) in those who received adjuvant chemotherapy versus 95.6 (95% CI, 92.7 to 97.4) in those who did not receive adjuvant chemotherapy.[11] Therefore, the additional benefit of adjuvant chemotherapy may be small in this group.
					High	
50-gene (PAM 50) (for node negative and 1-3 positive nodes)	Not determined	Yes	Other	2A	Node negative: Low (0-40)	For patients with T1 and T2 hormone receptor-positive, HER2- negative, lymph node-negative tumors, a risk of recurrence score in the low range, regardless of T size, places the tumor into the same prognostic category as T1a–T1b, N0, M0.[12]
					Node negative: Intermediate (41-60)	
					Node negative: High (61-100)	
					Node positive: Low (0-40)	In patients with hormone receptor-positive, HER2-negative, 1-3 positive lymph nodes with low risk of recurrence score, treated with endocrine therapy alone, the distant recurrence risk was less than 3.5% at 10 years [12] and no distant recurrence was seen at 10 years in TransATAC study in a similar group.[13]
					Node positive: High (41-100)	
12-gene (EndoPredict) (node negative and 1-3 nodes)	Not determined	Yes	Other	2A	Low (<3.3287)	For patients with T1 and T2 hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a 12-gene low-risk score, regardless of T size, places the tumor into the same prognostic category as T1a–T1b, N0, M0.[13] In ABCSG 6/8, patients in the low risk group has risk of distant recurrence of 4% at 10 years and in the TransATAC study, patients with 1-3 positive nodes in the low-risk group had a 5.6% risk of distant recurrence at 10 years.[13]
					High (>3.3287)	
Breast Cancer Index (BCI)	Not determined	Yes	Other	2A	Low risk of late occurrence (0-5)	For patients with T1 and T2 hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a BCI in the low-risk range, regardless of T size, places the tumor into the same prognostic category as T1a-T1b, N0, M0. There are limited data as to the role of BCI in hormone receptor-positive, HER2-negative, and lymph node-positive breast cancer.[13]
					High risk of late occurrence (5.1-10)	

Surrogate Histologic Markers and IHC in Clinical Practice

- Proliferation markers used to differentiate Luminal A from Luminal B
- Unlike ER and HER2 which show bimodal distribution with clear cutpoints, proliferation determined by several genes with continuous distribution

Surrogate Histologic Markers and Ki-67 IHC in Clinical Practice

- Tumor grade most widely used as a surrogate for proliferation
- Ki67 most widely used proliferation marker
- Use of Ki67 shifts some luminal A-like tumors to luminal B-like
- International Ki-67 working group (IKWG) developing guidelines
- Recently 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 $\geq$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

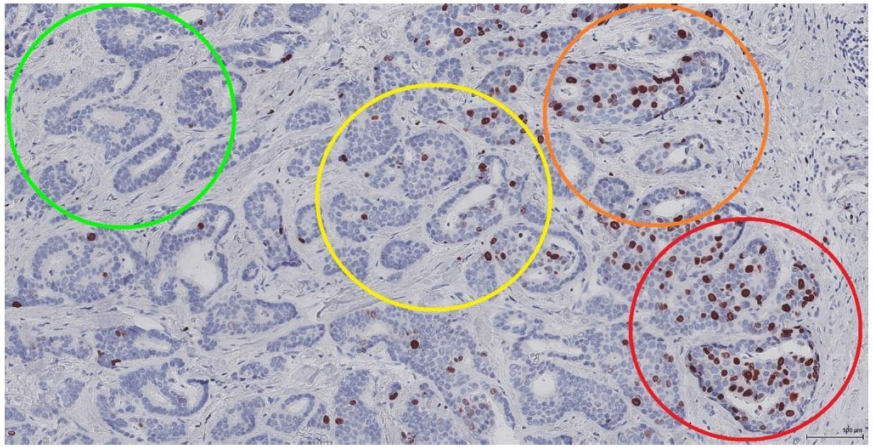
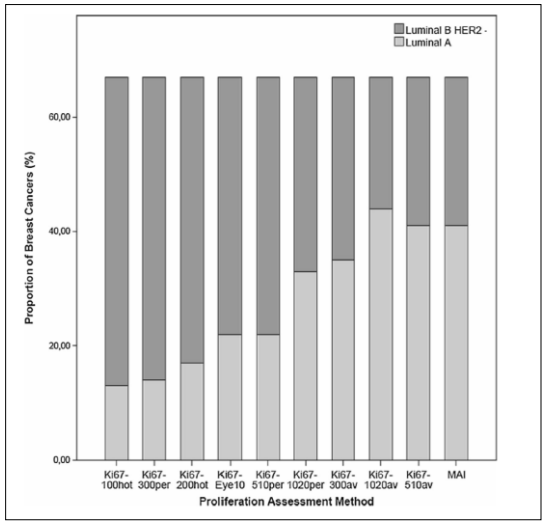


Fig. 1 Simplified example of a Ki67-labeled breast cancer showing hot spot (*red circle*), cold spot (*green circle*), periphery area (*orange circle*), and area of intermediate proliferation (*yellow circle*)

