Breast Cancer Biomarker Testing

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Making Cancer History®

Breast Cancer Biomarker Testing

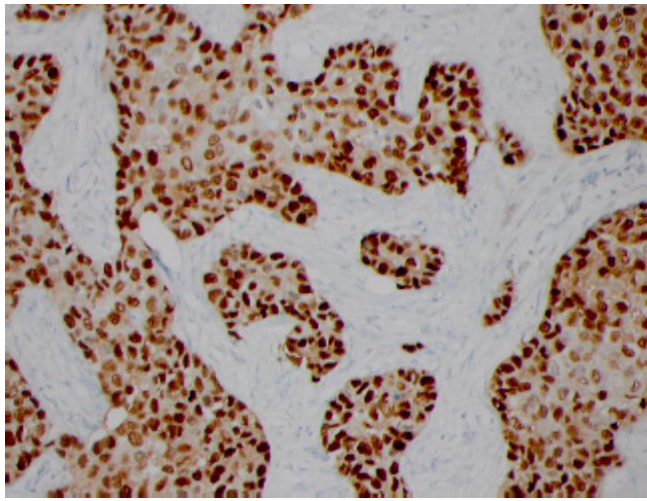
- ER/PR/HER2
  (targets and or indicators of effective therapy)
- Multigene predictors

Estrogen Receptor (ER)

- A nuclear transcription factor (activated by Estrogen
  ⟶ stimulates growth of normal epithelial cells)
- Proliferation of IBC expressing ER may be activated

ER Expression - testing

- Various methods used for almost 40 years
- **Currently, almost all testing is performed by IHC analysis using formalin-fixed, paraffin-embedded tissue sections, allowing morphologic correlation and semiquantitative scoring**
- IHC is a sensitive, specific, easy and inexpensive technique
- By IHC, about **80% of IBC express** nuclear ER (<1% to 100 + cells)
- PCR analysis of ER/PR mRNA (does not allow direct histologic correlation) recently used as part of multiplexed assays



ER as a predictive factor

- Strong predictive factor for response to hormonal therapy in ER-positive IBC

Tamoxifen (binds ER and blocks estrogen-stimulated growth)

Aromatase inhibitor (suppress the production of estrogen)

⟶ significant longer disease free and overall survival in pts with ER pos IBC

Level of ER expression in relation to response to hormonal therapy?

- Direct correlation between the likelihood of response and the levels of expression
- Even tumors with very low level of ER show a significant benefit far above that of entirely negative tumors.

Progesterone Receptor (PR)

- Expression is regulated by ER: presence of PR indicates a functional ER signaling pathway
- Expressed in 60-70% of IBC
- Correlation between levels of expression and response to hormonal therapies (even tumors with very low levels have a significant chance of responding)
- Correlation with ER expression imperfect: 4 phenotypes of combined expression

  ER +/PR+: most frequent (70%) associated with best rate of response

  ER-/PR-: next most common combination (25%), essentially unresponsive

  remaining 2, associated with intermediate response (ER-/PR+ tumors ?)

ER/PR

- When IHC replaced LBAs, no consensus as to optimal cutoff points and standardization of the technique
- Variety of cutoff points used clinically and in literature (1%, 5%, 10%)
- CBC NEWS FEB 15, 2010: a supreme court of Newfoundland and Labrador judge has approved a $17.5M settlement in flawed cancer tests, as some 425 victims suffered botched breast cancer tests, and 127 have died.

ASCO/CAP Recommendations for IHC Testing of ER/PR in Breast Cancer

- To improve the accuracy of hormone receptor testing and the utility of ER and PgR as prognostic and predictive markers for assessing in situ and invasive breast carcinomas.
- ASCO/CAP convened an international panel that conducted a systematic review and evaluation of the literature in partnership with Cancer Care Ontario and developed recommendation for optimal IHC ER/PgR performance

Guidelines

- Preanalytic, Analytic, and Postanalytic Variables
- Initial antibody validation
- Ongoing quality assurance and proficiency testing

Appropriate populations to be tested

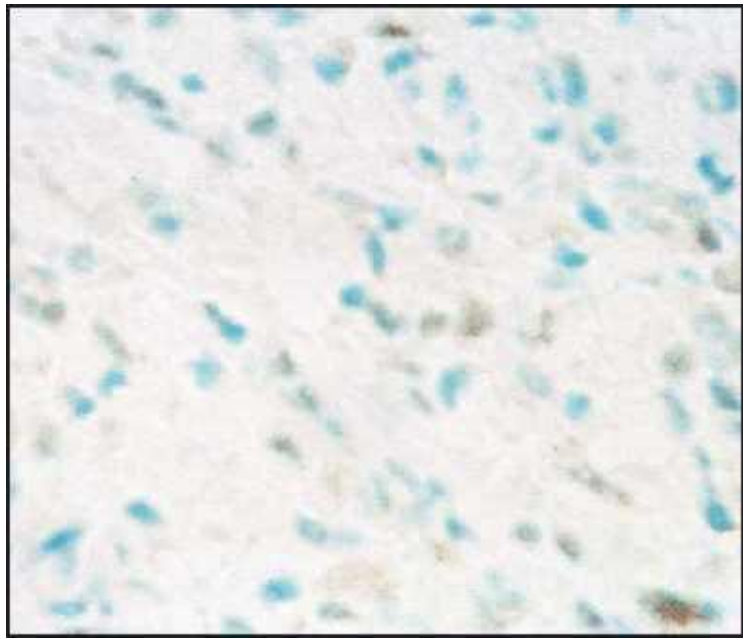
- All **newly diagnosed invasive breast cancers**
- For patients with multiple synchronous tumors, testing should be performed on at least **one of the tumors, preferably the largest. Tumors with differences in histology or grading.** Additional testing in smaller tumors for the biomarker not expressed in the index tumor (*Navale et al. Arch Pathol Lab Med. 2019;143: 190-196*)
- All **newly diagnosed DCIS**
- **Recurrent and metastatic cancers**

Preanalytic Standardization

1. **Tissue handling:** warm and cold ischemic times are important variables in the analysis of labile macromolecules such as proteins, RNA, and DNA from clinical tissue samples. (warm: time from interruption of blood supply to the tumor by the surgeon to excision; cold: time from excision to initiation of tissue fixation). Breast resection specimen should be fixed as quickly as possible in an adequate volume of fixative (optimally 10-fold greater than volume of the specimen). The time of tissue collection (the time that the tissue is handed from the surgical field) and the time the tissue is placed in fixative must be recorded on the tissue specimen requisition.

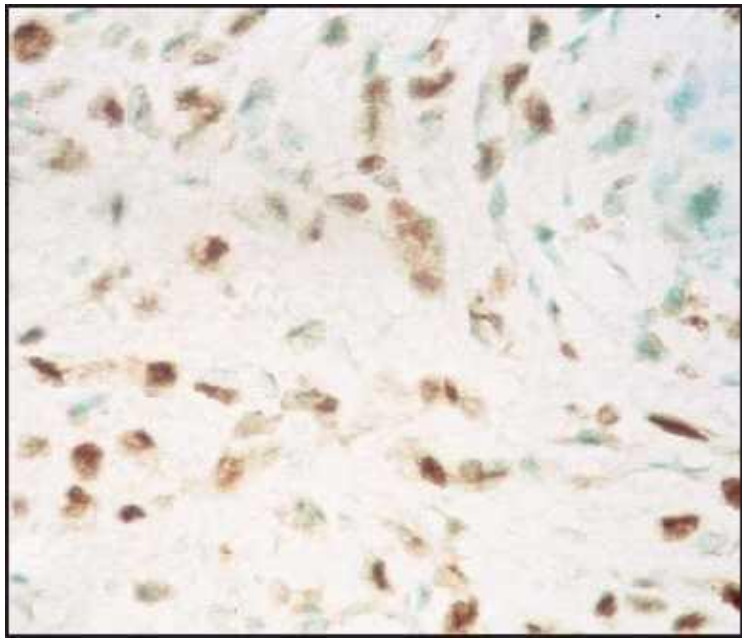
2. **Type of fixative.** Only 10% NBF should be used as the fixative for breast tissue specimens.

3. **Duration of tissue fixation.** No less than 6 hours and for not more than 72 hrs before processing.



