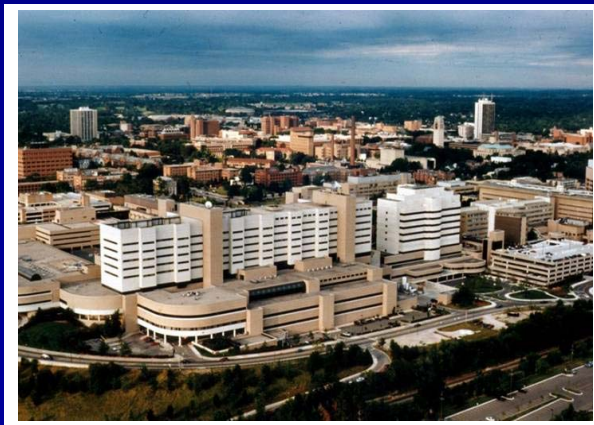


Measurement of HER2

Daniel F. Hayes, MD

Clinical Director, Breast Oncology Program

University of Michigan Comprehensive Cancer Center



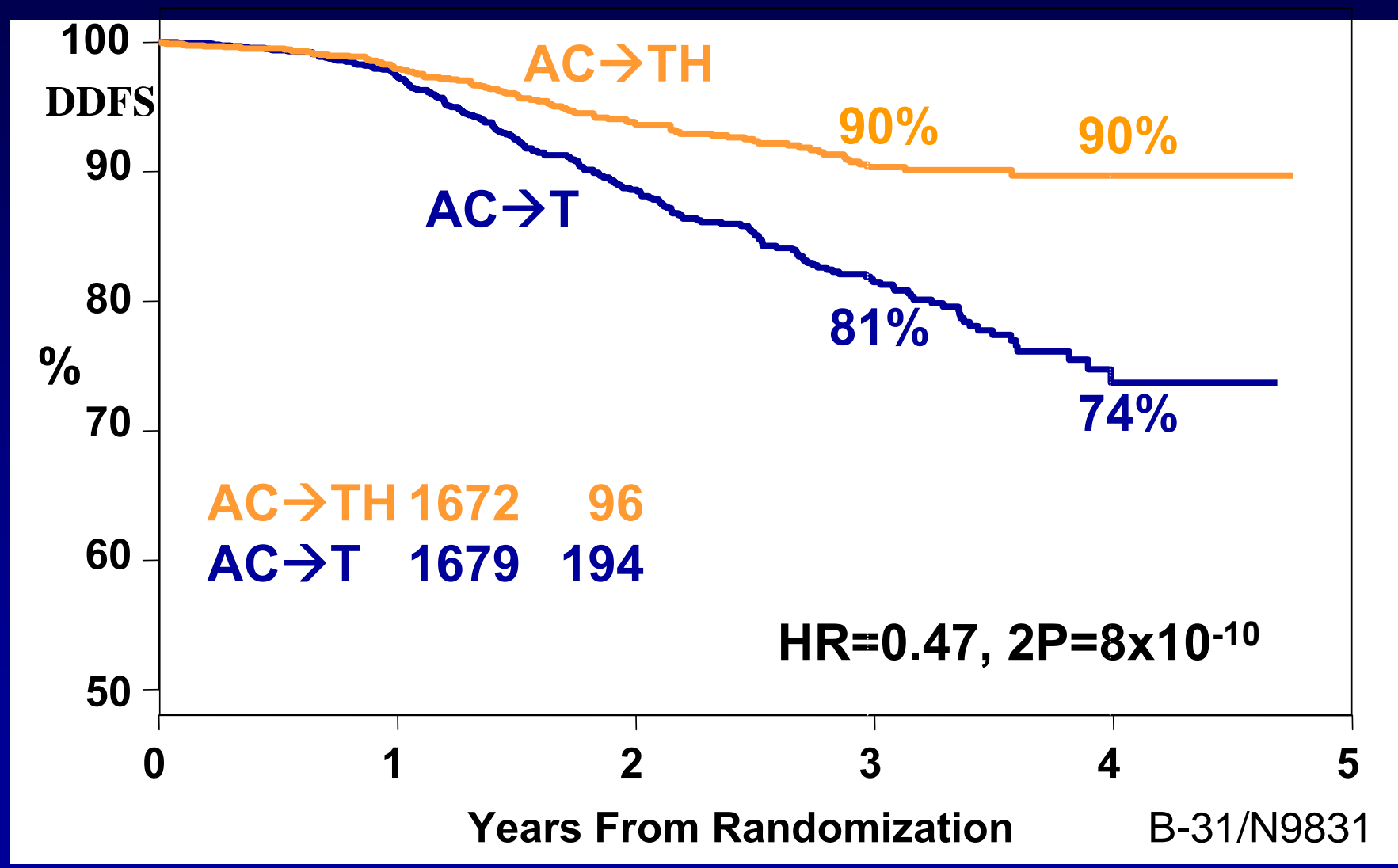
Why Test HER2?

- Trastuzumab
 - Metastatic
 - Adjuvant
- Lapatinib
 - Metastatic
 - Trials
- ? Selection of best or any chemotherapy
 - Adjuvant

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Adjuvant Trastuzumab: Combined Analysis NSABP B-31 / NCCTG N9831



Romond. *N Engl J Med.* 2005;353:1673. Copyright © [2005] Massachusetts Medical Society. All rights reserved.

Why Test HER2?

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Chemotherapy ± Lapatinib in MBC

HER2 FISH+ Patients Benefited From Lapatinib Therapy Regardless of Chemotherapy Used

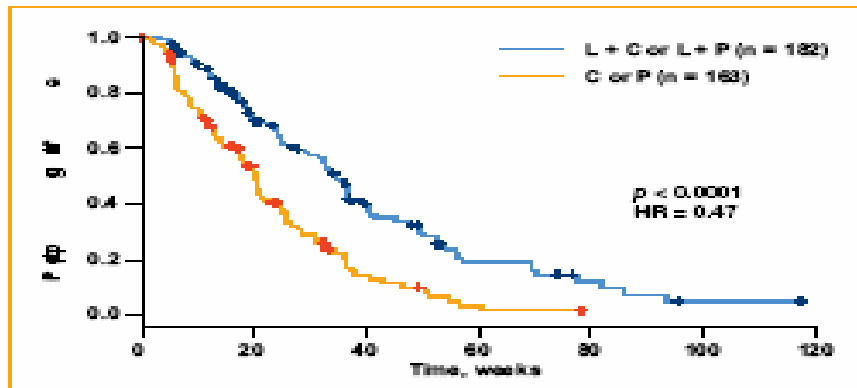


Figure 1. —FISH > 2: Combined analysis of PFS in EGF30001 and EGF100151 (N = 345).