Using <20% cut point to define luminal A tumors



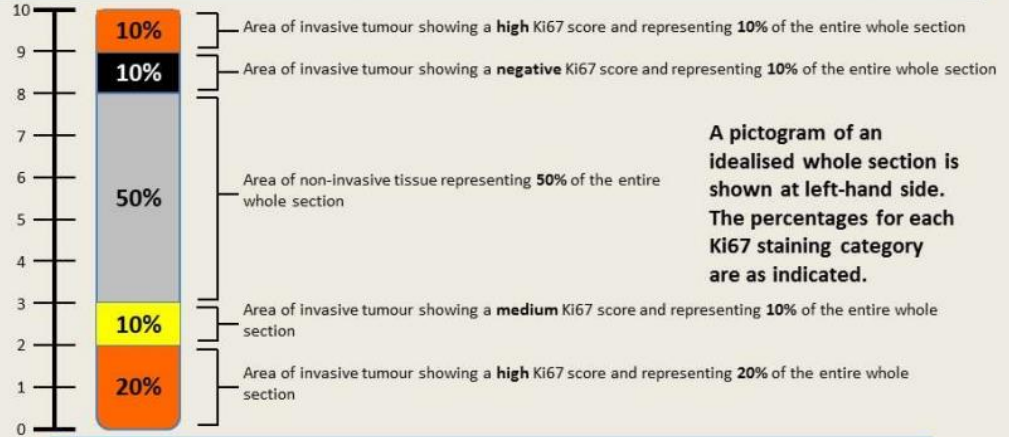
Focke, BCRT, 2016

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
- While ki-67 is prognostic, abemecliclib + ET benefit found to be independent of Ki-67 index (monarchE Trial: CDx Ki-67 IHC MIB-1 pharmDx (Dako Omnis, Carpinteria, CA)
- 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

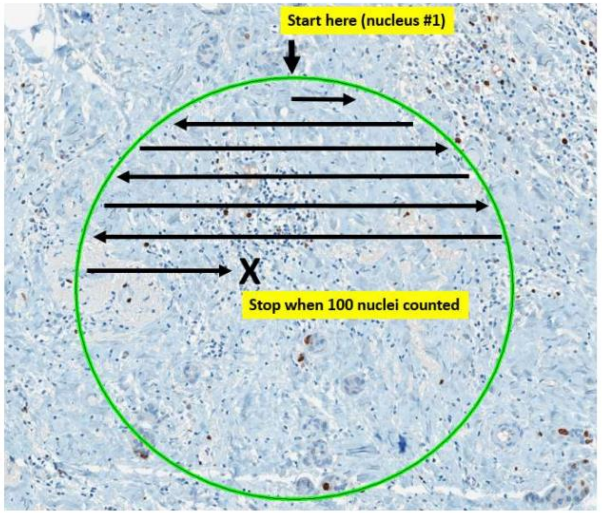
$$\text{Relative \% of invasive tumour nuclei in a particular Ki67 staining category} = \frac{\text{Total \% of invasive tumour nuclei in that category}}{\text{Total \% of all invasive tumour nuclei present}} \times 100$$

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.



