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# Application of the Concept of Intrinsic Subtypes to Clinical Decision Making

DESIGNED BY KATIE HOADLEY

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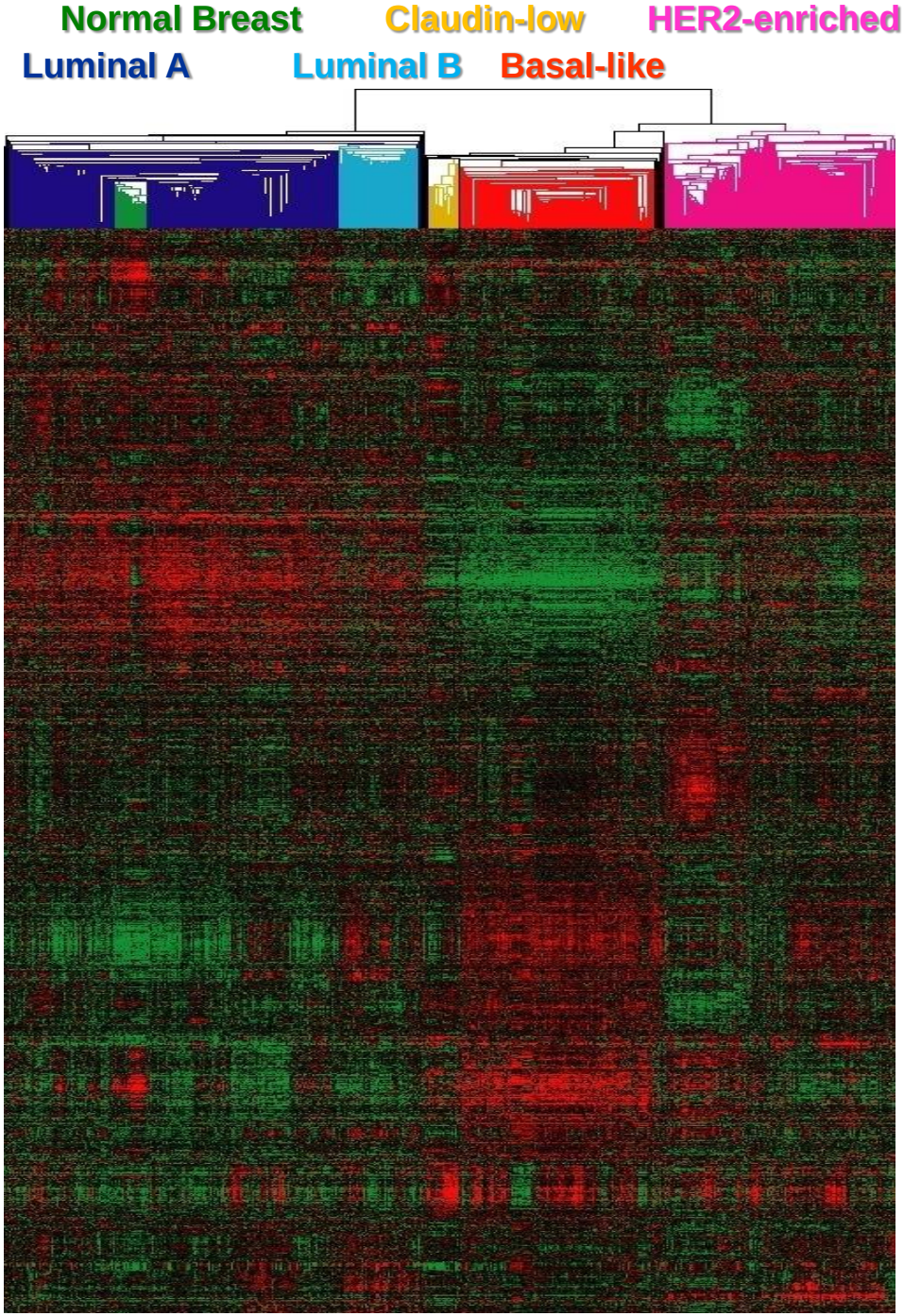
# Disclosures