HER2 IHC 3+ Patients Benefited From Lapatinib Therapy Regardless of Chemotherapy Used

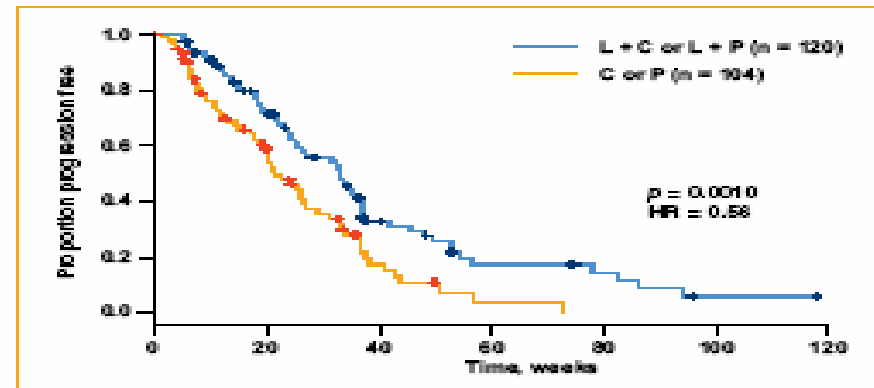


Figure 2. —IHC 3+: Combined analysis of PFS in EGF30001 and EGF100151 (N = 224).

HER2 FISH+ IHC ≤ 2 Patients Benefited From Lapatinib Therapy Regardless of Chemotherapy Used

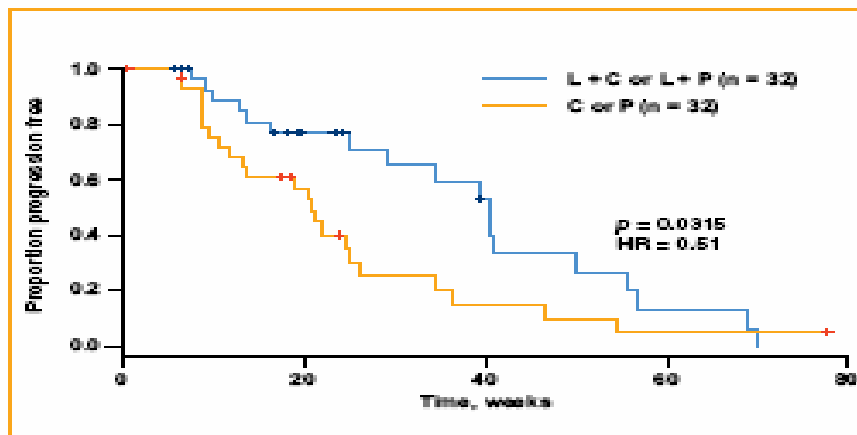


Figure 3. —FISH > 2 and IHC ≤ 2: Combined analysis of PFS in EGF30001 and EGF100151 (N = 64).

HER2 FISH-, IHC 1+ or 2+ Do Not Benefit From Lapatinib Therapy

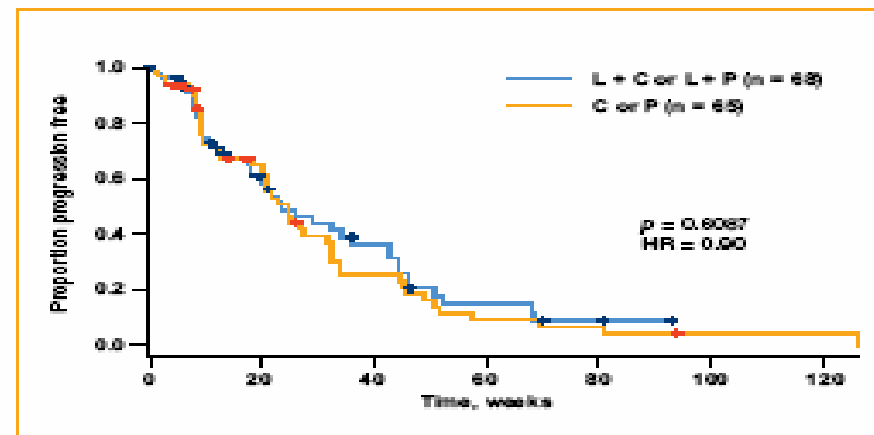
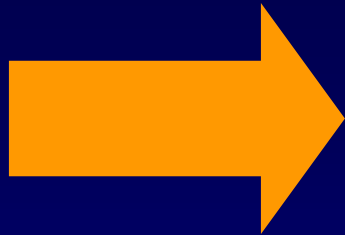


Figure 4. —FISH ≤ 2 and IHC 1+ or 2+: Combined analysis of PFS in EGF30001 and EGF100151 (N = 133).

Reproduced with permission from Press. ASCO. 2007 (abstr 51).

ALLTO Trial



Primary surgery: locally-determined HER2-positive
invasive breast cancer

Centrally-determined HER2 positive

Complete adjuvant chemotherapy
Complete adjuvant radiation therapy (if given)

LVEF $\geq 50\%$

Randomization

Trastuzumab
for 1 year

Lapatinib
for 1 year

Trastuzumab
for 3 months →
(washout)
Lapatinib for 3 months
(total 1 year)

Trastuzumab
plus lapatinib
for 1 year

Patients with ER- or PgR-positive tumors receive endocrine therapy
Selected according to menopausal status; administered concurrently with
biologics and continuing for at least 5 years

OPEN IN NORTH AMERICA, WINTER 2008, WE HOPE

Why Test HER2?

- Trastuzumab
 - Metastatic
 - Adjuvant
- Lapatinib
 - Metastatic
 - Trials
- ? Selection of best or any chemotherapy
 - Adjuvant

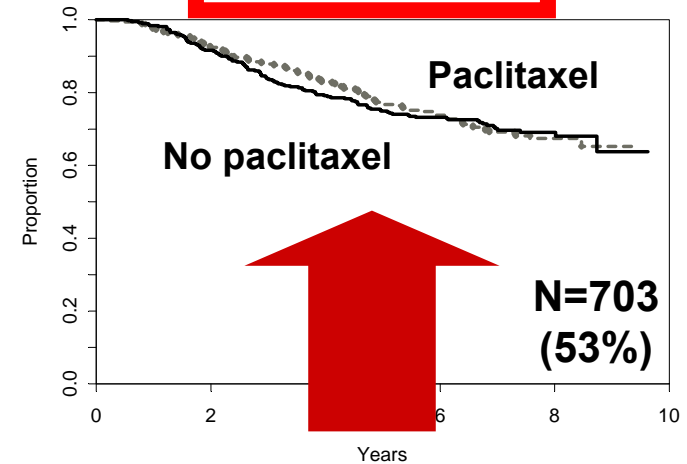
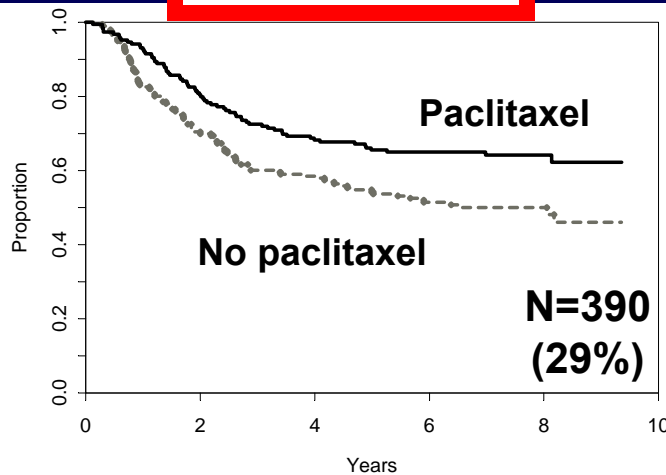
HER2 Is Predictive of Paclitaxel Benefit by Estrogen Receptor Disease-Free Survival