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

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

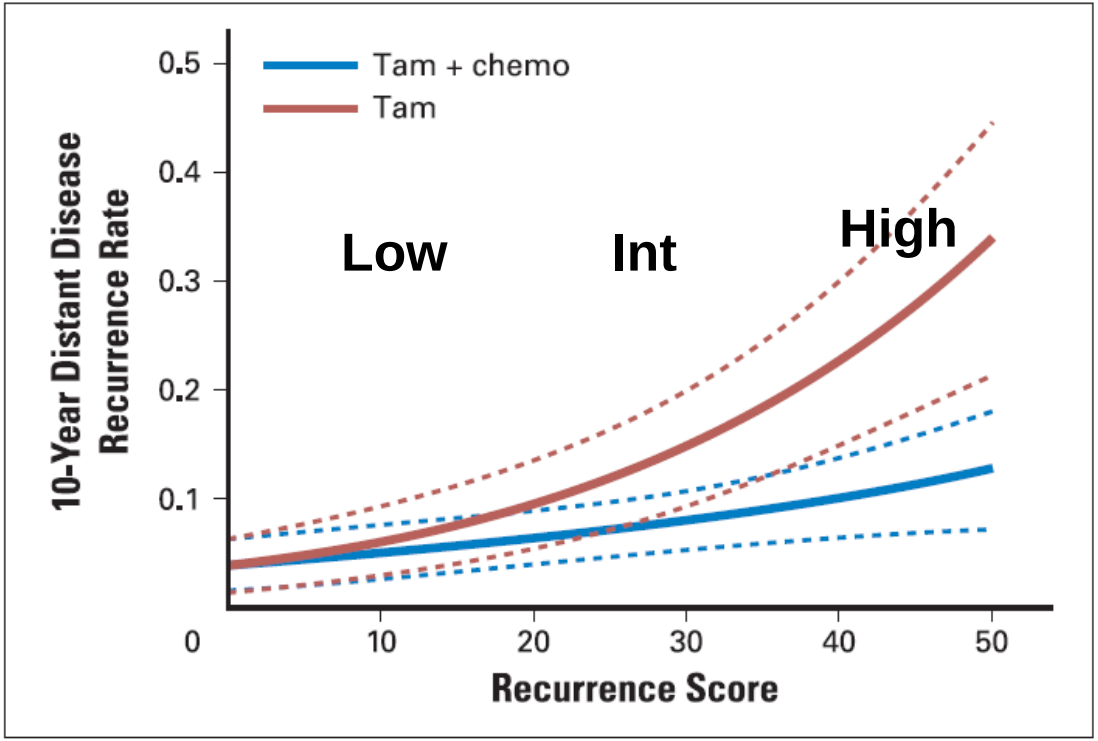
IKWG, website

Multigene Signatures
and
Predictive Factors

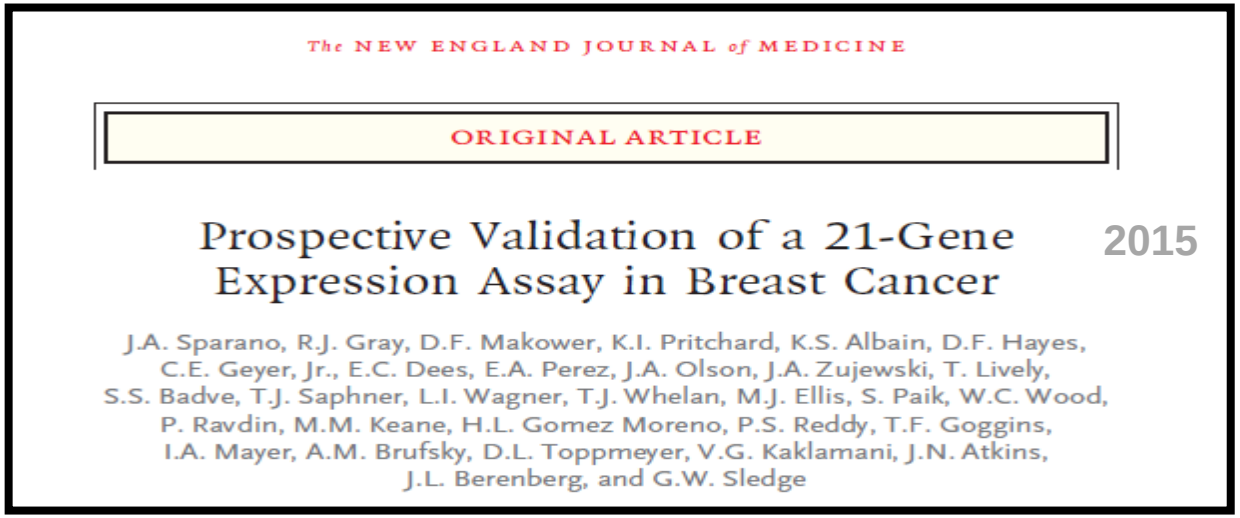
Multigene Assays for Consideration of Adjuvant Systemic Therapy in addition to Endocrine Therapy					
Test	**Predictive**	**Prognostic**	**NCCN category of preference**	**NCCN category of evidence**	**Recurrence Risk**
21 gene assay (OncotypeDX) Node negative	YES	Yes	Preferred	1	Low Intermediate High
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



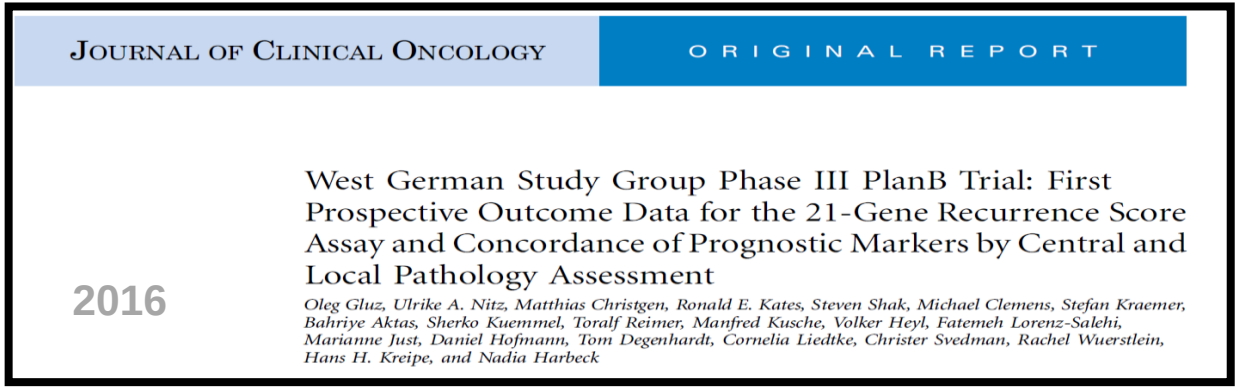
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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