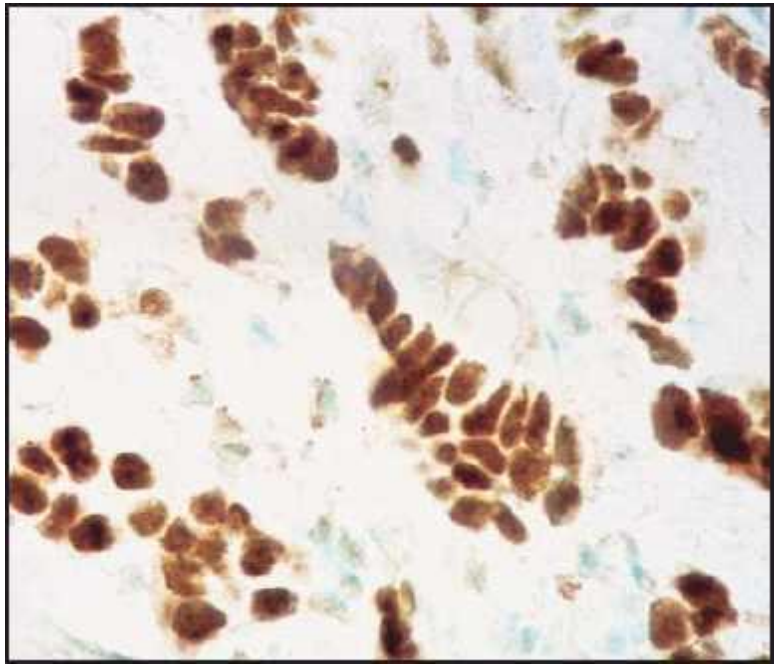
Fixation, 3 h;

antigen retrieval, 40 min



Fixation, 6h;

antigen retrieval, 40 min



Fixation, 8 h;

antigen retrieval, 40 min

Goldstein, AJCP, 2003:120:86-92

Analytic Standardization

1. Antibody selection for ER and PgR testing.
2. Control samples for ER and PgR IHC assays.

1. Antibody selection for ER testing:

Should be restricted to those reagents that have well-established specificity and sensitivity and have been clinically validated (should be at least 90% concordant with those of the clinically validated assay for the ER- and PgR-positive category and 95% concordant with those for the ER- or PgR-negative category)

ER –α: clones 1D5, 6F11, SP1 (rabbit monoclonal), and 1D5+ER.2.123 (cocktail)

PgR: clones 1294, 1A6, 636, 312, and 1E2 (rabbit monoclonal)

2. Control samples for ER and PgR IHC assays

Positive and negative controls should be included with every batch run

Acceptable batch controls include cell lines with defined receptor content varying from high positive to negative and including one intermediate level of receptor content. Other acceptable external controls include endometrial tissue with known receptor content.

On-slide external controls and internal normal epithelial elements should be used to help ensure that all reagents were dispensed onto the slide containing a test sample and that the assay is performing properly.

To ensure that there has not been analytic drift because of subtle differences in technique or dilution, controls with intermediate reactivity or controls covering a spectrum of expression should be scored and recorded daily (percent positive tumor cells and intensity of staining). It is not appropriate to use a single strong positive control tissue to evaluate assay performance. If an external or internal control does not produce the expected reaction, the assay should be repeated with the standard reagents under the standard conditions until acceptable ER and/or PgR reactivity of control material is achieved.

Postanalytic Standardization:

1. Interpretation of IHC assays for ER and PgR.
2. Reporting of ER/PR Results
3. Internal quality control and validation

Small and cytology samples: minimum 100 cells on slide

1. Interpretation of IHC assays for ER and PgR

Review controls (external standard and internal normal breast epithelium if present). If not as expected, the test should be repeated and not interpreted.

1. Interpretation of IHC assays for ER and PgR- continue

- **Provide an interpretation of the assay as receptor positive, receptor negative, or receptor uninterpretable.**
- **Positive interpretation requires at least 1% of tumor cells showing positive nuclear staining of any intensity.**
- **Receptor negative is reported if < 1% of tumor cells show staining of any intensity.**
- **Receptor uninterpretable is reported if the assay controls are not as expected or the preanalytic or analytic conditions do not conform to the guideline and there is no tumor cell staining in the absence of normally stained intrinsic epithelial elements.**

2. Reporting of ER/PR Results (Tables 5 and 6)

Three required result elements:

1. Receptors positive (cutoff of a minimum of 1% of tumor cells of any intensity); receptors negative; receptors uninterpretable
2. Percentage of tumor cells with nuclear staining
3. Intensity of nuclear staining as weak, moderate, or strong

Two optional results elements

May provide a composite score (H score, Allred score, or quick score)

Cautionary statement--

Table 5- Elements to Be Included in Accession Slip for ER and PgR Assays

Patient identification information

Physician identification

Date of procedure

Clinical indication for biopsy

Specimen site and type of specimen

Collection time

Time sample placed in fixative

Type of fixative

Fixation duration

Table 6- Reporting Elements for ER and PgR IHC assays

- Patient identification information
- Physician identification
- Date of procedure
- Clinical indication for biopsy
- Specimen site and type of specimen
- Specimen identification (case and block number)
- Time sCollection time
- ample placed in fixative
- Type of fixative
- Fixation duration
- Staining method used : primary antibody and vendor, references supporting validation
- Results as to % and intensity
- Interpretation as to positive , negative and uninterpretable
- External and internal control
- Standards assay conditions met/notmet--

3. Internal Quality Control and Validation

- CAP Laboratory Accreditation Elements Requiring Documentation (Table 7–such as validation, QC, reporting)

What is the regulatory frame work that allows for increased scrutiny?

The Clinical Laboratory Improvement Act of 1998 (CLIA 88)

provides stringent quality standards for highly complex tests, which include all predictive cancer factor assays.

What Are the Optimal External Quality Assurance Methods to Ensure Ongoing Accuracy in ER/PgR Testing?

- CAP laboratory accreditation program (listed in Table 7)
- Proficiency testing

CAP Proficiency Testing

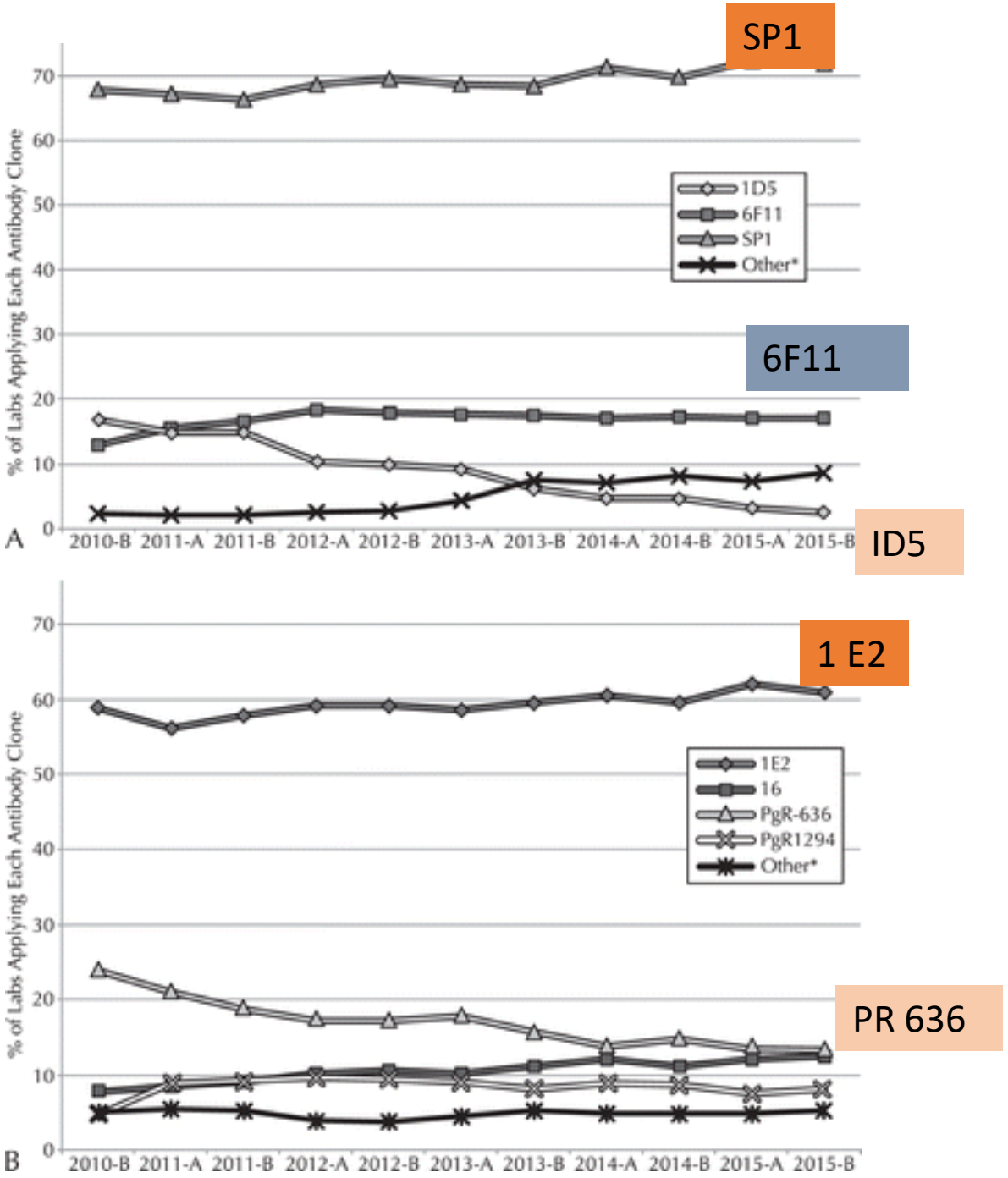
- Unstained slides cut from the same tissue microarrays block. Two10-core slides for ER and also for PR mailed twice/year (total 40 ER and 40 PR cores /year)
- Laboratories are instructed to stain the provided slides by using their clinically validated protocols (assuming a negative control is run and is appropriately negative) and score them according to the ASCO/CAP guidelines. Laboratories transmit their resultant scores to CAP; stained slides are not submitted for review

Comparison of Estrogen and Progesterone Receptor Antibody Reagents Using Proficiency Testing Data.

Troxell ML, et al. Arch Pathol Lab Med. 2017 Oct;141(10):1402-1412

CAP proficiency testing data from 80 ER and PR cores by antibody clone from >1200 lab (staining results from 2014 and 2015 were analyzed by antibody; data on clone utilization from a longer time period were reviewed (2010–2015).