N=1322

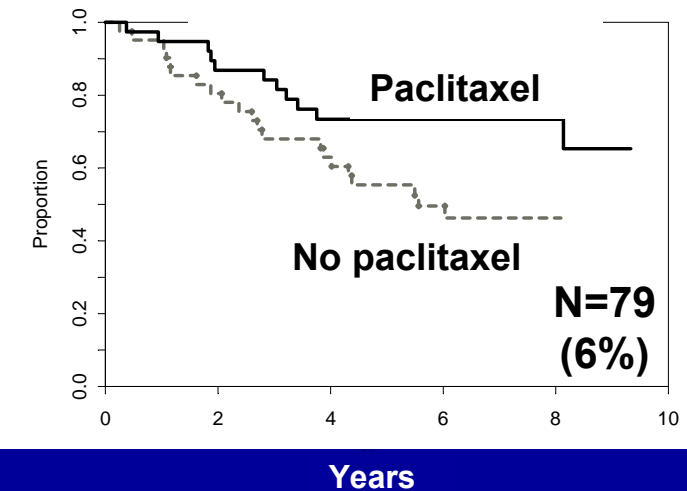
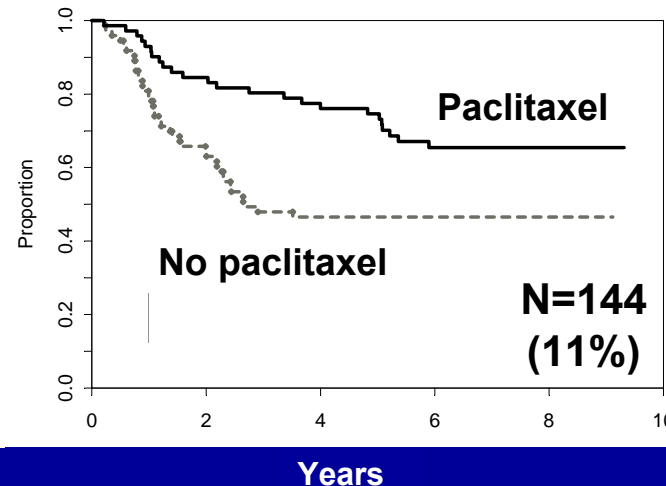
ER Negative

ER Positive

HER2
Negative



HER2
Positive



What Is Different About HER2 (and Predictive Markers in General)?

- HER2 test is not a simple adjunct to anatomic pathology to confirm a tissue diagnosis
 - Assays are being used as the sole determinant of treatment selection
 - Although HER2 predicts benefit, the big issue is **NO BENEFIT**:
 - *Should we withhold therapy from a group of patients in whom it might improve survival?*

A Recipe for Problems

- HER2 testing has been done in a decentralized fashion
- US FDA allows individual anatomic path labs to develop and use their own “home brew” assays if:
 - Use FDA-approved analyte specific reagents
 - Lab is CLIA approved
- Assay validation is not the norm
- Ongoing proficiency testing is not the norm

Problem?

- Would you give (or take!) a drug that:
 - Was made in a laboratory next to your clinic?
 - You were not sure of the dose?
 - You were not sure of what it was mixed in?
 - It seems close to the drug that has been tested, but the laboratory that made the one you are going to use has never validated that their drug works as well (or at all)?

HER2 Testing Concordance in N9831

Concordance Central vs Local Lab

	JNCI 2002 (total N=119)	ASCO 2004 (total N=976)	JCO 2006 (total N=2535)
IHC 3+ (HercepTest)	74% →	79.5% →	82%
FISH + (PathVysion)	67% →	85% →	88%

ASCO-CAP HER2 Initiative

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 - Pre-analytical handling
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JOURNAL OF CLINICAL ONCOLOGY

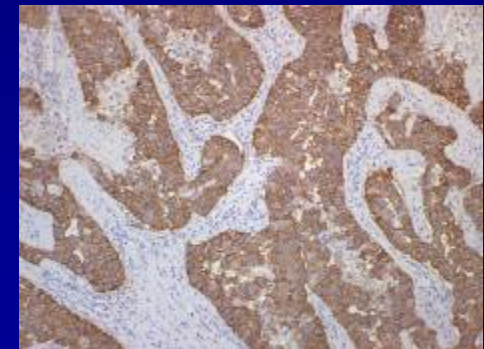
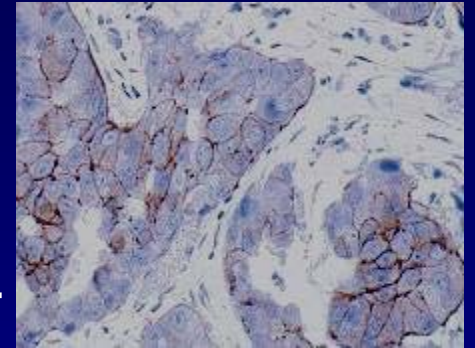
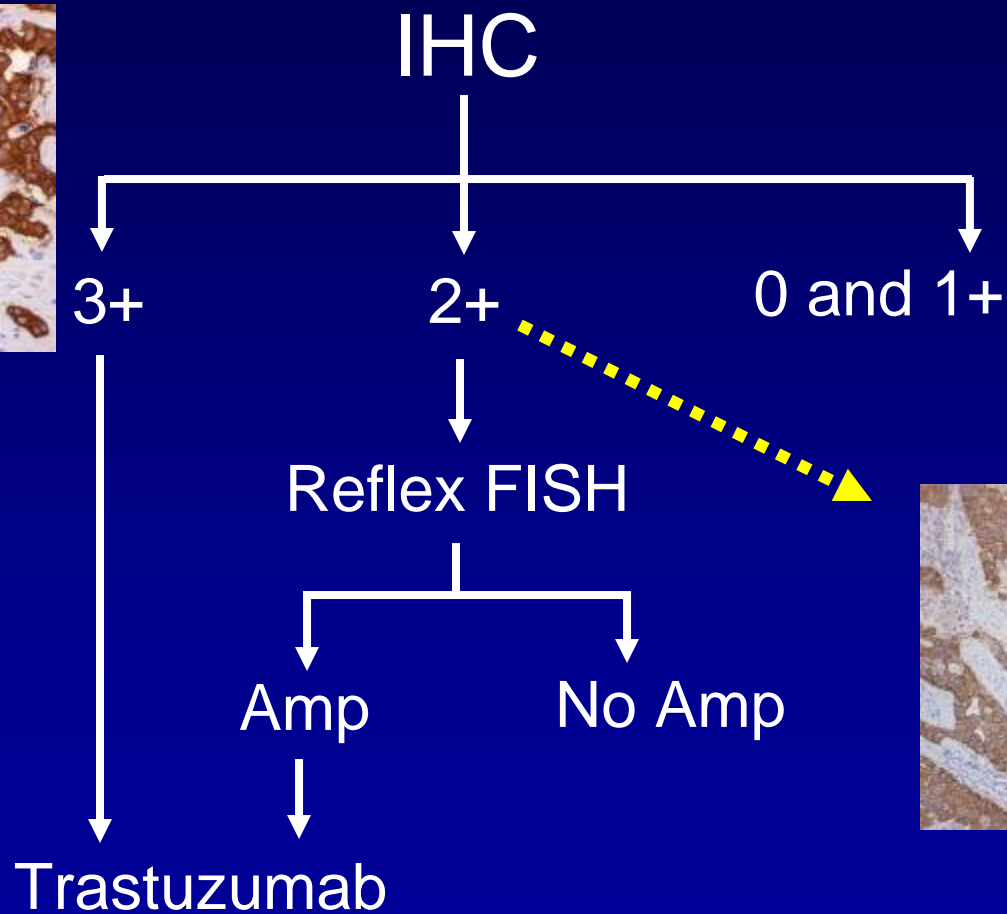
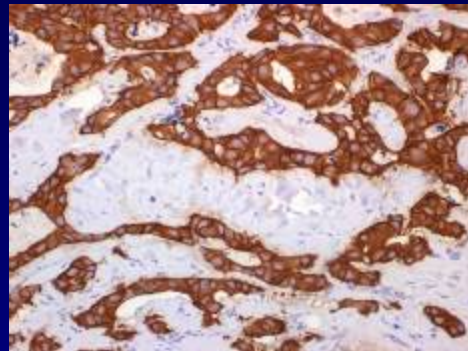
ASCO SPECIAL ARTICLE