2015

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



JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

2016

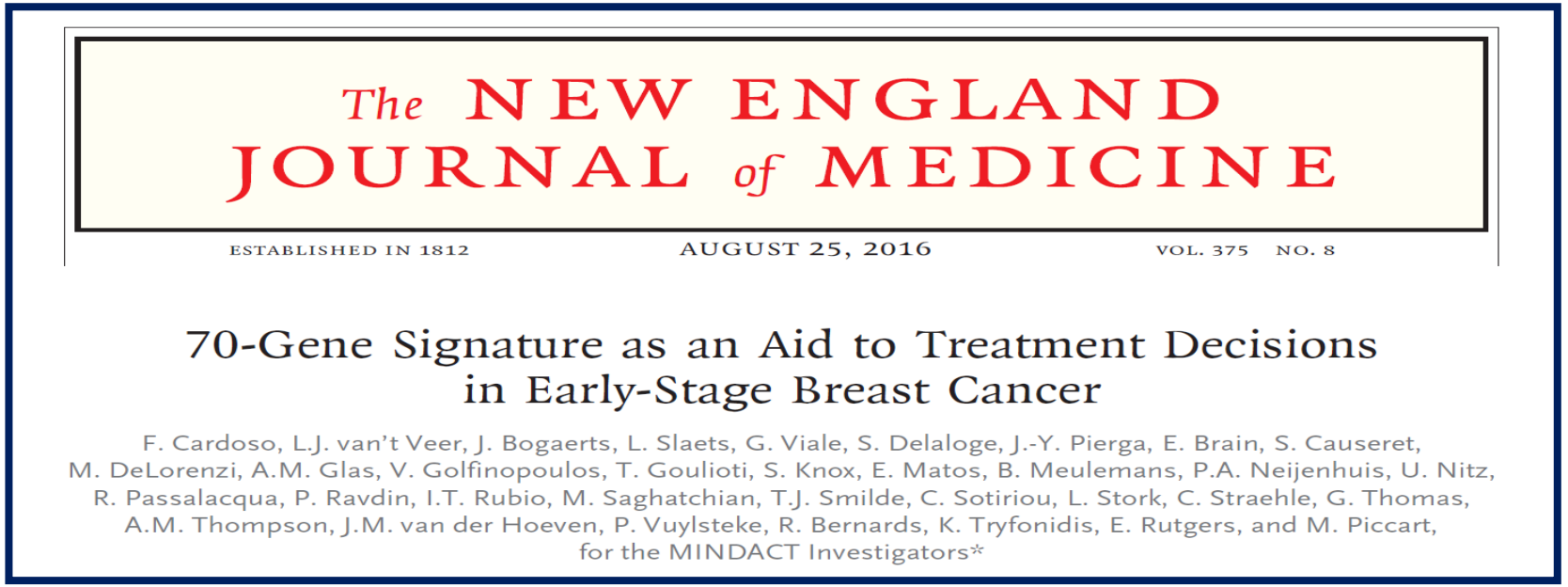
West German Study Group Phase III PlanB Trial: First Prospective Outcome Data for the 21-Gene Recurrence Score Assay and Concordance of Prognostic Markers by Central and Local Pathology Assessment

Oleg Gluz, Ulrike A. Nitz, Matthias Christgen, Ronald E. Kates, Steven Shak, Michael Clemens, Stefan Kraemer, Bahriye Aktas, Sherko Kuemmel, Toralf Reimer, Manfred Kusche, Volker Heyl, Fatemeh Lorenz-Salehi, Marianne Just, Daniel Hofmann, Tom Degenhardt, Cornelia Liedtke, Christer Svedman, Rachel Wuerstlein, Hans H. Kreipe, and Nadia Harbeck

Both studies have shown very low rates of recurrence 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 to be made for use of alternate algorithms, such as Magee Equation (or variations thereof) which demonstrate $100M in cost savings to the health care economy
- In a recent study of 1396 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

Chemotherapy Benefit?

- Three prospective randomized trials-MINDACT, TAILORx and RxPONDER- are testing the usefulness of gene signatures in predicting benefit from adjuvant chemotherapy in patients with ER+ breast cancer in the intermediate risk groups
- Results demonstrate 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

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)