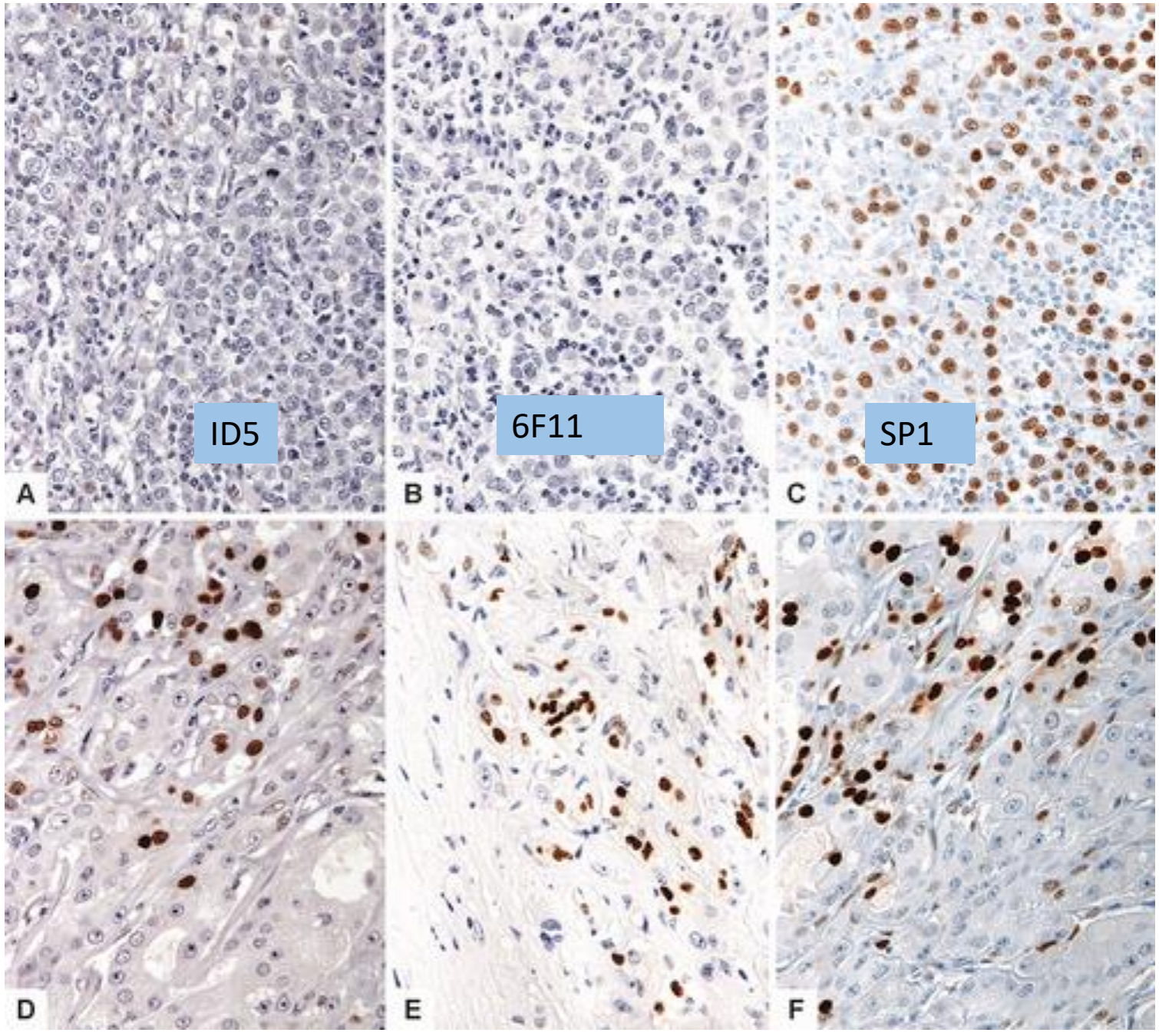
Changes in Antibody Clone Utilization Over Time 2010-2015



Difference in Number of Laboratories Reporting Negative ER Interpretations by Antibody Clone (total 17 of 80 cores)

Clone Comparison	No. of Cores With Statistically Different Estrogen Receptor Results
1D5 versus 6F11	4
1D5 versus SP1	14
6F11 versus SP1	12
SP1 different from both 1D5 and 6F11	9
1D5 different from both SP1 and 6F11	4
Different for all 3 pairs of clones	2

Comparison of 3 estrogen receptor antibodies. A through C, 1D5 and 6F11 show negativity, whereas SP1 shows positivity:. D through F, Estrogen receptor expression is heterogeneous with all 3 antibodies



Conclusion of study:

- Significant differences in the number of lab reporting negative results by antibody clone for 17 of 80 tested cores for ER, with the 3 most widely applied antibodies, all of which were considered by ASCO/CAP to have been validated in prior clinical studies. SP1 resulted in greater number of positive interpretation.
- There may not be universal implementation of various elements of the ASCO/CAP 2010 guidelines, even among lab enrolled in PT.

Tips: Avoiding false positive/negative results

- False positive results

– very rare entrapped normal cells, cytoplasmic positivity, mistaking control on same slide for patient sample, transcription error

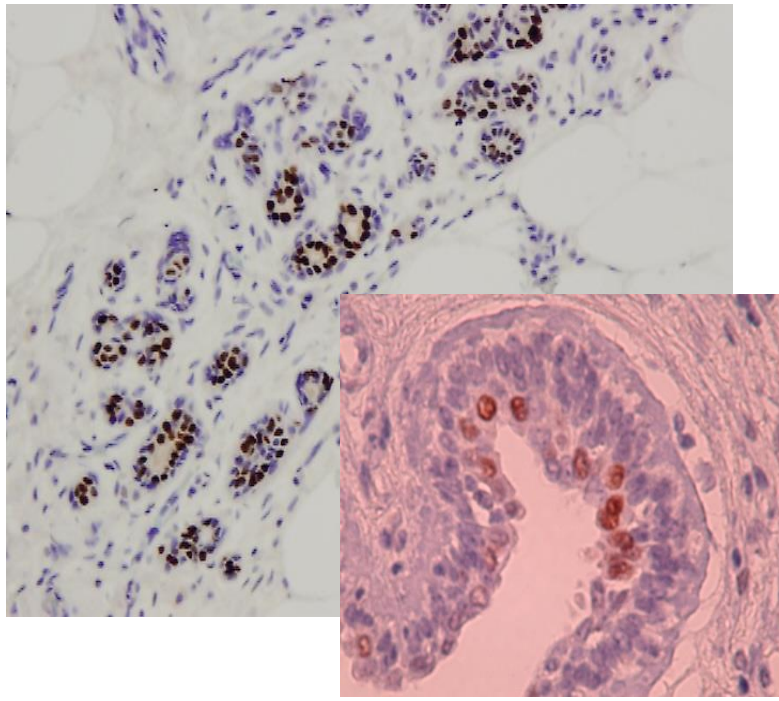
- False negative results

– more common problems with the tissue cautery, decalcification, prolonged ischemic time, poor fixation technical problems, interpretative problems cut-off threshold

ER/PR: Avoiding false negative results

- Always check that the normal breast epithelium is positive
- If not, repeat on the same or different specimen – especially if both ER and PR are negative
- Correlate with histology. Some cancers are almost always ER-positive:

  →mucinous carcinoma →tubular carcinoma →low-grade ductal carcinoma →grade I and II lobular carcinomas



ER/PR: other methods?

- Molecular analysis from scraped tissues: --ER concordant in 99%, PR concordant in 94%
- Most common type of discrepancy: false negative --low cellularity carcinomas (esp lobular) --small cancers with large biopsy sites
- IHC is the correct result in 70% of discordant cases

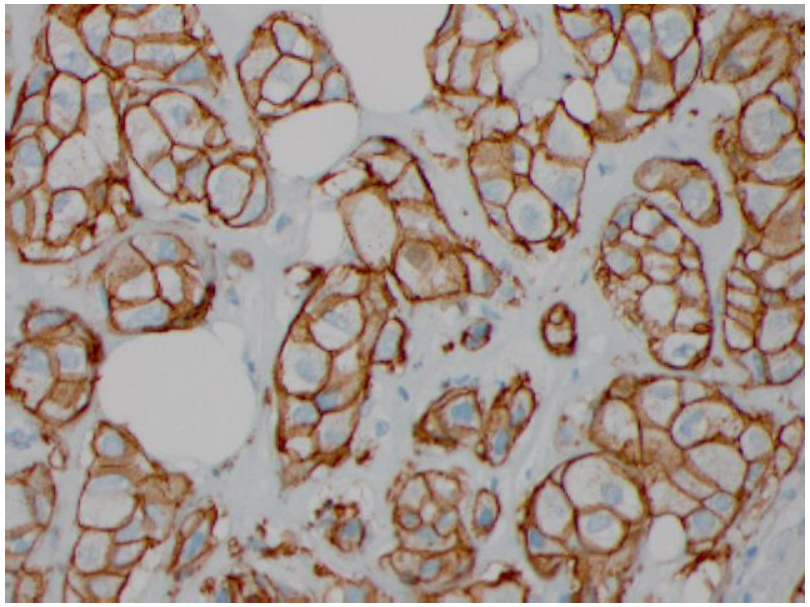
HER2

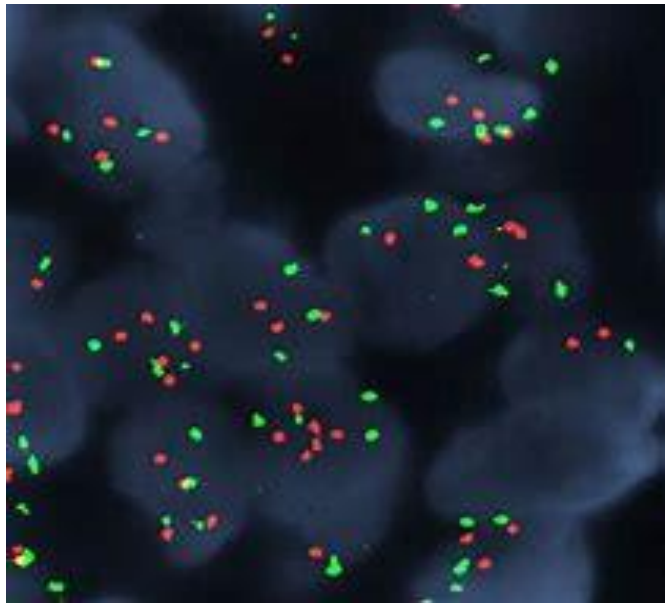
- HER2 gene located on Ch 17, encodes a growth factor receptor on the surface of normal breast epithelial cells
- Amplification is highly correlated with protein overexpression