- Equity Interest = Bioclassifier LLC, and GeneCentric Diagnostics
- Board of Directors = Bioclassifier LLC, and GeneCentric Diagnostics
- Consulting = G1 Therapeutics, Ionis Pharmaceuticals, NanoString Technologies, Bioclassifier LLC, and GeneCentric Diagnostics
- Intellectual Property = licensed IP to Bioclassifier LLC and NanoString Technologies, and to GeneCentric Diagnostics




# Intrinsic Subtypes and the ROR Score provide valuable information for:

1. The biology of breast cancer
2. Baseline prognosis
3. Prognosis/prediction for endocrine therapy treated patients
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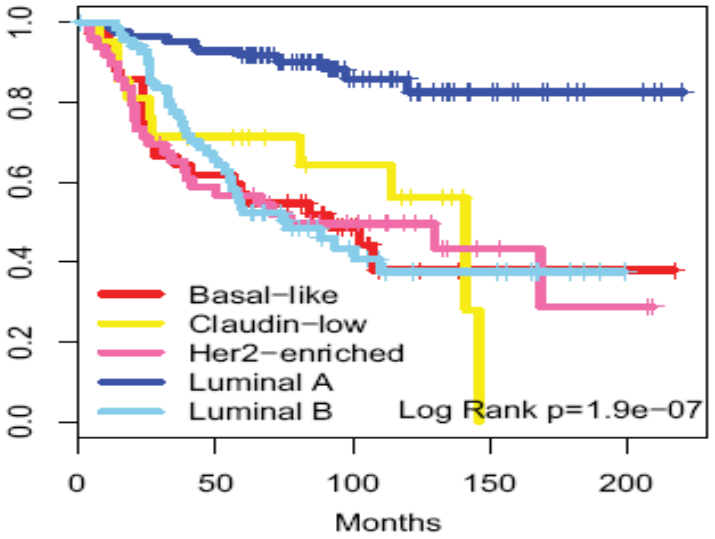
5. Prediction of response to HER2-targeting



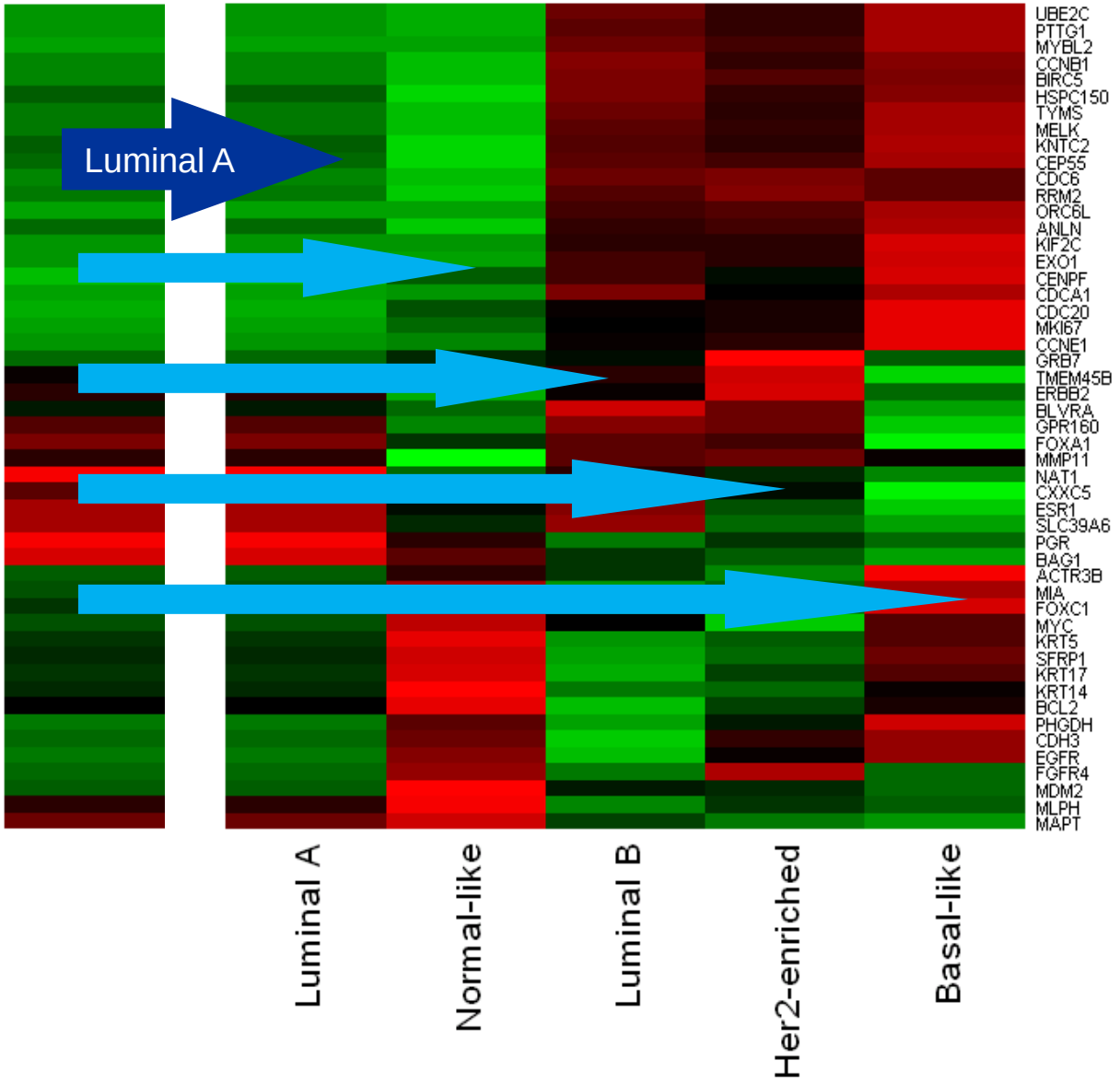
# Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes ("PAM50")