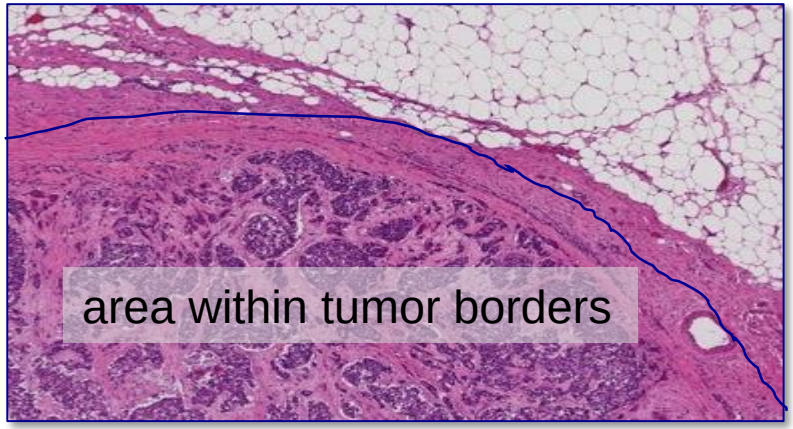
Salgado, Ann Oncol, 2015

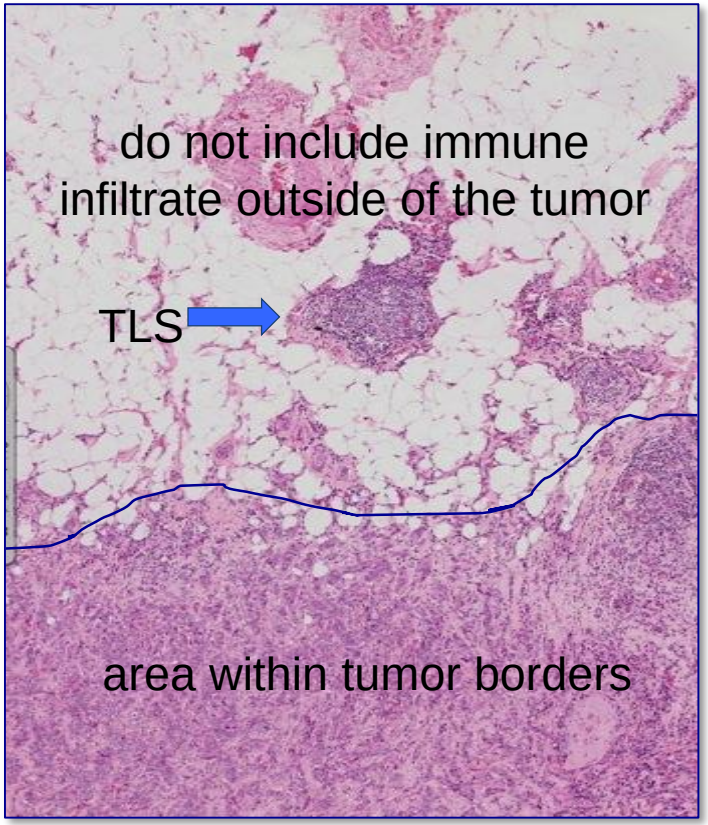
Step 1: Define area for TIL evaluation

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

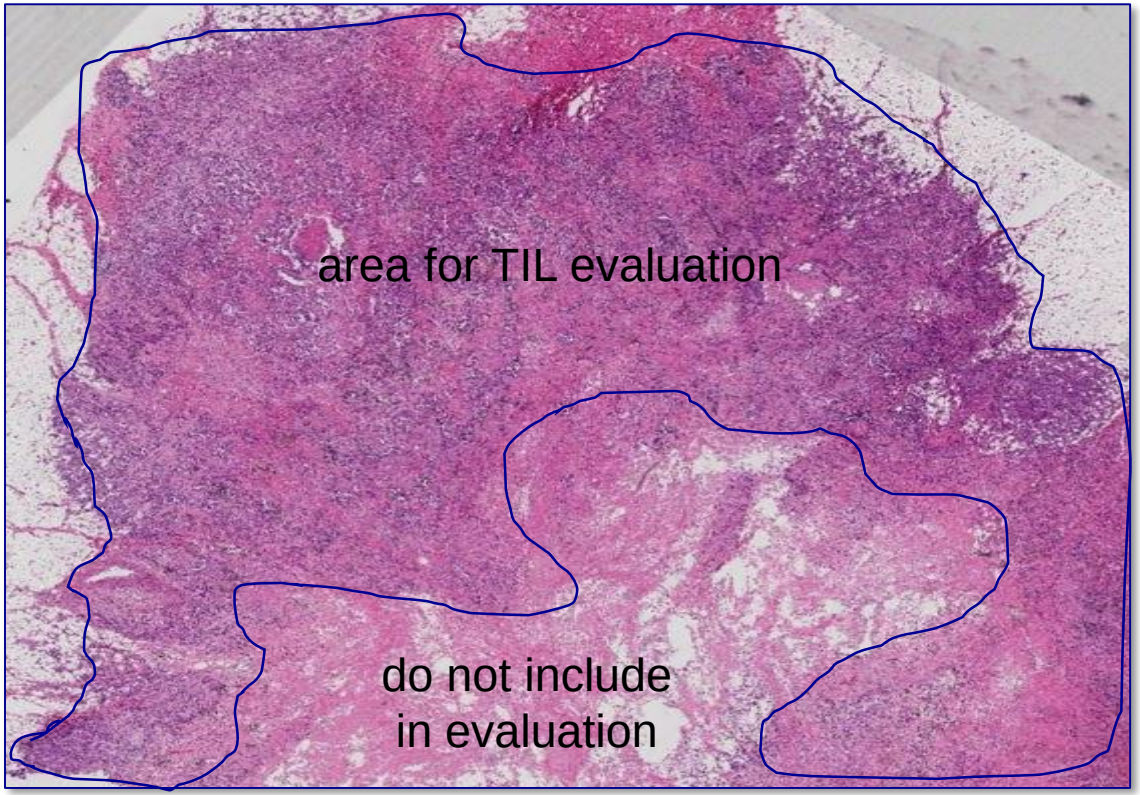