American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

Annals C. Wolff, M. Elizabeth A. Mamon, Jerod M. Schwartz, Karen L. Hegarty, D. Craig Allred, Richard J. Coe, Mitchell Dowson, Patrick L. Fitzgibbon, Wafar M. Hwang, Amy Langer, Lisa M. McShane, Soonyoung Park, Mark D. Jayaram, Eulida A. Perez, Michael J. Press, Anthony Rhoads, Catherine Saubers, Sheila E. Zujewski, Raymond Zujewski, Carl M. Vlastakis, Marc van de Vijver, Thomas M. Wheeler, and David F. Hayes

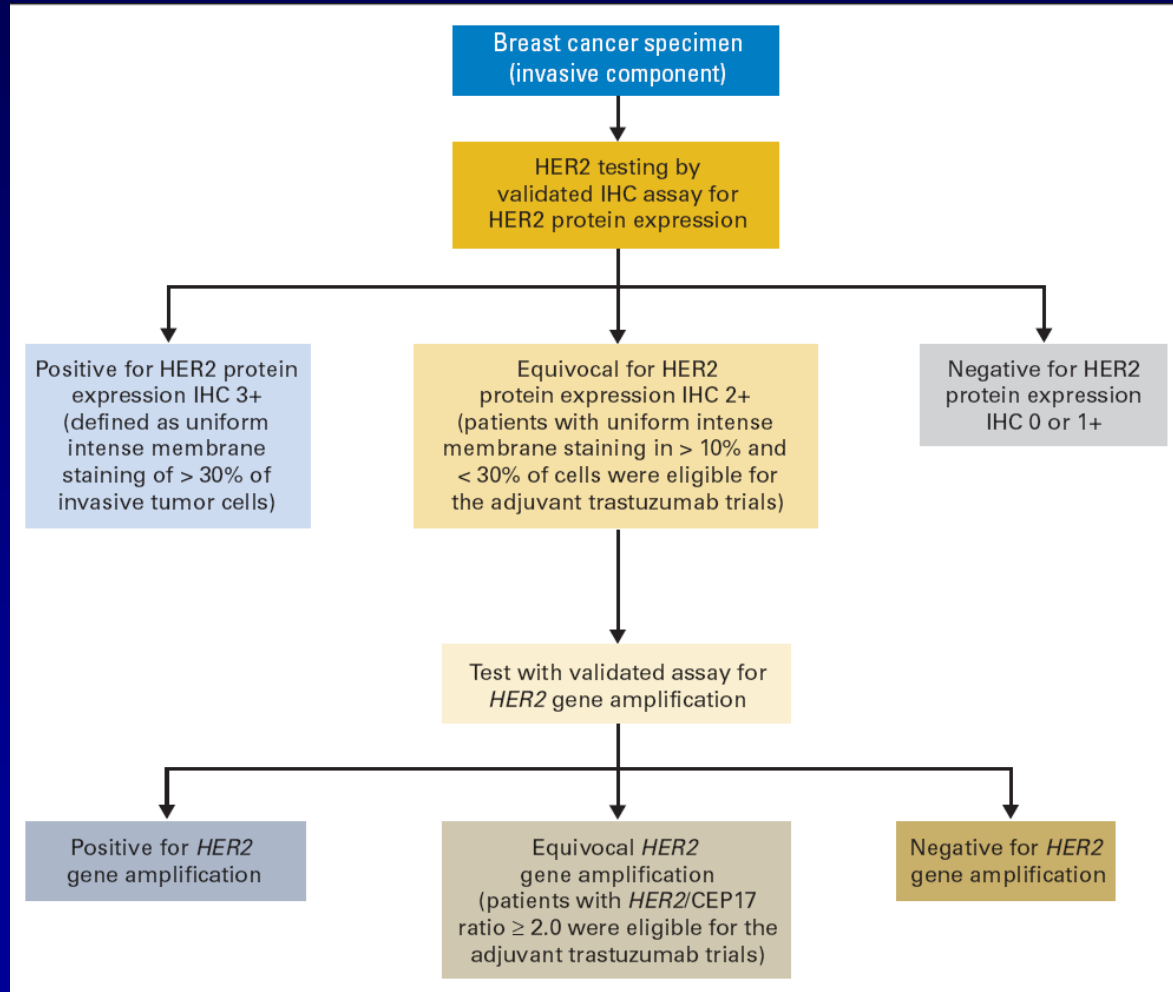