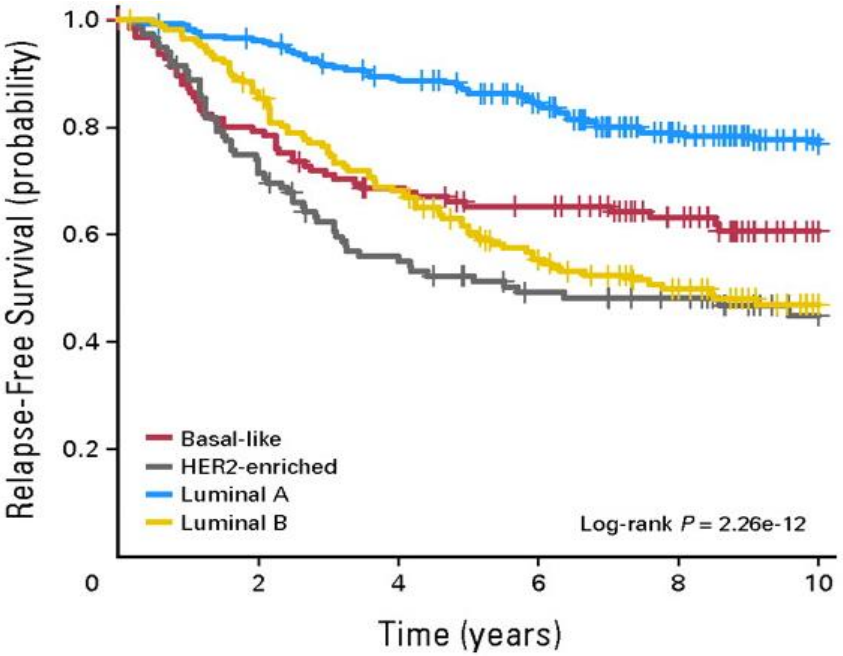
**Parker et al., JCO, 2009 (PMID:19204204)**