From TILs website www.tilsinbreastcancer.org

Step 1: Define area for TIL evaluation

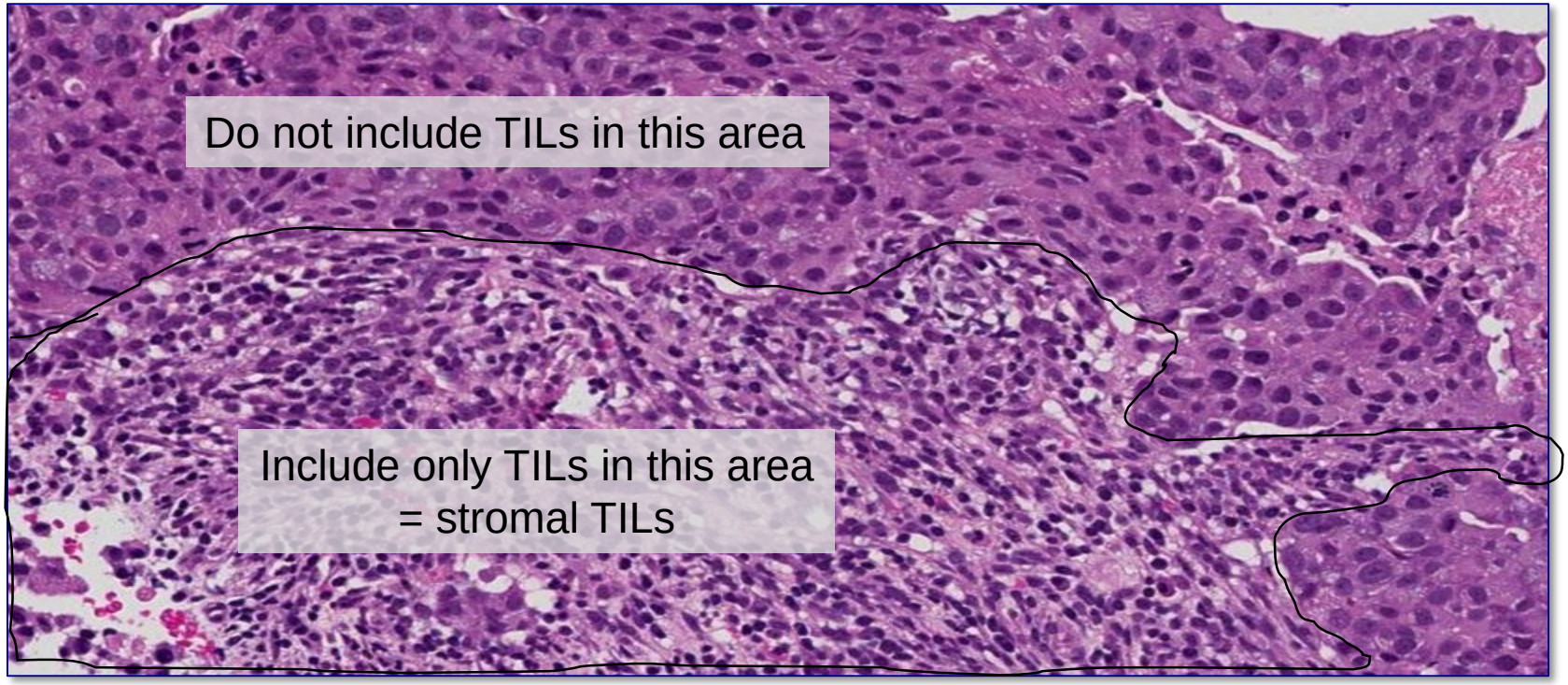
Large areas of central necrosis or fibrosis are not included in the evaluation