- The first cancer biomarker to be targeted by a therapeutic Ab (1998)

HER2

- Amplified and overexpressed in 15% of invasive breast cancers
- Evaluate HER2 using IHC and/or FISH





Updated (2018) ASCO/CAP Guidelines for HER2 Testing in Breast Cancer

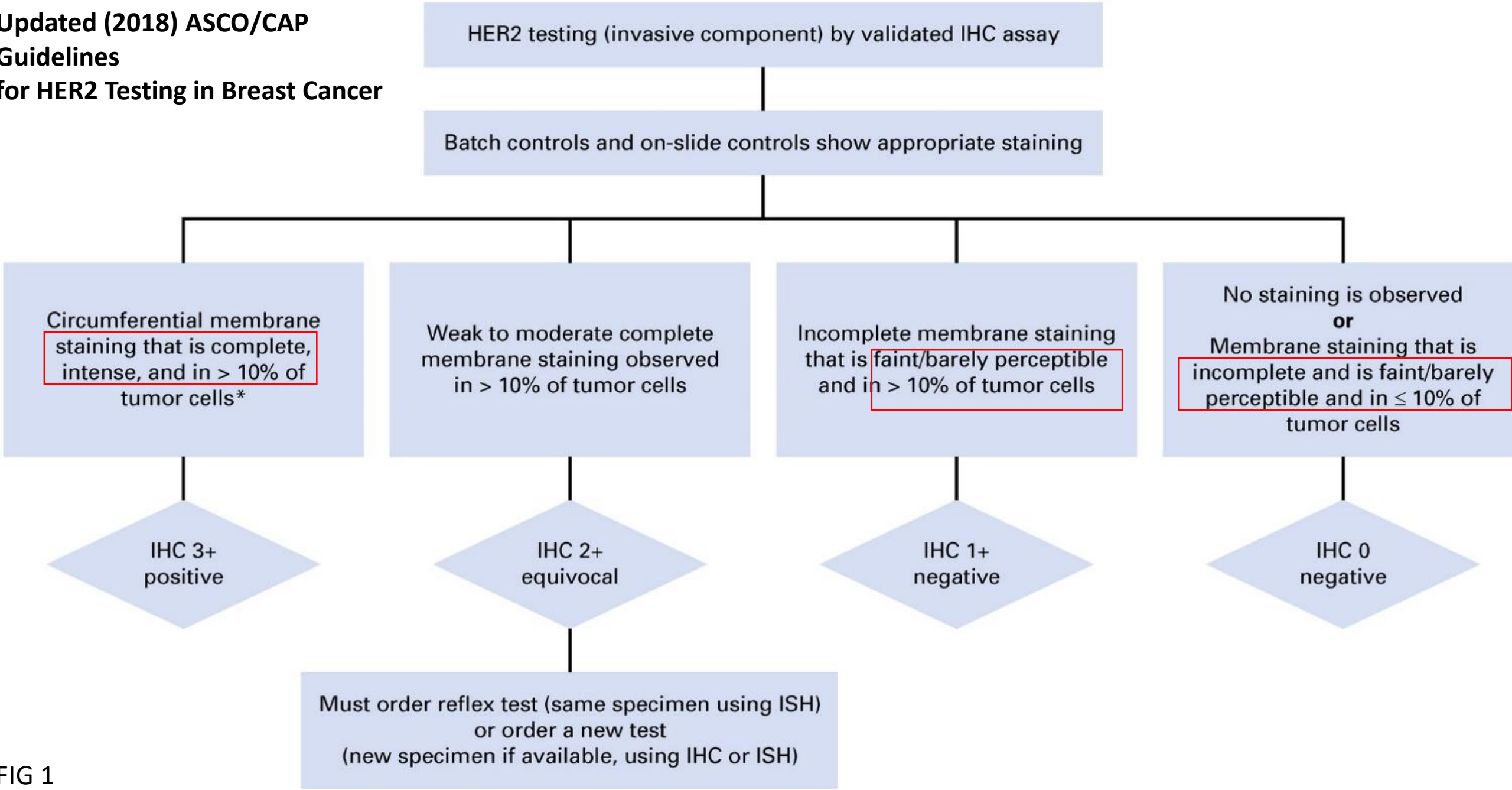
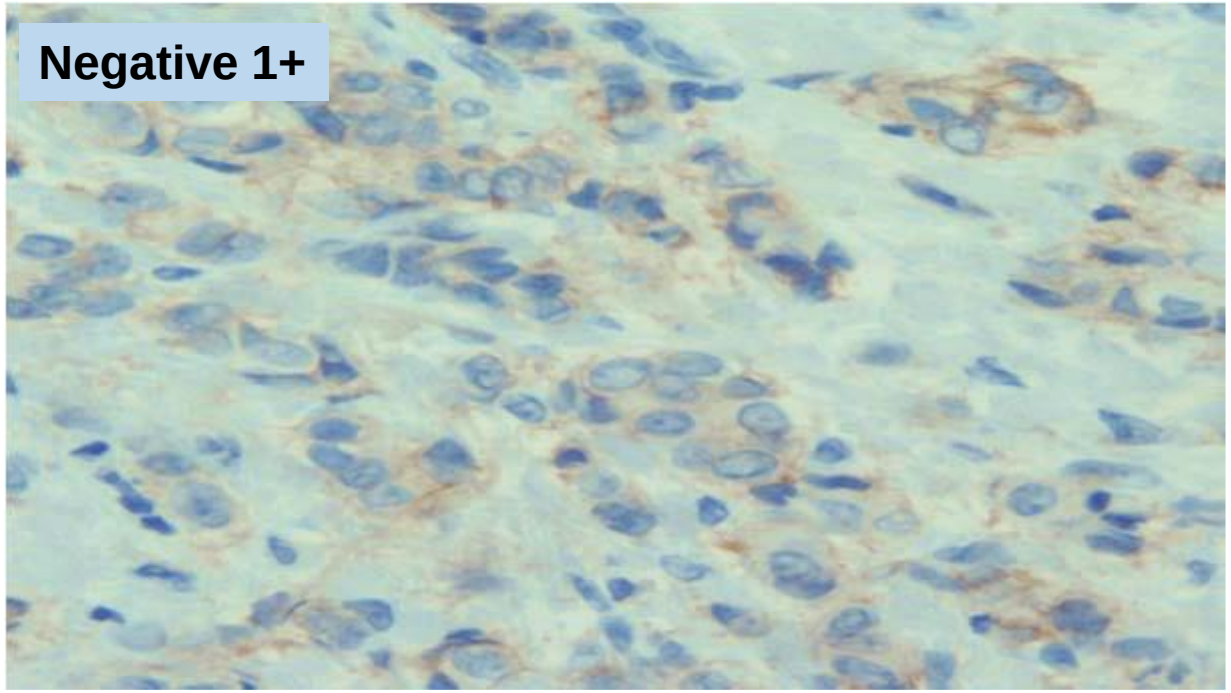
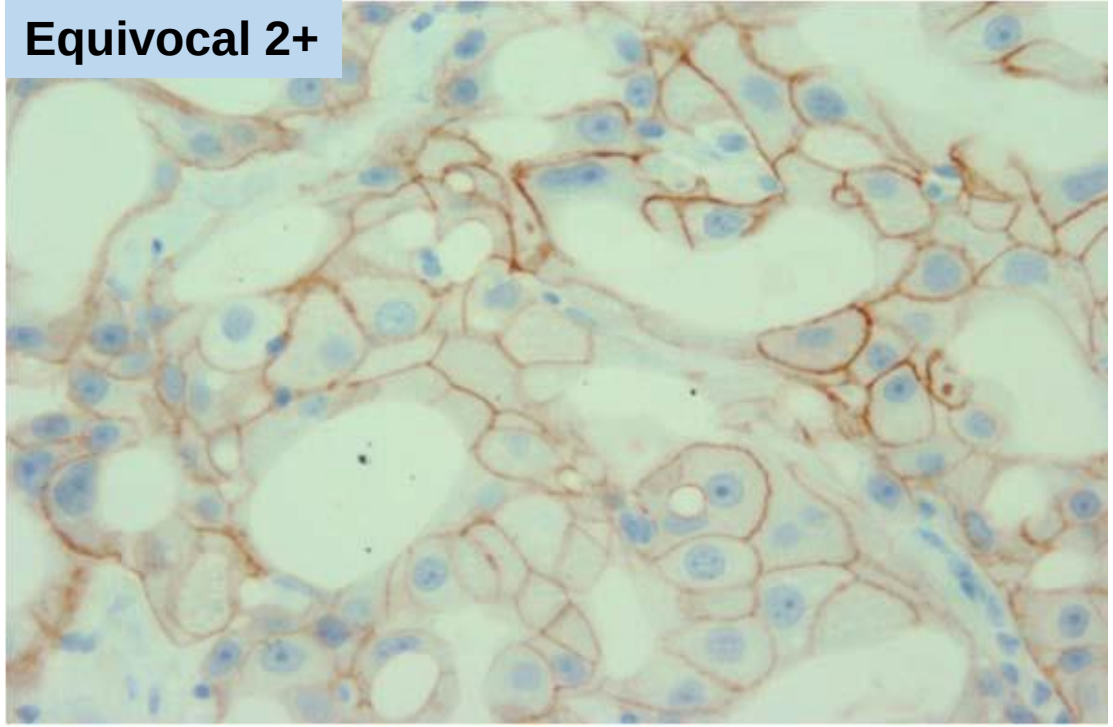