**Elaine Mardis**

**Joel Parker**

**Torsten Nielsen** **Phil Bernard** **Matthew Ellis**





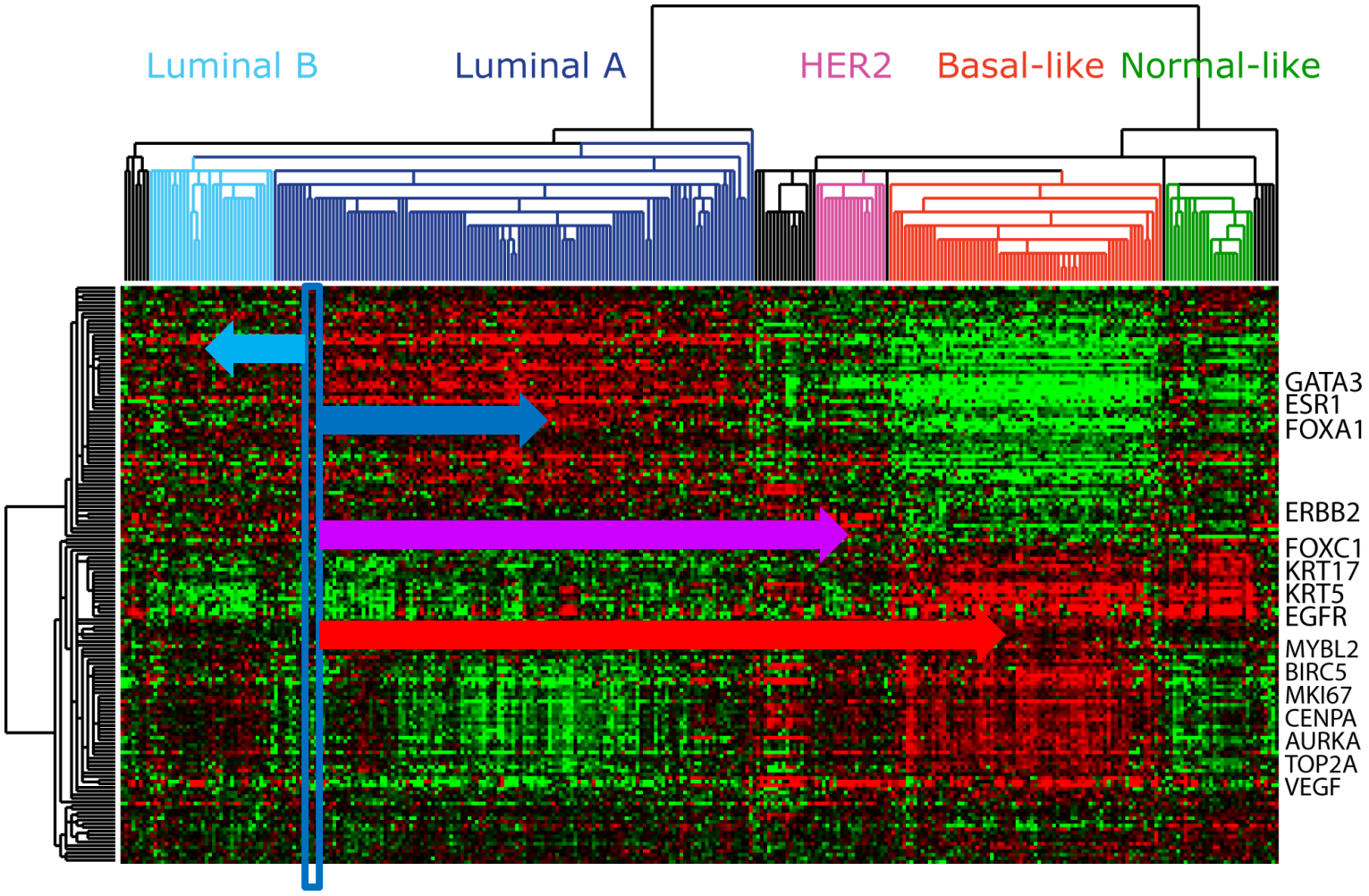

**710 node negative breast cancer patients who received no systemic adjuvant therapy as a test set (pure prognosis)**

**Table 2. Models of Relapse Free Survival (untreated)**

| Model | A | | B | | C | |
|---|---|---|---|---|---|---|
| Variable | Hazard Ratio | p-value | Hazard Ratio | p-value | Hazard Ratio | p-value |
| Basal-like* | 1.33 | 0.330 | ***1.79*** | ***0.030*** | 1.58 | 0.066 |
| HER2-enriched* | ***2.53*** | ***0.00012*** | ***3.25*** | ***<0.0001*** | ***2.90*** | ***<0.0001*** |
| Luminal B* | ***2.43*** | ***<0.0001*** | ***2.88*** | ***<0.0001*** | ***2.54*** | ***<0.0001*** |
| ER Status~ | 0.83 | 0.38 | 0.83 | 0.34 | 0.83 | 0.32 |
| Tumor Size† | ***1.36*** | ***0.034*** | ***1.43*** | ***0.012*** | ***1.57*** | ***0.001*** |
| Node Status‡ | ***1.75*** | ***0.035*** | ***1.72*** | ***0.041*** | - | - |
| Histologic Grade^ | ***1.40*** | ***0.0042*** | - | - | - | - |
| Full vs Subtype≈ | | ***<0.0001*** | | ***<0.0001*** | | ***<0.0001*** |