From TILs website www.tilsinbreastcancer.org

Step 2: Focus on stromal TIL

In the diagnostic setting, only stromal TILs are relevant



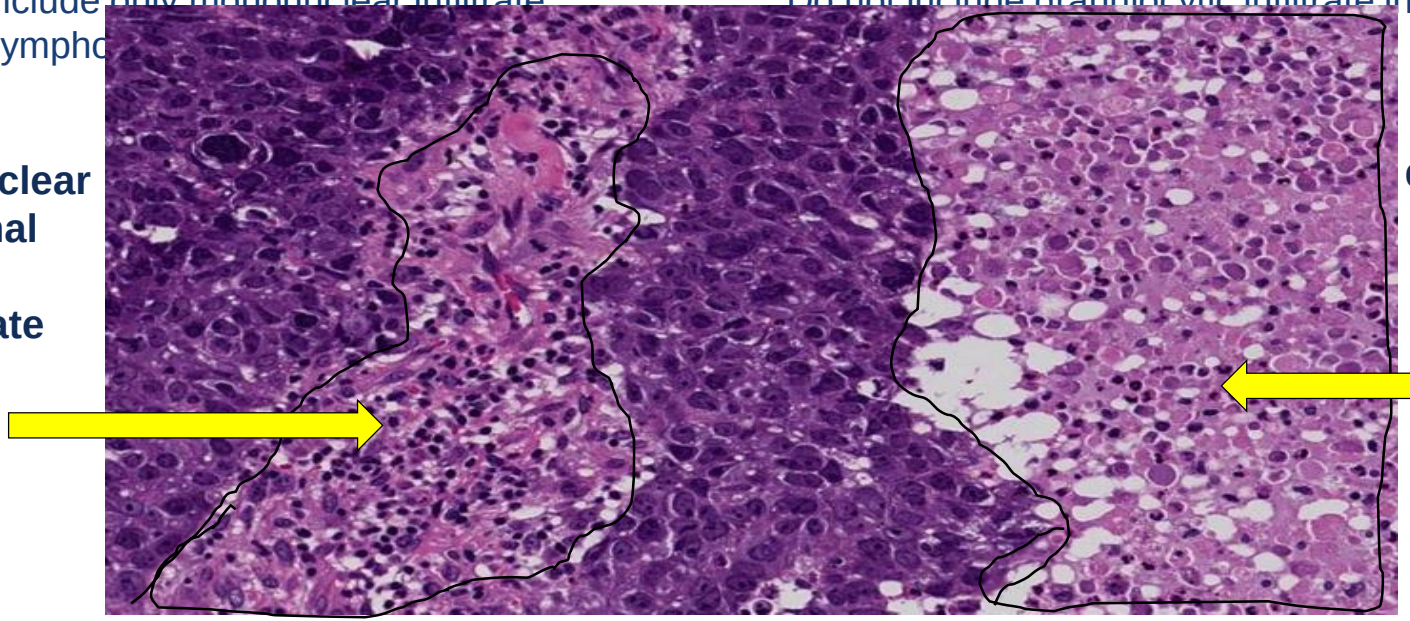
From TILs website www.tilsinbreastcancer.org

Step 3: Determine type of inflammatory infiltrate

Include only mononuclear infiltrate (lympho

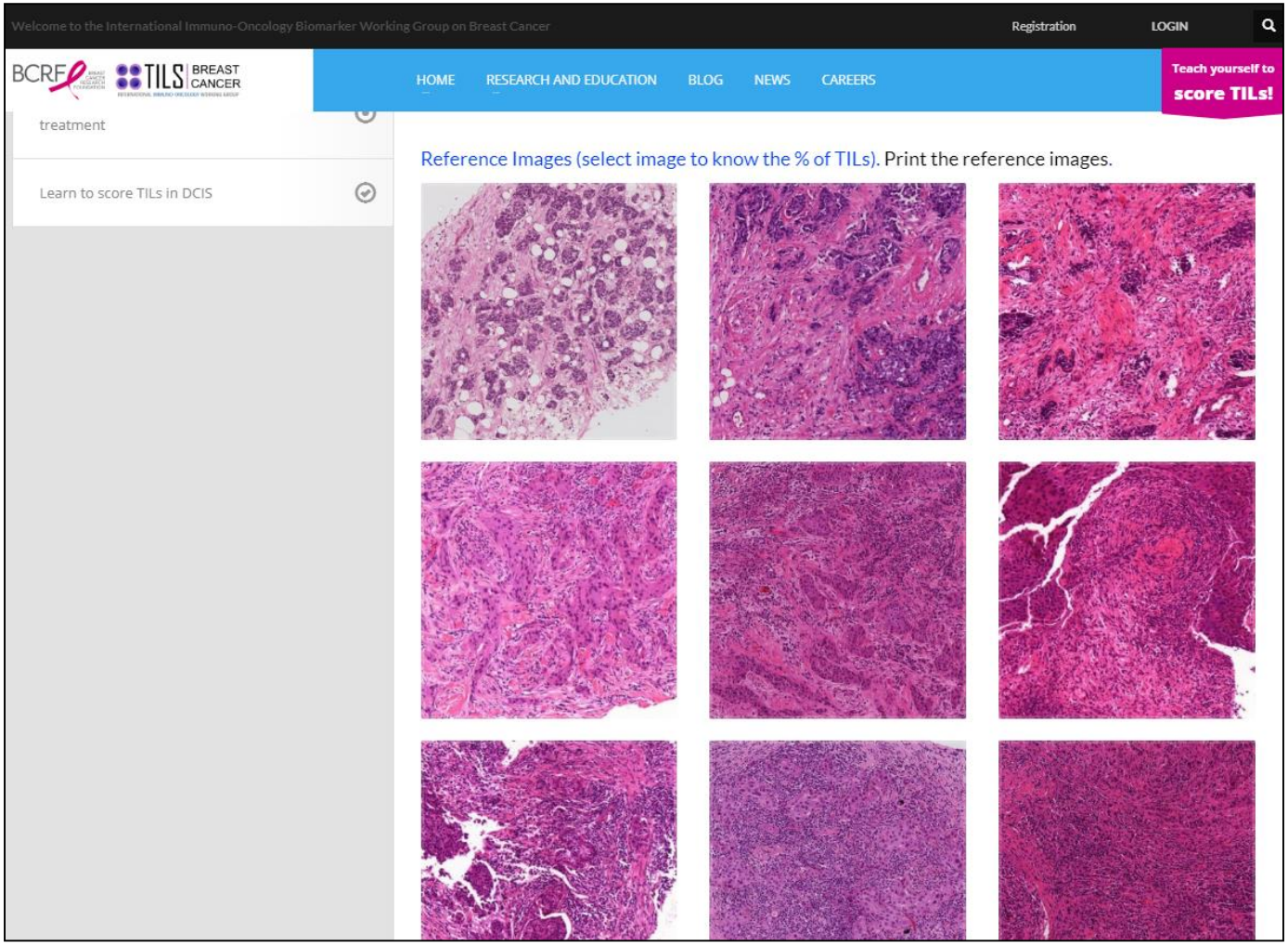
Do not include granulocytic infiltrate in areas of