HER2 Testing Algorithm

Adjuvant Trials and Clinical Practice



ASCO/CAP Testing Algorithms

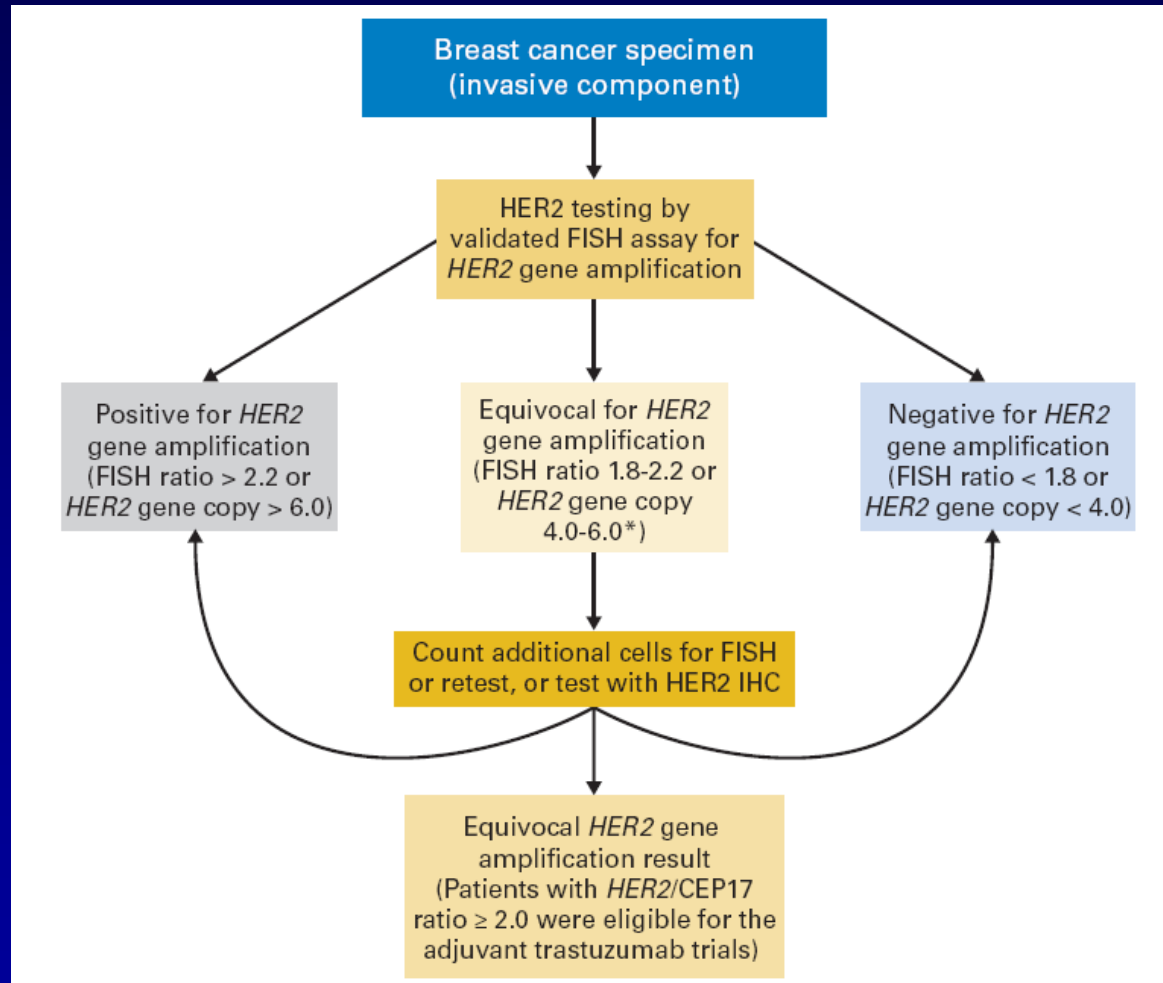
Immunohistochemistry



Wolff. *J Clin Oncol.* 2007;25:4021. Reprinted with permission from the American Society of Clinical Oncology.

ASCO/CAP Testing Algorithms

Fluorescent In Situ Hybridization



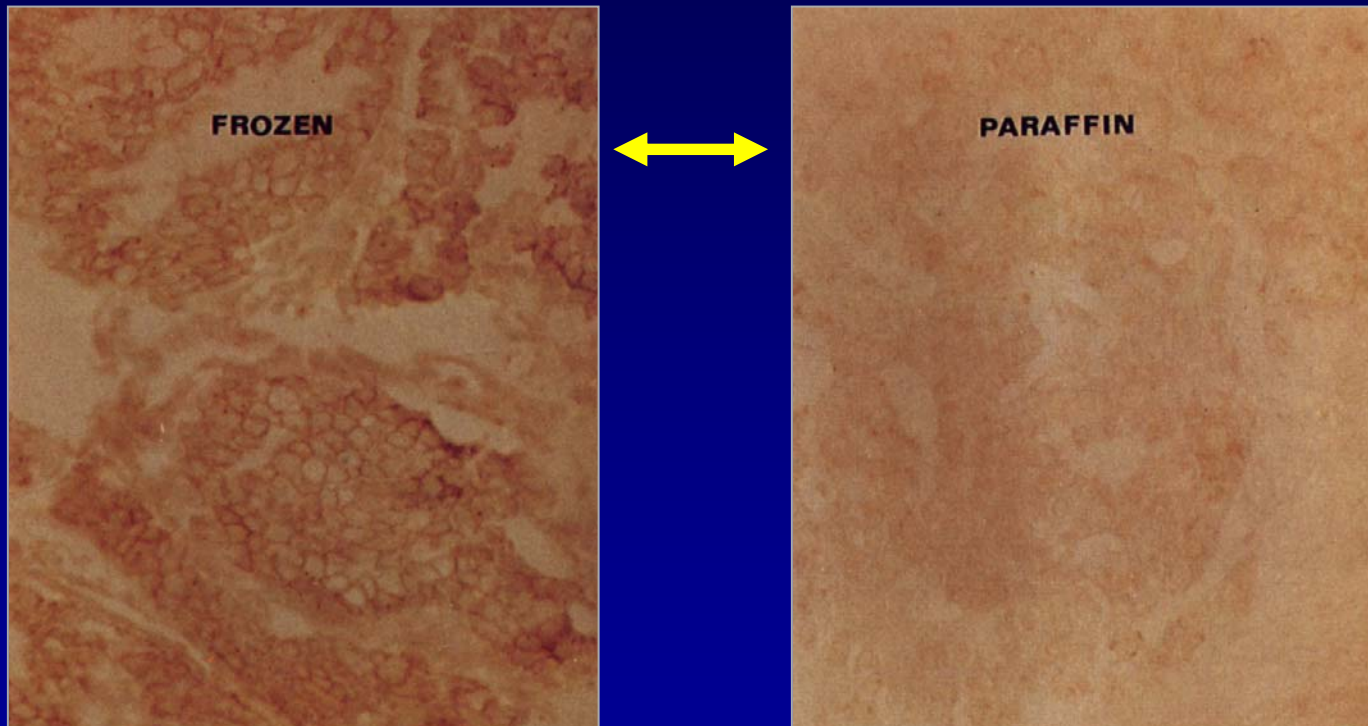
Wolff. *J Clin Oncol.* 2007;25:4021. Reprinted with permission from the American Society of Clinical Oncology.