FIG 1

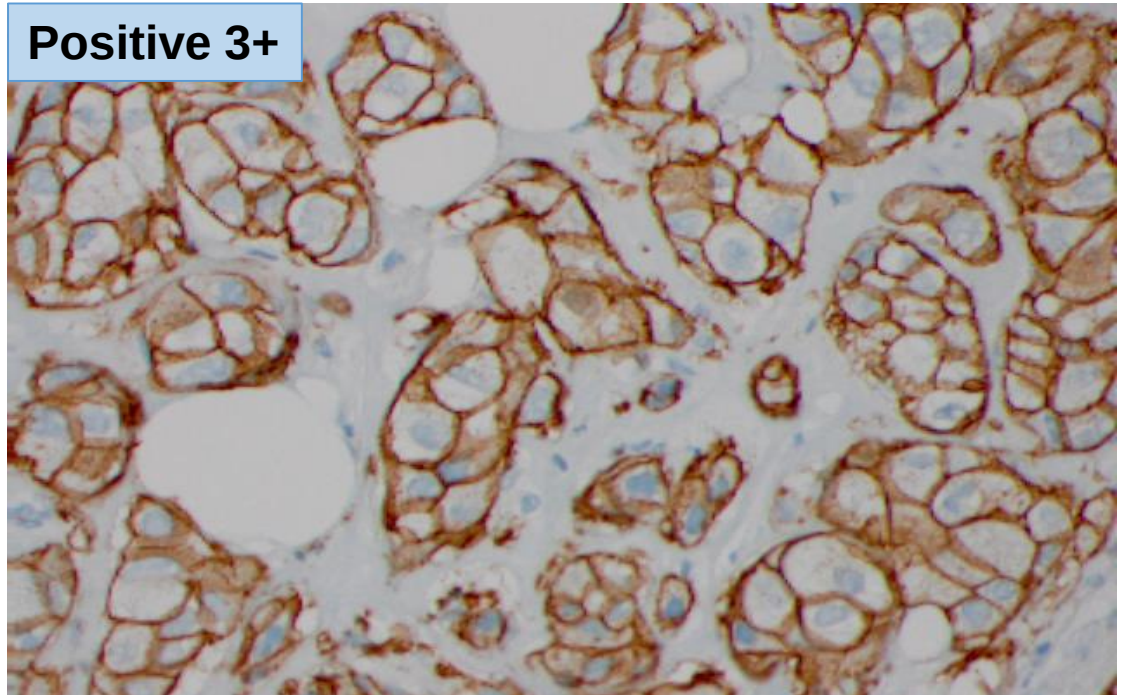
Negative 1+



Equivocal 2+



Positive 3+



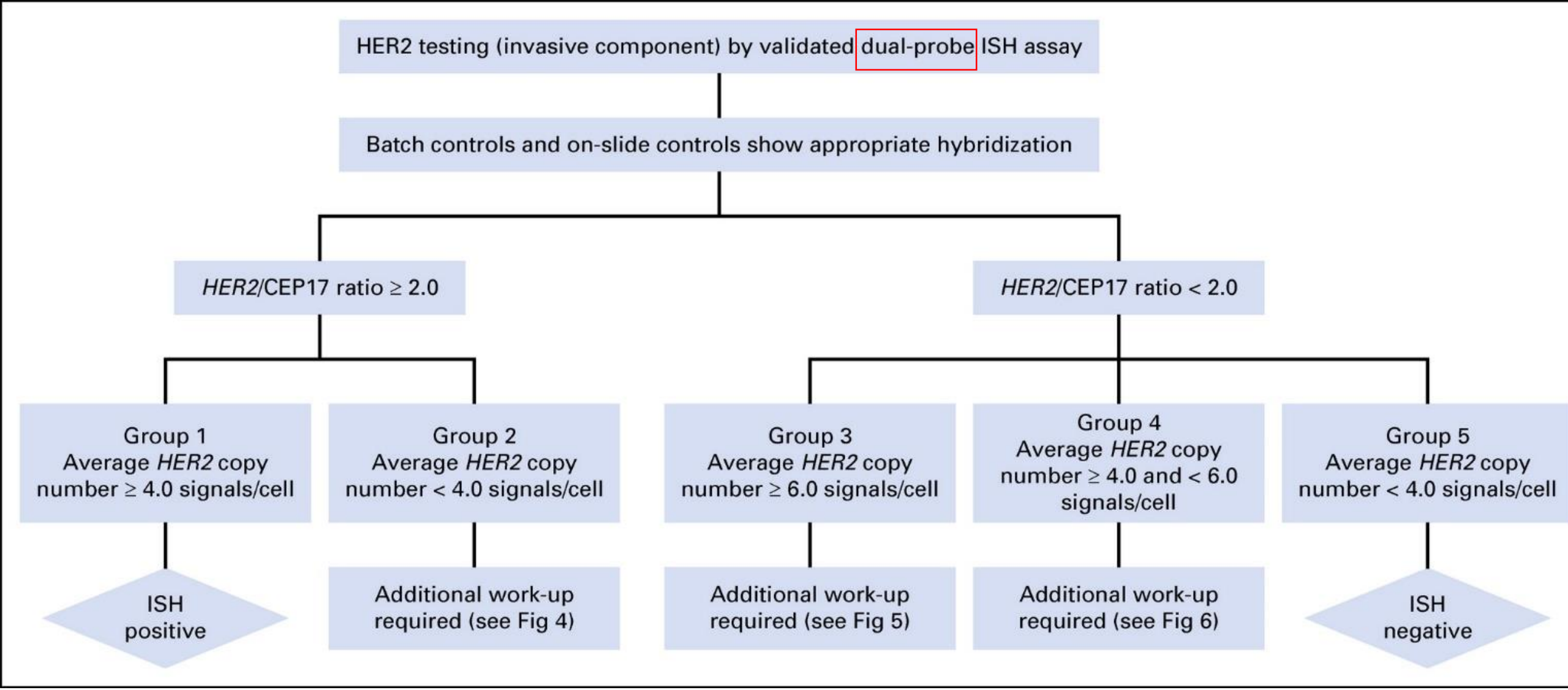


FIG 3
Group 2 monosomy
Group 3 polysomy
Group 4 old equivocal

Previously classified as Positive (group 2)
Positive by ratio but not by copy number ratio >2.0
HER2 CN<4.0

- cases with loss of CEP17 (monosomy)

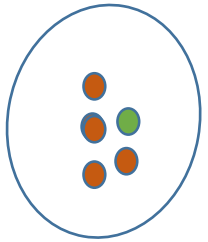
→Negative by HER2 CN

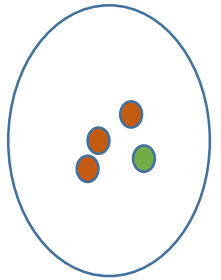
→Ratio is >2 due to loss of CEP17

3+ IHC→ POS

2+ IHC→ NEG with comment

0/1+ IHC→NEG with comment





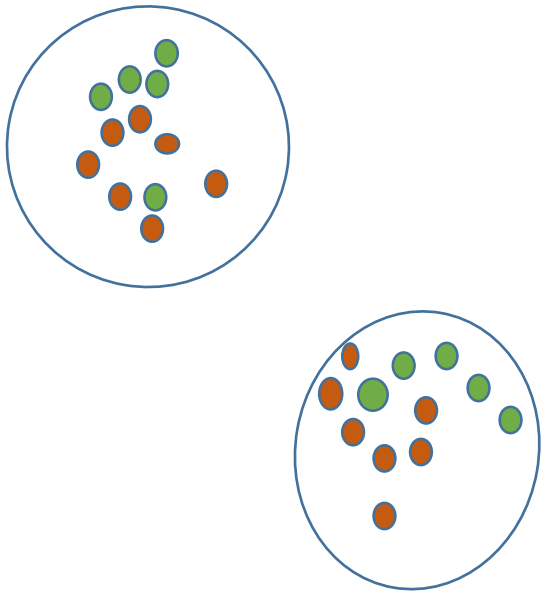
Previously classified as Positive (group 3) (average HER2 CN > 6 but not by ratio

- CEP17 coamplification/polysomy

  --Positive by copy number

  HER2 CN >6

  --Ratio <2



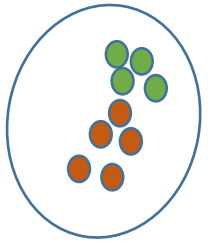
3+ IHC→ POS

2+ IHC→ POS with comment*

0/1+ IHC→ NEG with comment

Previously classified as Equivocal ratio <2.0 with HER2 >4.0 and <6.0 (group 4)

- Slightly elevated HER2 CN (4-6)
- Ratio is <2
- 5% of all cases
  - 3+ IHC→POS
  - 2+ IHC→NEG with comment*
  - 0/1+ IHC→NEG with comment
- *FISH recount 2nd observer same