| Full vs Clinical¥ | | ***<0.0001*** | | ***<0.0001*** | | ***<0.0001*** |

**Parker et al. J Clin Oncol; 27:1160-1167 2009 (PMID:19204204)**

# Heterogeneity within Subtypes




**Distance of a tumor to each centroid/subtype as a genomic summary feature**

# Prognostic Risk Classification Strategy (ROR)

- **Similarity to the subtypes are used as variables in the prognostic model where the outcome is Risk of Recurrence (ROR):**

**(Model 1) ROR-S** $= \beta_{a*}\text{Basal} + \beta_{b*}\text{HER2} + \beta_{c*}\text{LumA} + \beta_{d*}\text{LumB}$

**(Model 2) ROR-T** $= \beta_{e*}\text{Basal} + \beta_{f*}\text{HER2} + \beta_{g*}\text{LumA} + \beta_{h*}\text{LumB} + \beta_{i*}\text{Size}$

**(Model 3) ROR-PT** $= \beta_{j*}\text{Basal} + \beta_{k*}\text{HER2} + \beta_{l*}\text{LumA} + \beta_{m*}\text{LumB} + \beta_{n*}\text{Size} + \beta_{o*}\text{Proliferation}$

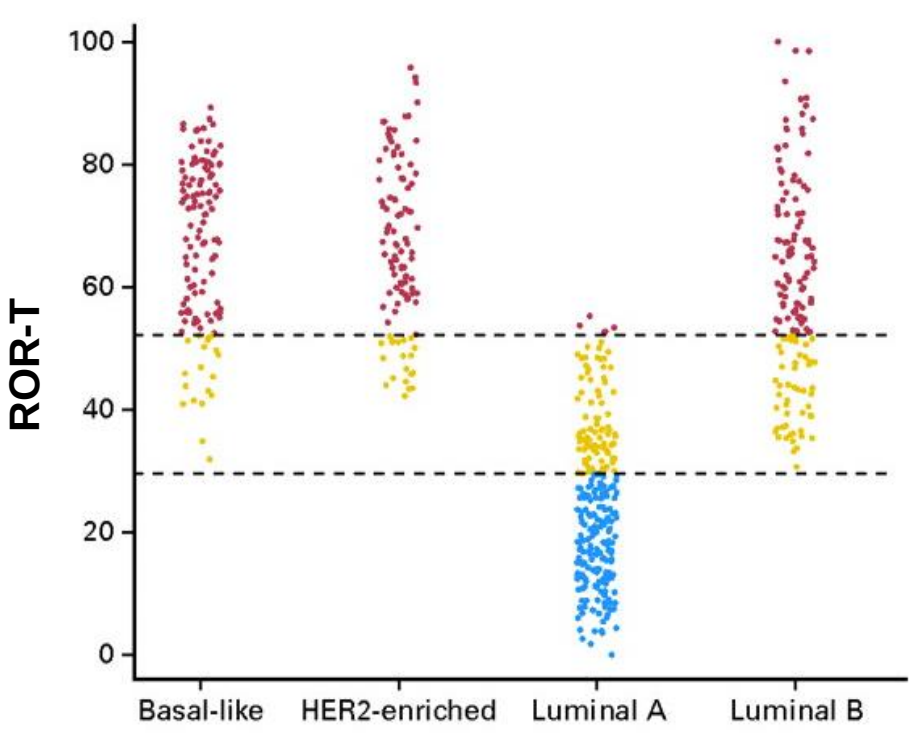
- **Weights (β coefficients) for each feature are learned from a training data set using a Cox Proportional Hazards model with Ridge Regression[1]**