ASCO-CAP HER2 Initiative

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HER2 Antigen Becomes “Hidden” With Formalin Fixation/Paraffin Embedding

HER2 specimen amplified 2- to 5-fold by Southern Hybridization

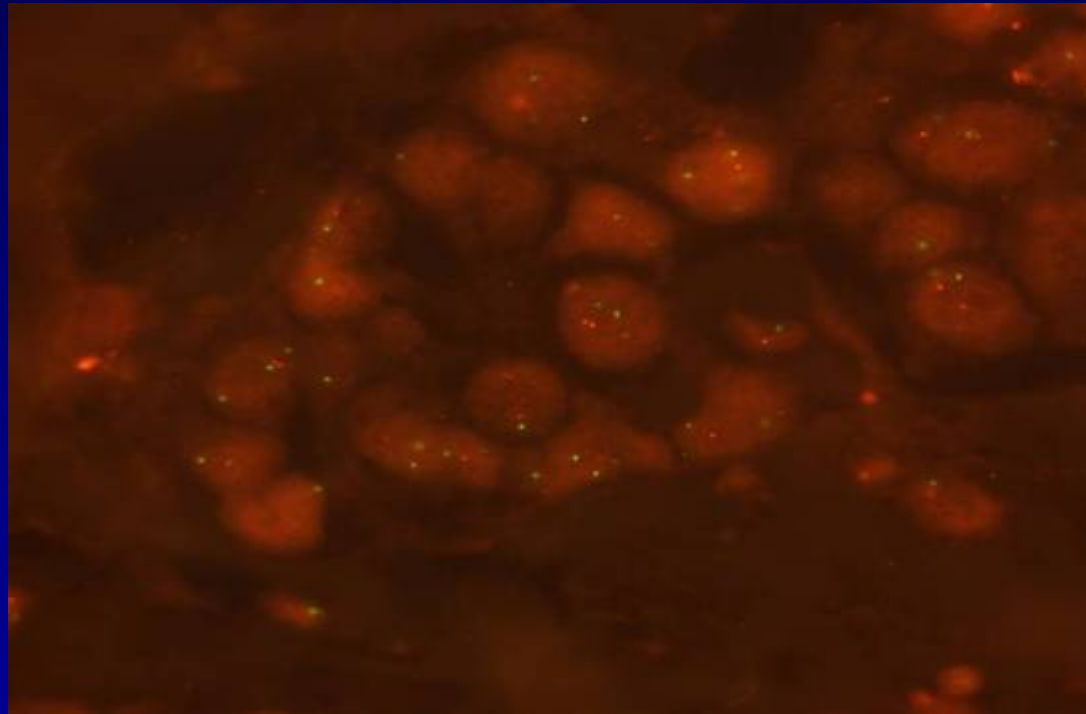


HER2 “negative” status is a fixation artifact that requires antigen retrieval for “correction”

Photos courtesy of Michael Press.

Slamon. *Science*. 1989;244:712.

FISH Can Also Be Affected by Formalin Overfixation

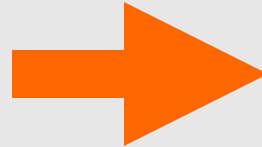


FISH not interpretable
(sample fixed in formalin over the weekend...)

Sources of HER2 Testing Variation

Preanalytic

- Time to fixation
- Method of tissue processing
- Time of fixation
- Type of fixation



Guideline Recommendation:
“...samples for HER2 testing are fixed in neutral buffered formalin for 6-48 hours.”

Analytic

- Assay validation
- Equipment calibration
- Use of standardized laboratory procedures
- Training and competency assessment of staff
- Type of antigen retrieval
- Test reagents
- Use of standardized control materials
- Use of automated laboratory methods

Postanalytic

- Interpretation criteria
- Use of image analysis
- Reporting elements
- Quality assurance procedures
 - Laboratory accreditation
 - Proficiency testing
 - Pathologist competency assessment

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ASCO-CAP HER2 Initiative

The panel recommends that HER2 testing be done in a **CAP-accredited laboratory** or in a laboratory that meets the accreditation and proficiency testing requirements set out by this document

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“The Panel recommends that HER2 testing be done in a CAP-accredited laboratory or in a laboratory that meets the accreditation and proficiency requirements set out by this document”

HER2 Testing

So... now we have solved everything, right?

NO

What About HER2 Subsets?

- HER2 test discordants? (~4% of specimens)
 - IHC positive / FISH negative
 - IHC negative / FISH positive
- HER2 test negative? (80% of specimens)
- HER2 polysomy (~8% of specimens)
 - Polysomy chromosome 17
 - Low levels of protein expression

Mortality by FISH Positive vs Negative (relative risk, 95% CI)

H648g	2+ and 3+	3+	2+
All	0.80 (0.64-1.00) N=469	0.70 (0.51-0.90) N=349	1.26 (0.82-1.94) N=120
FISH positive	0.70 (0.53-0.91) N=325	0.67 (0.51-0.89) N=293	1.31 (0.53-3.27) N=32
FISH negative	1.06 (0.70-1.63) N=126	0.88 (0.39-1.98) N=43	1.11 (0.68-1.82) N=83

Retrospective, unplanned.

Treatment Outcome in N9831 as a Function of HER2 Overexpression or Amplification

HER2 Assay Result*	Number of Patients	HR for DFS [†] (95% CI)
IHC 3+		
FISH Positive	1170	0.42 (0.27-0.64)
FISH Negative	51	0.71 (0.04-11.79)
FISH Unknown	51	0.69 (0.09-5.14)
IHC 0, 1+, or 2+		
FISH Positive	174	1.01 (0.18-5.65)

*IHC by Herceptest, FISH by PathVysion as performed at a central laboratory.

[†]Hazard ratio: risk of recurrence, second primary malignancy, or death in the trastuzumab plus chemotherapy vs the chemotherapy arm; estimated by Cox regression stratified by number of positive nodes and hormone receptor status.

Herceptin® [package Insert]. South San Francisco, CA: Genentech, Inc.; November 2006.