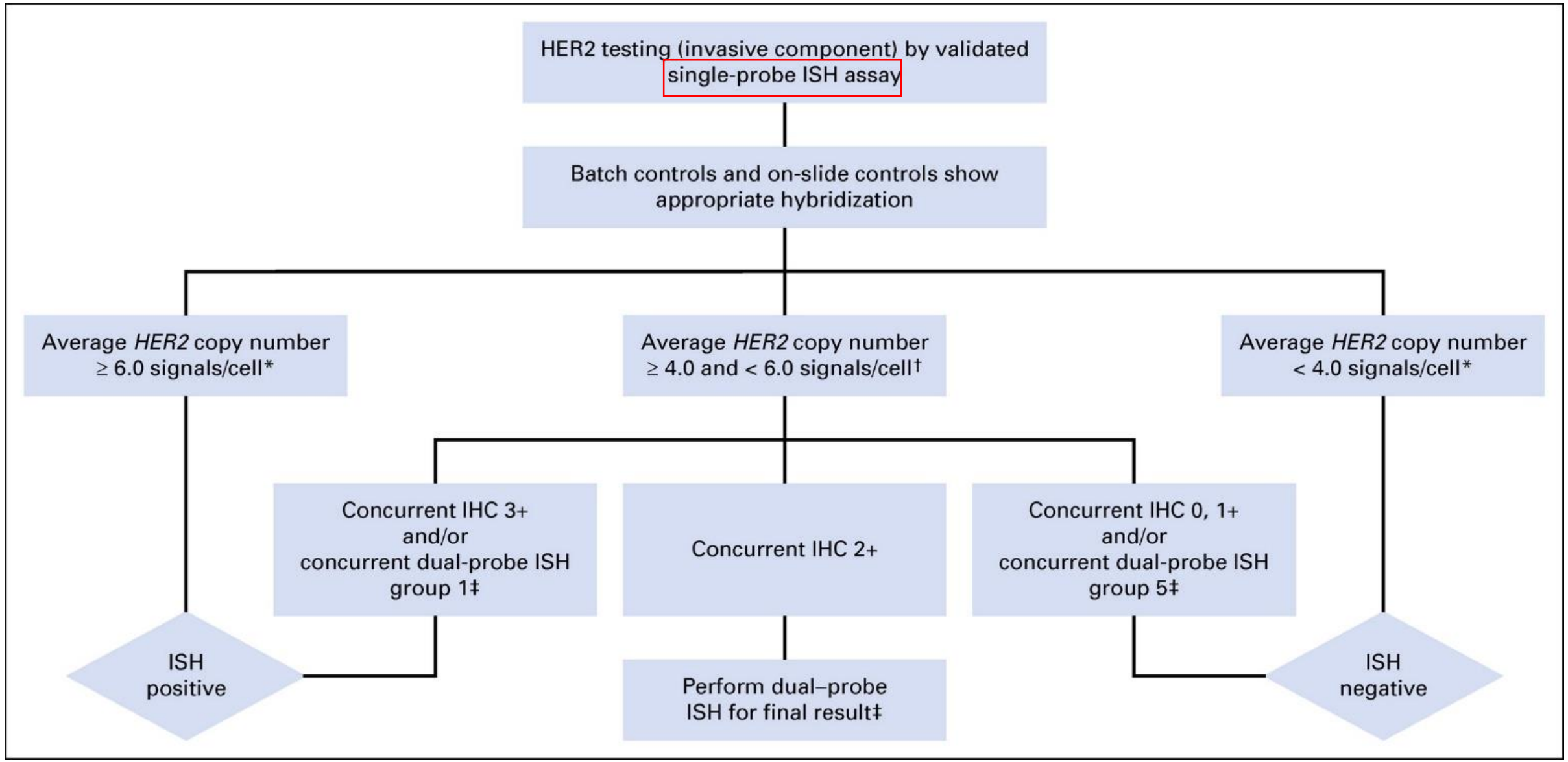

HER2 testing (invasive component) by validated single-probe ISH assay
Batch controls and on-slide controls show appropriate hybridization
Average HER2 copy number ≥ 6.0 signals/cell*
Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell†
Average HER2 copy number < 4.0 signals/cell*
Concurrent IHC 3+ and/or concurrent dual-probe ISH group 1‡
Concurrent IHC 2+
Concurrent IHC 0, 1+ and/or concurrent dual-probe ISH group 5‡
ISH positive
Perform dual–probe ISH for final result‡
ISH negative

Fig 2

HER2 –Appropriate population to be tested

- All newly diagnosed IBC
- Recurrent and metastatic breast cancers

- IHC is subject to preanalytic, analytic and postanalytic variables

Table 2. Histopathologic Features Suggestive of Possible HER2 Test Discordance

Criteria to Consider*
A new HER2 test should not be ordered if the following histopathologic findings occur and the initial HER2 test was negative:
Histologic grade 1 carcinoma of the following types:
Infiltrating ductal or lobular carcinoma, ER and PgR positive
Tubular (at least 90% pure)
Mucinous (at least 90% pure)
Cribriform (at least 90% pure)
Adenoid cystic carcinoma (90% pure) and often triple negative
Similarly, a new HER2 test should be ordered if the following histopathologic findings occur and the initial HER2 test was positive:
Histologic grade 1 carcinoma of the following types:
Infiltrating ductal or lobular carcinoma, ER and PgR positive
Tubular (at least 90% pure)
Mucinous (at least 90% pure)
Cribriform (at least 90% pure)
Adenoid cystic carcinoma (90% pure) and often triple negative
If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test ***may*** be ordered on the excision specimen if one of the following is observed:
Tumor is grade 3
Amount of invasive tumor in the core biopsy specimen is small
Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core
Core biopsy result is equivocal for HER2 after testing by both ISH and IHC
There is doubt about the handling of the core biopsy specimen (long ischemic time, short time in fixative, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error

NOTE. Adapted from 2013 ASCO/CAP HER2 Testing Guideline.[1]

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; PgR, progesterone receptor.

*Criteria to consider if there are concerns regarding discordance with apparent histopathologic findings and possible false-negative or false-positive HER2 test result.

HER2 IHC: Causes of false positive 3+

- Overstaining – normal breast tissue should be negative (except apocrine metaplasia which can be 1+ to 2+)
- Edge artifact – lobular carcinomas can appear falsely positive in edges or between cells
- Cytoplasmic positivity – only membrane positivity should be scored.
- Overinterpretation – moderate complete or granular membrane expression

HER2 IHC: Avoiding false positive 3+

- Have a very high threshold for interpreting a cancer as 3+.
- Should have strong crisp complete membrane positivity throughout (rarely, contiguous 10% focus).
- Have a low threshold for confirming by ISH in uncertain cases.

Scoring HER 2 FISH

Probe signals are counted in a minimum of 20 tumor cell nuclei

Exclude DCIS

Calculate: avg HER2 signals/cell

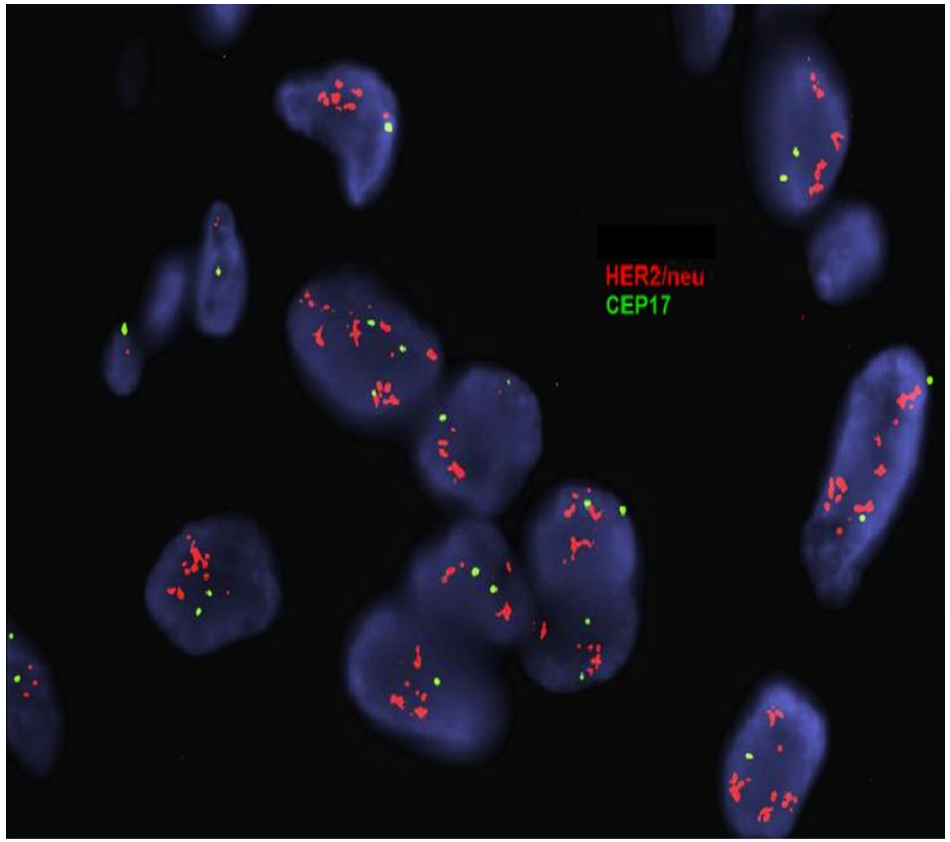
avg CEP17 signals/cell

ratio of *HER2* signals to *chr17* centromere signals

90% of all breast cancers have classical results

Ballard et al. Mod Pathol 30:227-235, 2017

- 78% classic non-amplified
- 12% Classic amp
- 5%
- 5%



FISH - HER2 amplified

Heterogeneity

- Discrete contiguous second population of cells with IHC 3+
- Rare, <5% of total IBC
- Discrete second population may be a source of “resistant” disease
- Oncologists may wish to tailor therapy to include both the positive and negative areas, especially if triple negative.