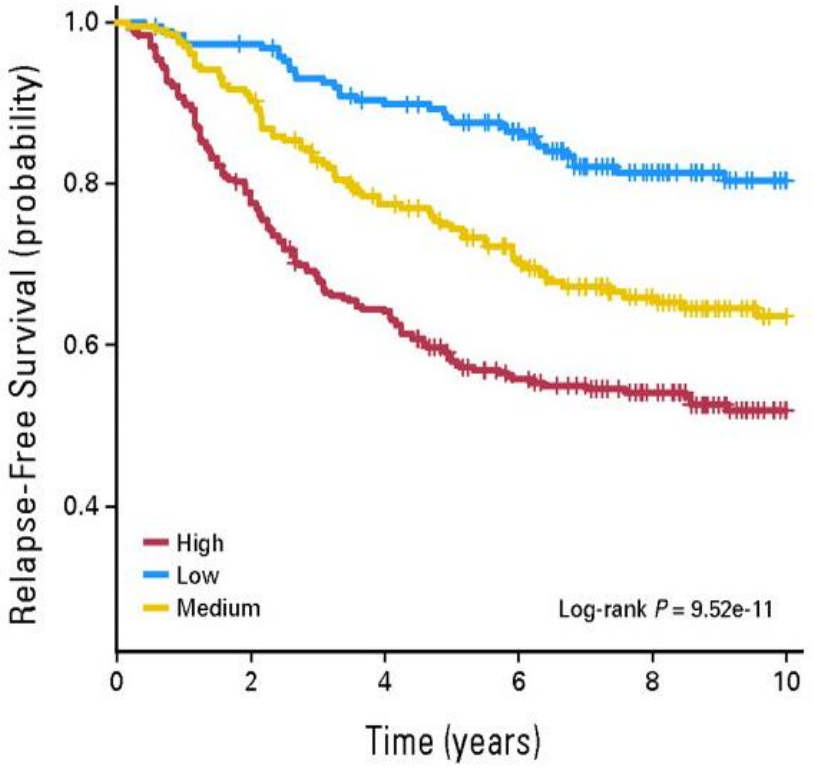
[1]Ridge regression with Cox model: Tibshirani, Statistics in Medicine 1997

- **The weighted sum is assigned as the ROR score for a test case and a threshold is applied for risk class assignment (low-intermediate-high risk)**