ASCO 2007 Oral Presentations

- Updated results of the combined analysis of NCCTG N9831 and NSABP B-31 adjuvant chemotherapy with/without trastuzumab in patients with HER2-positive breast cancer (Perez, Abstract 512)
- Benefit from adjuvant trastuzumab may not be confined to patients with IHC 3+ and/or FISH-positive tumors: Central testing results from NSABP B-31 (Paik, Abstract 511)
- CALGB 150002: Correlation of HER2 and chromosome 17 copy number with trastuzumab efficacy in CALGB 9840, paclitaxel with or without T in HER2-positive and HER2-negative metastatic breast cancer (Kaufman, Abstract 1009)

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HER2 Central Testing: N9831

Results of IHC/FISH and DFS

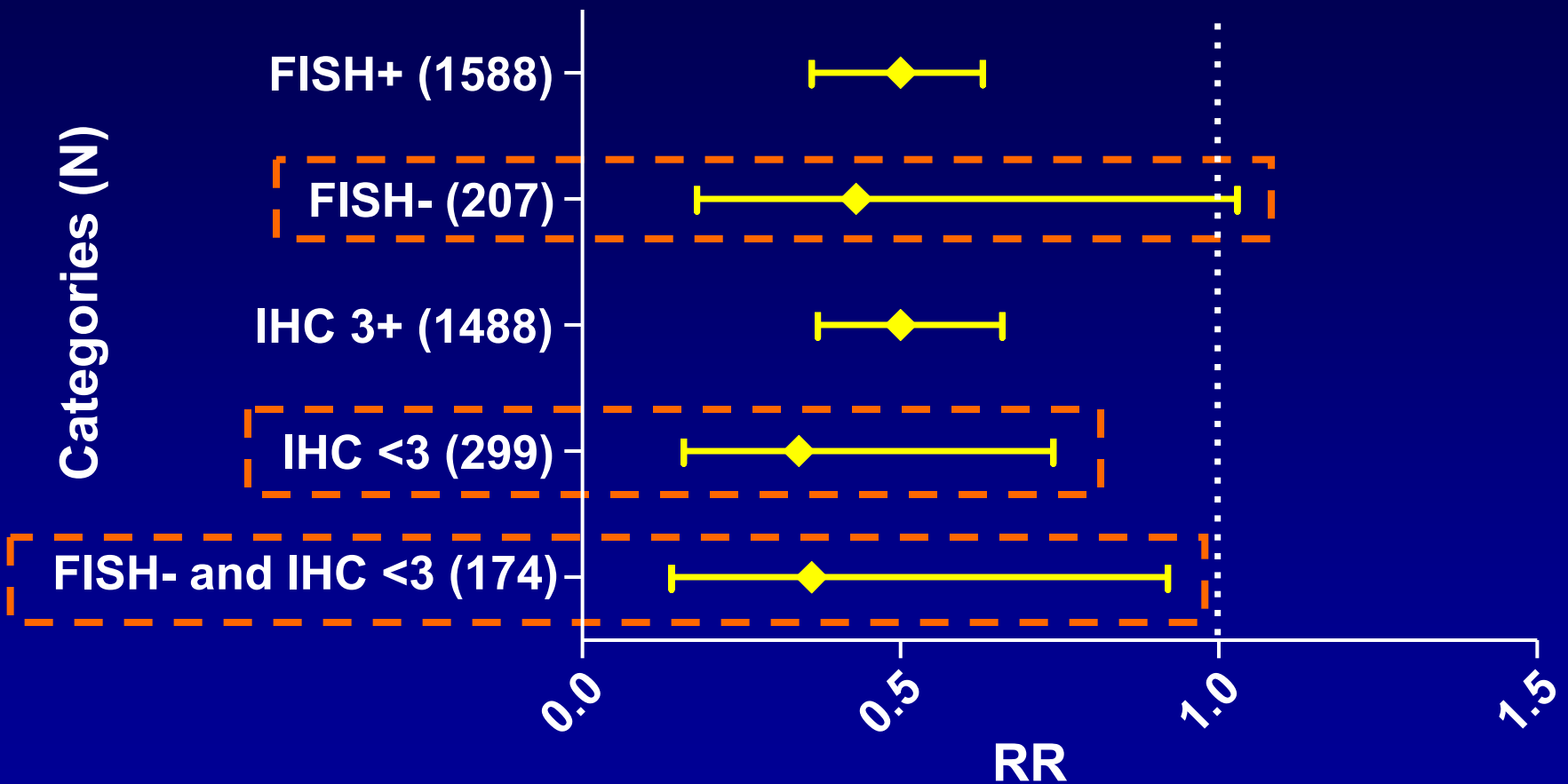
IHC Result	FISH Ratio	N=1842		Events (N)		HR	95% CI	P
		A	C	A	C			
3+	≥2.0	715	690	116	58	0.47	0.34-0.66	<0.0001
	<2.0	30	23	5	3	0.61	0.11-3.29	0.57
0, 1, 2+	≥2.0	95	123	7	9	0.98	0.33-2.91	0.97
	<2.0	44	59	14	9	0.51	0.21-1.2	0.13

HER2 Central Testing: N9831

Results of IHC/FISH and DFS

IHC Result	FISH Ratio	N=1842		Events (N)		HR	95% CI	P
		A	C	A	C			
3+	≥2.0	715	690	116	58	0.47	0.34-0.66	<0.0001
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	<2.0	44	59	14	9	0.51	0.21-1.2	0.13

RR of ACTH/ACT for RFI (NSABP B-31)



Reproduced with permission from Paik. ASCO. 2007.

Does Trastuzumab Work in HER2 Low or Negative Patients??

- Possible answers:
 - Yes
 - No
 - Retrospective, unplanned, partial subset analysis
 - All of these patients were “POS” somewhere
 - Biological plausibility?

Tumor Marker Development: The Problems and Pitfalls of Translating Laboratory Observations to Clinical Utility: It Isn't Easy!

2007 ASCO Extended Educational Session

**“If you torture the data long enough
it will confess to anything”**

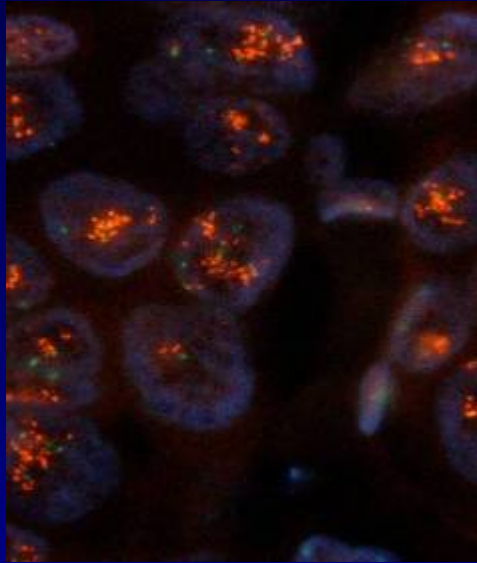
Lisa McShane, PhD

Explanations: chance, technical, biological?