mononuclear stromal TIL infiltrate



do not include granulocytes in necrotic areas

From TILs website www.tilsinbreastcancer.org


Welcome to the International Immuno-Oncology Biomarker Working Group on Breast Cancer
Registration
LOGIN
BCRF
TILS BREAST CANCER
HOME
RESEARCH AND EDUCATION
BLOG
NEWS
CAREERS
Teach yourself to score TILs!
treatment
Learn to score TILs in DCIS
Reference Images (select image to know the % of TILs). Print the reference images.

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

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

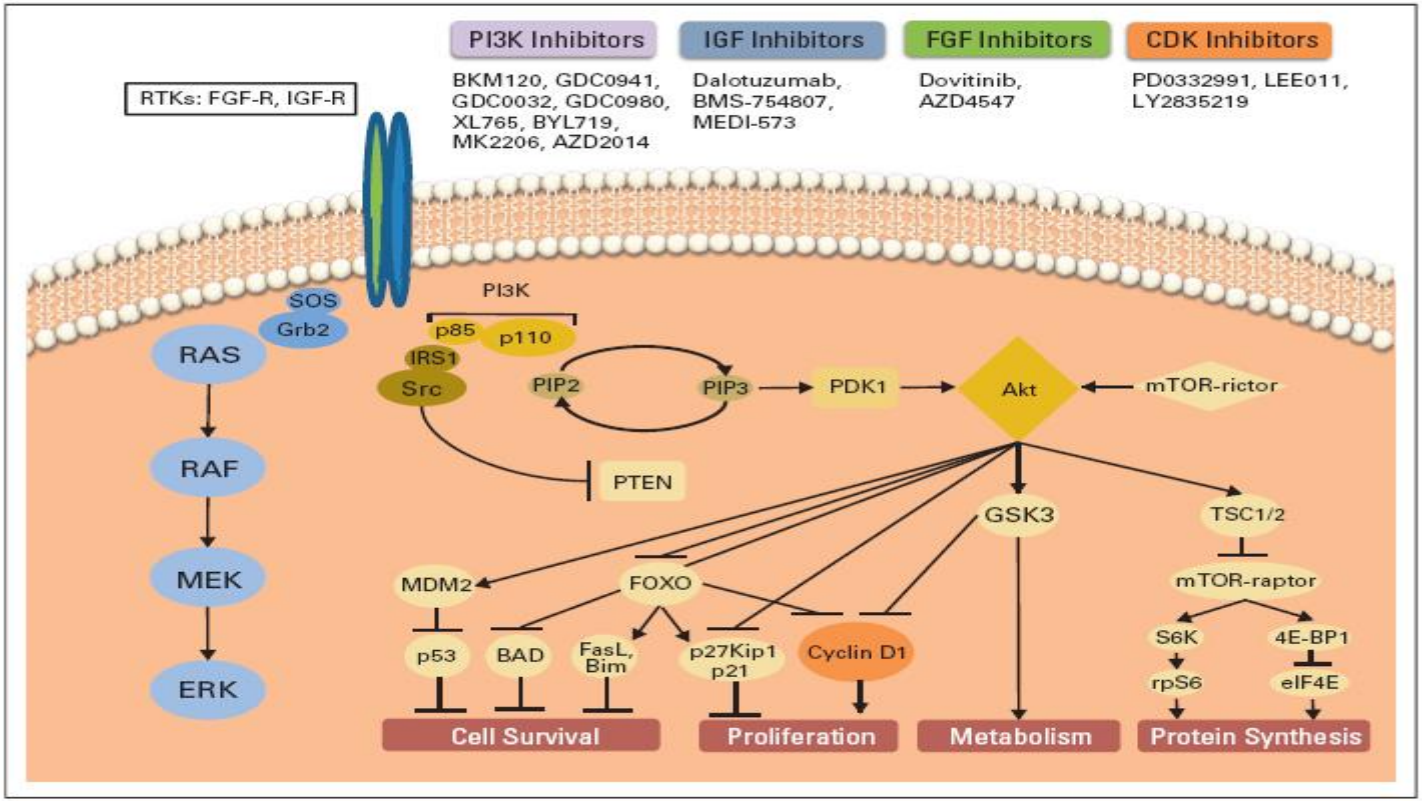
- PD-L1 testing in advanced TNBC used to predict benefit from pembrolizumab
- 22C3 antibody (companion diagnostic to pembrolizumab) is scored using the combined positive scoring system (CPS) [positive $\geq$ 10%]
- PDL-1 testing with SP142 no longer indicated [atezolizumab withdrawn for this indication]
- 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 increasingly utilized in patients with ER+, HER2, pN0 –pN1a to determine need for adjuvant chemotherapy