Carcinomas are classified as HER2 positive if >10% of the cancer is positive (IHC/ISH). The cells must be “observed in a homogeneous and contiguous population” (i.e. not scattered)

HER2: Summary

- The majority of breast cancers can be classified into pos and neg groups.
- False positive results on IHC most often are due to overstaining or overcalling cases with moderate immunoreactivity. When in doubt, back down to 2+.
- Heterogeneity: a discrete, contiguous population of HER2 positive tumor cells in a HER2 negative background →Rare. Report HER2 status for both populations.

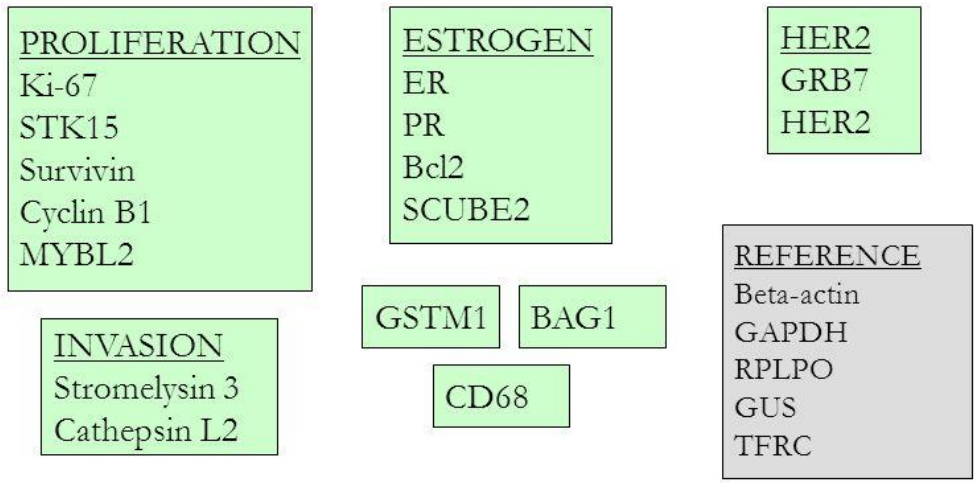
Breast cancer

- Standard-of-care markers ER/PR/HER2
- Multigene predictors
    1. Who gets chemotherapy (ER+ node neg)
    2. Prognostic stage

Oncotype DX™

Oncotype uses 21 genes to calculate a recurrence score

16 Cancer and 5 Reference Genes From 3 Studies

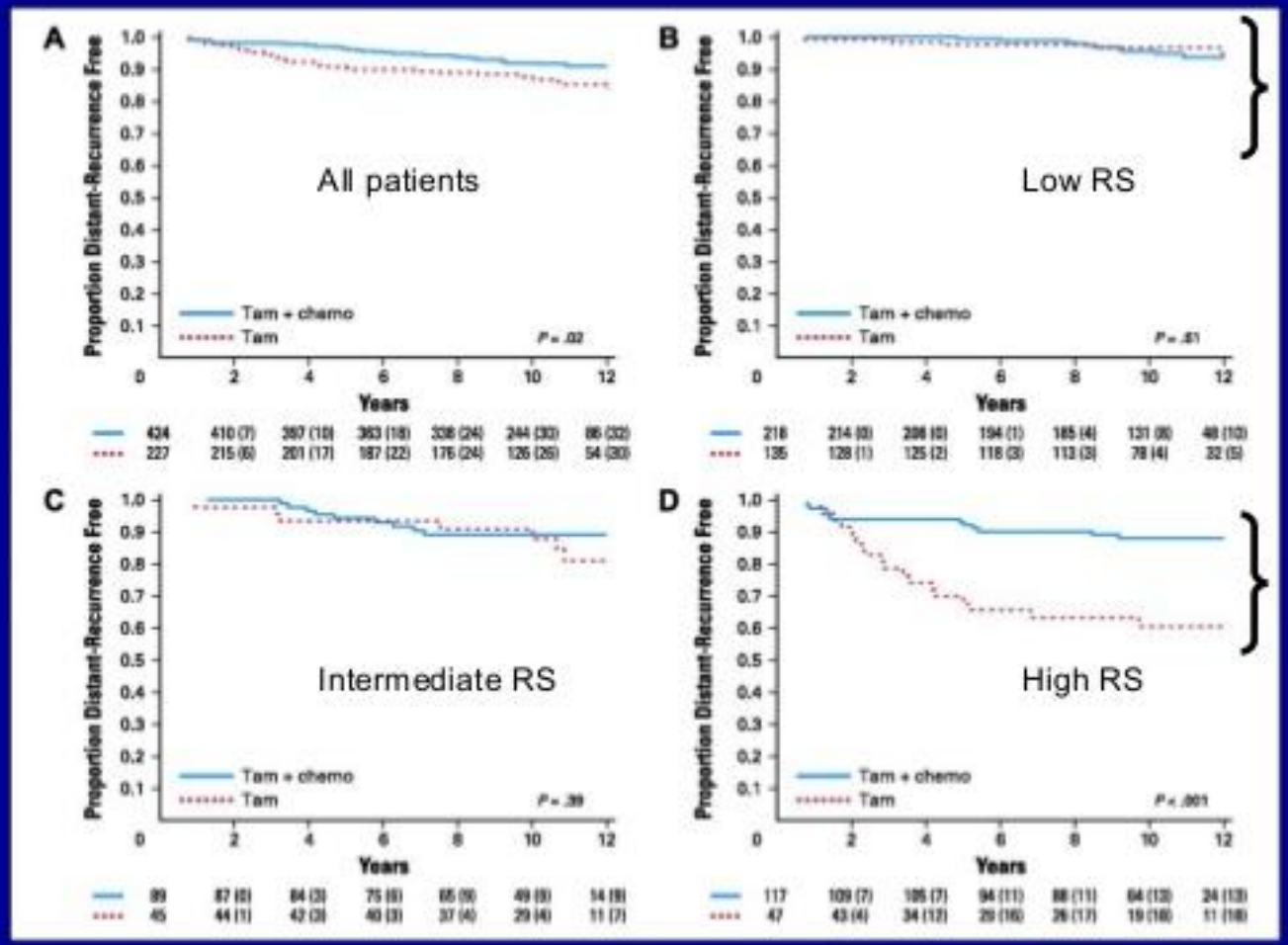


Paik et al. *N Engl J Med.* 2004;351: 2817-2826

Oncotype DX™

- Marketed by Genomic Health, Inc. after retrospective validation in tumors from NSABP B14 and B20 trials.
- FFPE tissue to predict recurrence (RS was superior to that of age, T size and grade) and chemotx benefit in ER+ breast cancer.
- Magnitude of benefit from chemotherapy: ≥ to 31 had a substantial absolute benefit from chemo; 18-30 did not appear to receive a substantial benefit; <18 derived minimal if any benefit.

The Oncotype DX® Assay: Patients Do Not Benefit Equally from Chemotherapy



Little, if any, benefit

28% Absolute Benefit

Paik et al. *J Clin Oncol*. 2006;24:3726-3734

TAILORx Trial

Clinical utility of Oncotype Dx testing in women with HR– positive, HER2-, LN-negative invasive breast cancer