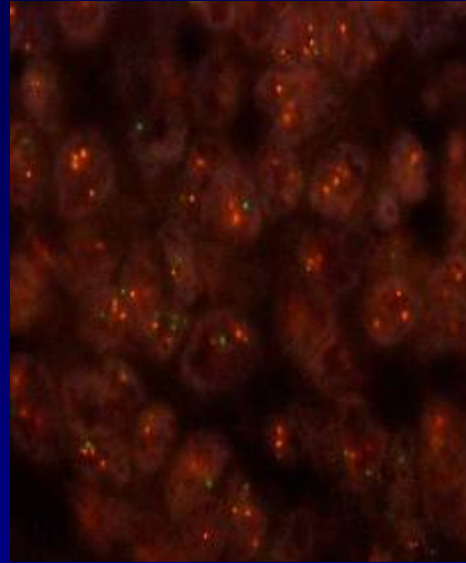
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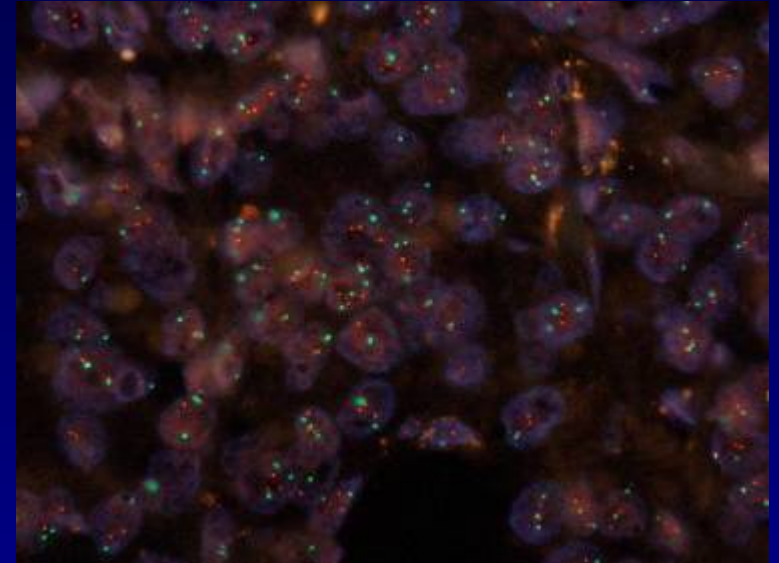
HER2 FISH Patterns



**FISH
Amplified**



**FISH
Not amplified**



**Aneusomy
Not amplified**

CALGB 9840: Paclitaxel Every 1 vs 3 Weeks; Trastuzumab vs Nil in “HER2 Negative”

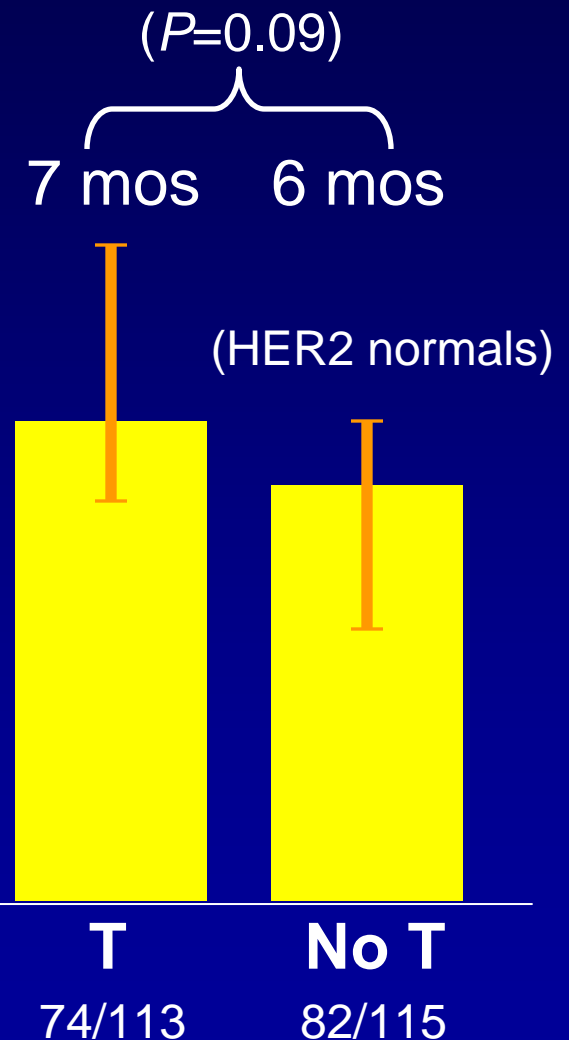
- Weekly vs 3-weekly paclitaxel
- 288 patients with HER2 negative randomized: trastuzumab vs not

Possible explanations:

- 1) Not real?
- 2) False negative HER2 assay?
- 3) Change in HER2 status?

Months

12
11
10
9
8
7
6
5
4
3
2
1



N (events/pts) =

Reproduced with permission from Seidman et al.
Proc Am Soc Clin Oncol. 2004;23:6s (abstr 512).

CALGB 9840: Central Testing

HER2-negative subjects in C9840	585
Tissue blocks available for C150002	303
HER2:CEP17 FISH ratio <2	192
CEP17 copy number > 2.2 (polysomy)	38
IHC 0-2+ / IHC 3+	34 / 3

RESPONSE in FISH Ratio <2	PAC	PAC + Trastuzumab	P Value
No polysomy	18/50 (36%)	19/53 (36%)	NS
Polysomy	5/19 (26%)	12/19 (63%)	<i>P</i> =0.048

Questions Raised by These Data

- Do patients with HER2-negative disease benefit from trastuzumab? **Hypothesis**
 - Does it represent undetected HER2 heterogeneity in primary tumor? Clonal evolution in the metastasis?
- Is polysomy associated with protein expression? **Yes**
- Does polysomy predict treatment benefit? **Hypothesis**
- Is one measure of HER2 (gene or protein) superior to the other? **No (a few disagree)**
- What about discordant results (~4% of all specimens)? **Larger numbers needed, may not matter**

Next Steps

- Retrospective subset analyses regarding benefit from trastuzumab in patients with HER2-negative disease (including those with HER2 polysomy) are hypotheses-generating
- Data from individual adjuvant trials should be pooled to improve precision and test reproducibility of these initial observations
- If confirmed, prospective randomized trials targeting well-defined patient subgroups should be considered to test these intriguing hypotheses

For now, status quo (ie, high-quality testing/reporting)

Cancer Therapy: Take Aim...

Stephen Jeffrey



The Economist, June 7, 2007.

Acknowledgements: Joint CAP/ASCO HER2 Panel

- **Antonio C. Wolff (co-Chair)**
- **Elizabeth H. Hammond (co-Chair)**
- **Jared Schwartz (co-Chair)**
- **Karen Hagerty**
- **D. Craid Allred**
- **Richard J. Cote**
- **Mitchell Dowsett**
- **Patrick L. Fitzgibbons**
- **Wedad M. Hanna**
- **Amy Langer**
- **Lisa McShane**
- **Soonmyung Paik**
- **Mark D. Pegram**
- **Edith A. Perez**
- **Michael F. Press**
- **Anthony Rhodes**
- **Catharine Sturgeon**
- **Sheila Taube**
- **Raymond Tubbs**
- **Gail H. Vance**
- **Marc van de Vijver**
- **Thomas M. Wheeler**