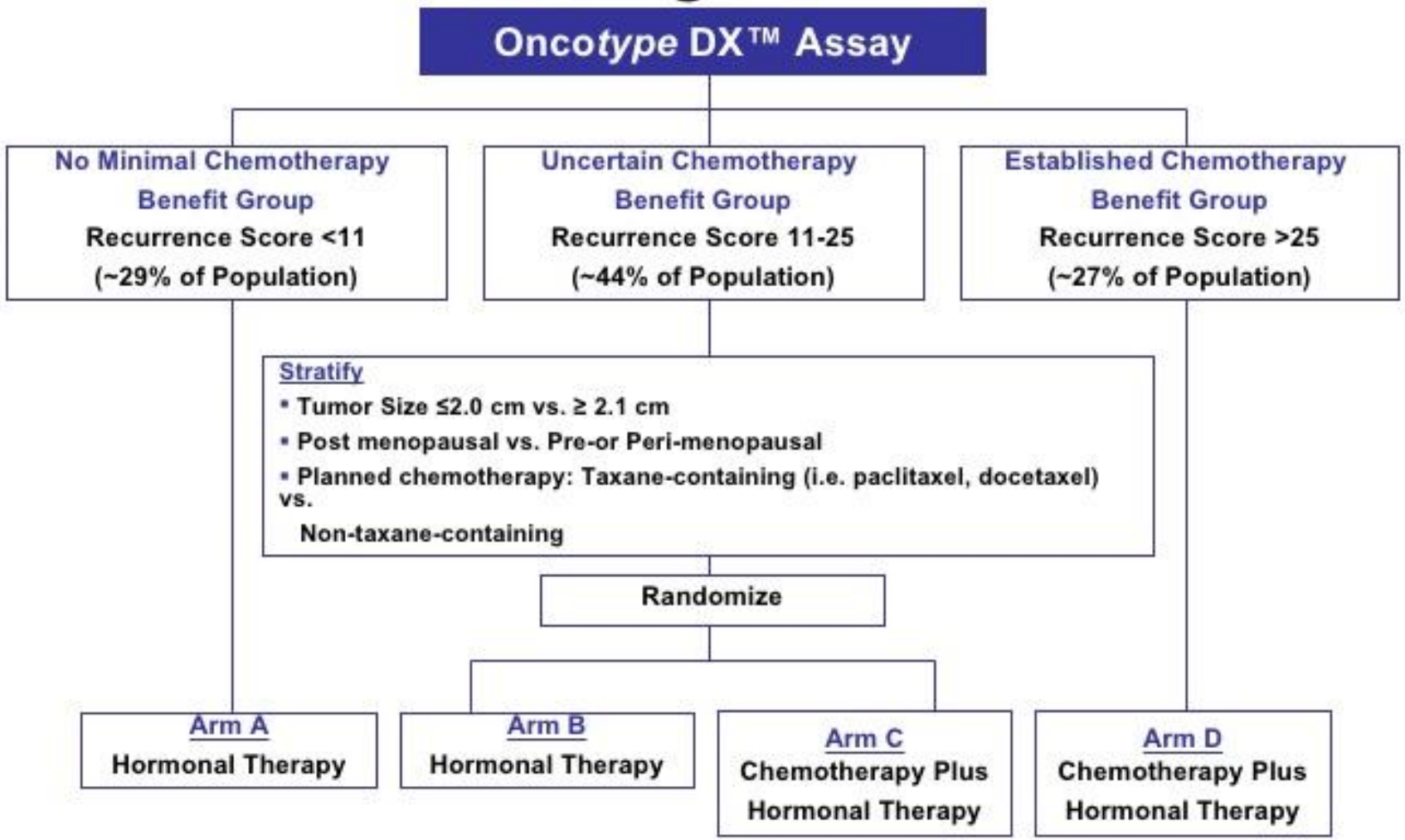
Enrolled >10,000 pts

RS 0-10 got endocrine tx only

11-25 got randomized to chemo/no chemo

26+ got chemo

Oncotype DX™ - TAILORx - Study Design



TAILORx ClinicalTrial

- At 9 years, the 2 treatment groups had similar rates of DFS (83.3% in the endocrine therapy group and 84.3% in the chemoendocrine-therapy group), with some benefit of chemotherapy found in women 50 years of age or younger with a recurrence score of 16 to 25.

Saprano et al, NEJM, 2018

Many Women With the Most Common Form of Breast Cancer Can Skip Chemotherapy (up to 70%). *Cancer, Aug 2018*

Chemotherapy can be avoided in those who are diagnosed when they are:

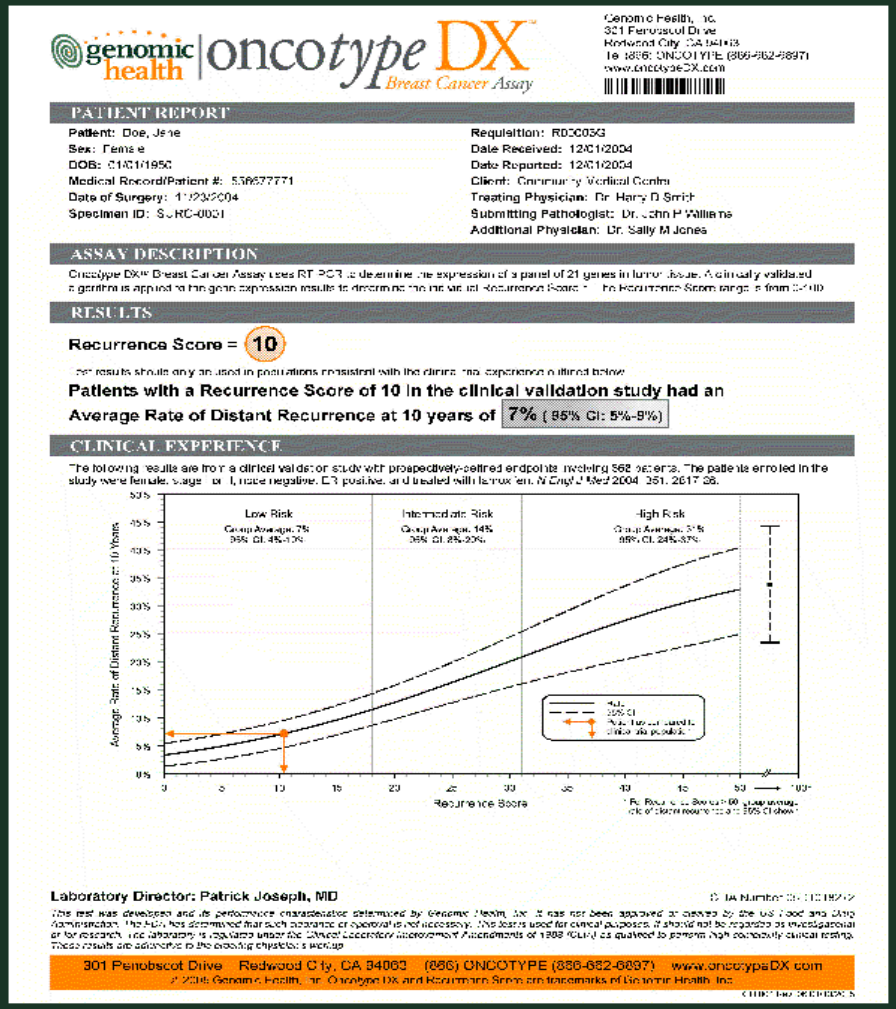
- Older than 50 years with a RS of 11 to 25 (45% of all cases);

  At any age with a recurrence score of 0 to 10 (16% of all cases);

  Fifty years old or younger with a recurrence score of 11 to 15 (8% of all cases).
- Chemotherapy should still be considered for the remaining 31% of women with hormone receptor–positive, *HER2*-negative, node-negative breast cancer who are diagnosed when they are:
- At any age with a recurrence score of 26 to 100 (17% of all cases);
- Fifty years old or younger with a recurrence score of 16 to 25 (14% of all cases).

Oncotype DX™ in Clinical Practice: Patient Report

- The patient report includes
  - Recurrence Score (RS)
  - Average 10-year distant recurrence rate for that RS (with 95% CI)
  - Graph of 10-year recurrence risk as a function of RS in LN-, ER+ tamoxifen-treated patients
- The report is sent to
  - Treating physician
  - Submitting pathologist



genomic health | oncotype DX Breast Cancer Assay

Genomic Health, Inc.
301 Penobscot Drive
Redwood City, CA 94063
Tel: (866) ONCOTYPE (866-662-6897)
www.oncotypeDX.com

PATIENT REPORT

Patient: Doe, Jane
Sex: Female
DOB: 01/01/1950
Medical Record/Patient #: 536677771
Date of Surgery: 11/23/2004
Specimen ID: S-RC-0001

Requisition: RDC0053
Date Received: 12/01/2004
Date Reported: 12/01/2004
Client: Community Medical Center
Treating Physician: Dr. Harry D Smith
Submitting Pathologist: Dr. John P Williams
Additional Physician: Dr. Sally M Jones

ASSAY DESCRIPTION

OncoType DX® Breast Cancer Assay uses RT-PCR to determine the expression of a panel of 21 genes in tumor tissue. A clinically validated algorithm is applied to the gene expression results to determine the individual Recurrence Score® (RS). The Recurrence Score ranges from 0-100.

RESULTS

Recurrence Score = 10

Test results should only be used in populations consistent with the clinical trial experience outlined below.

Patients with a Recurrence Score of 10 in the clinical validation study had an Average Rate of Distant Recurrence at 10 years of 7% (95% CI: 5%-9%)

CLINICAL EXPERIENCE

The following results are from a clinical validation study with prospectively-defined endpoints involving 668 patients. The patients enrolled in the study were female, stage I or II, node negative, ER positive, and treated with tamoxifen. *N Engl J Med* 2004; 351: 2817-26.

Laboratory Director: Patrick Joseph, MD

CLIA Number: 05D1092972

This test was developed and its performance characteristics determined by Genomic Health, Inc. It has not been approved or cleared by the US Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical testing. These results are advisories to the attending physician's workup.

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Other Multigene Predictors

- MammaPrint®
- Prosigna® (PAM50)
- EndoPredict®
- Breast Cancer Index (BCI)

These assays differ from each other and from Oncotype Dx

- in the technological platforms used
- the specific genes included
- the patient populations used for their development/validation

Oncotype DX Distinguished as Only Multi-Gene Test for Staging Breast Cancer

- Fundamental change recently announced by AJCC* for breast cancer staging
- First time molecular features (Oncotype DX, ER, PR, and HER2) have been added to anatomic criteria with Level 1 evidence
- CAP/NCCN developing protocols for January 1, 2018 implementation
- New rules expected to influence adoption and future treatment guidelines

COLLEGE of AMERICAN PATHOLOGISTS

NCCN National Comprehensive Cancer Network®

AJCC
American Joint Committee on Cancer
Validating science. Improving patient care.

"Based on the best available evidence at this time, the Expert Panel determined that it was appropriate to incorporate the Oncotype DX score into staging for the subgroup of patients defined by Arm A of the TAILORx study."

Inclusion of multigene panels (when available) as stage modifiers—21-gene recurrence score (Oncotype Dx)

- For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a 21-gene (Oncotype Dx) recurrence score less than 11, regardless of T size, places the tumor into the same prognostic category as T1a-T1b N0 M0, and the tumor is staged using the AJCC prognostic stage group table as stage I.