**Parker et al. J Clin Oncol; 27:1160-1167 2009 (PMID:19204204)**

# Prognostic Risk Classification Strategy (ROR)



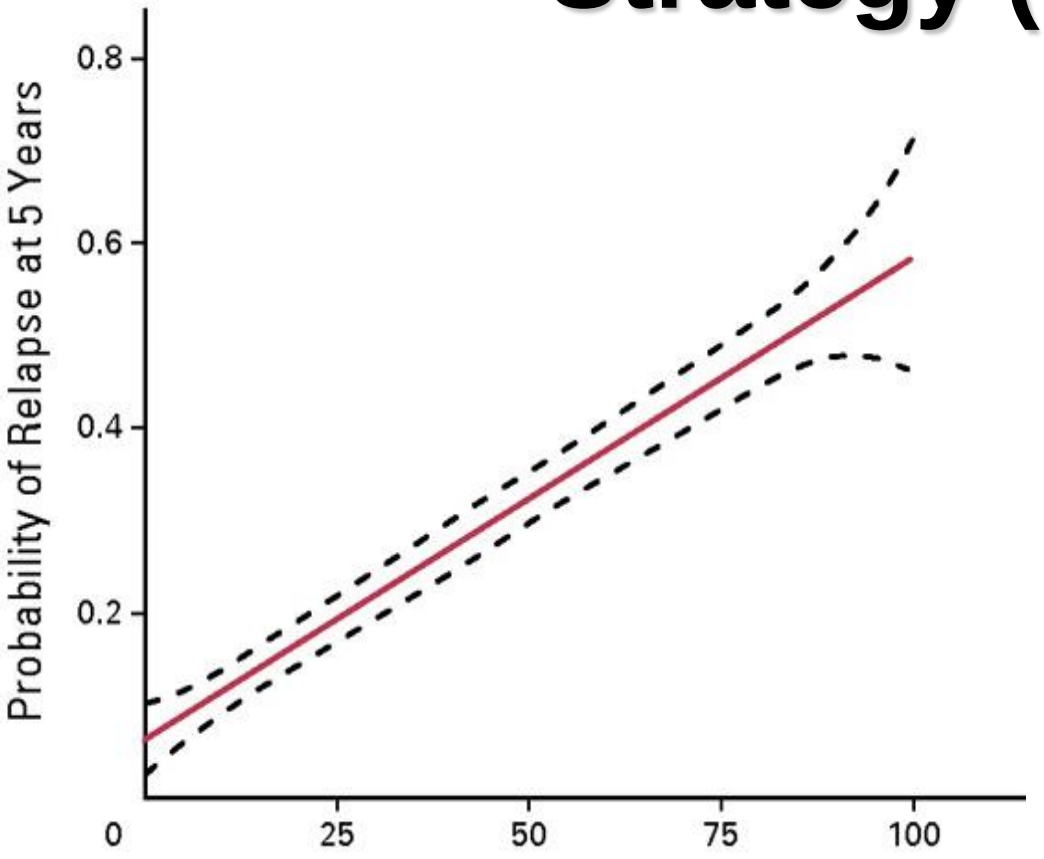



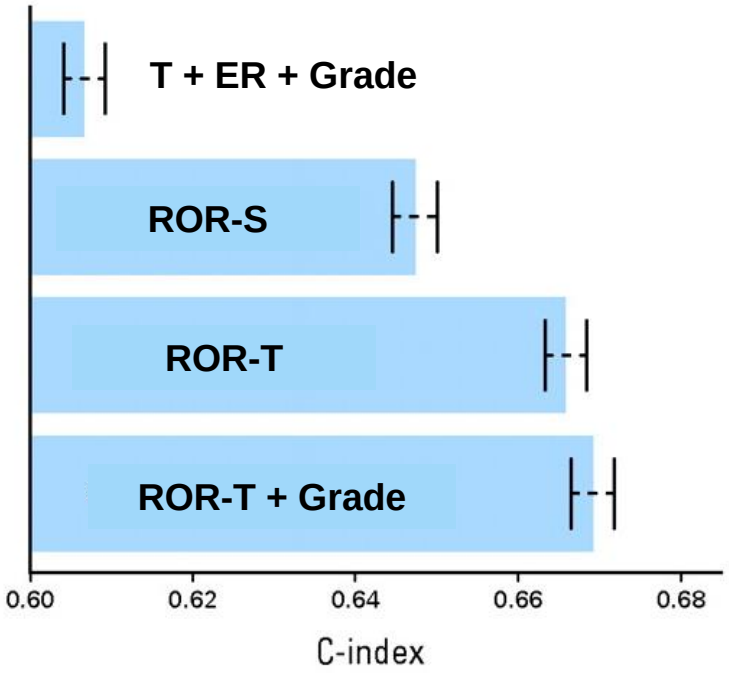

**N=558 no adjuvant systemic therapy and node negative test cases**

**ROR-T thresholds determined from training cases**

**Parker et al. J Clin Oncol; 27:1160-1167 2009 (PMID:19204204)**

# Prognostic Risk Classification Strategy (ROR)



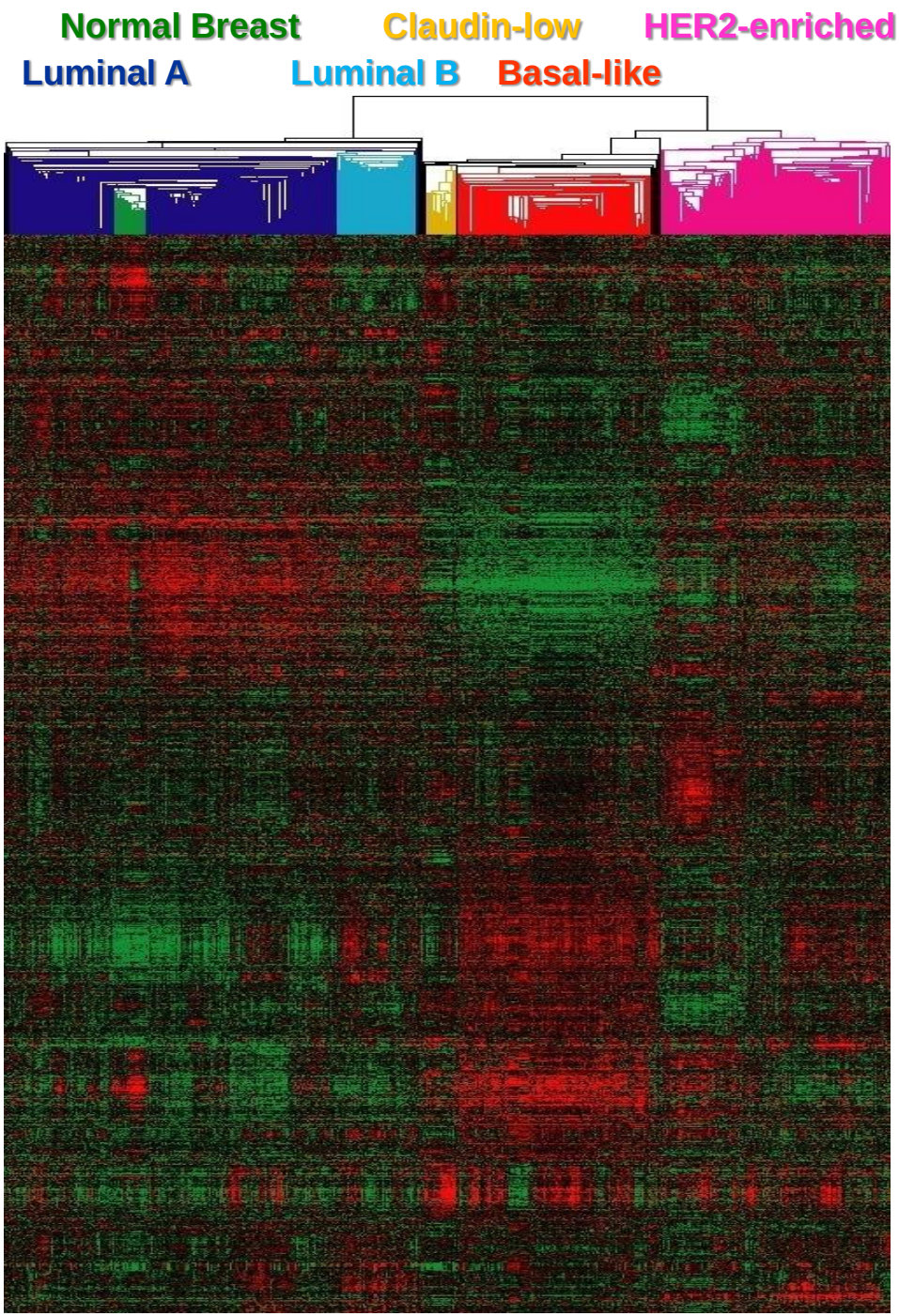




**C-index: Harrell et al., JAMA, 1982 (PMID:7069920)**

**"The c-index is the proportion of all pairs of subjects whose survival time can be ordered such that the subject with the higher predicted survival is the one who survived longer" (taken from Harrell, Regression Modeling Strategies, Springer Series in Statistics).**

**N=558 no adjuvant systemic therapy and node negative test cases**

**Parker et al. J Clin Oncol; 27:1160-1167 2009 (PMID:19204204)**

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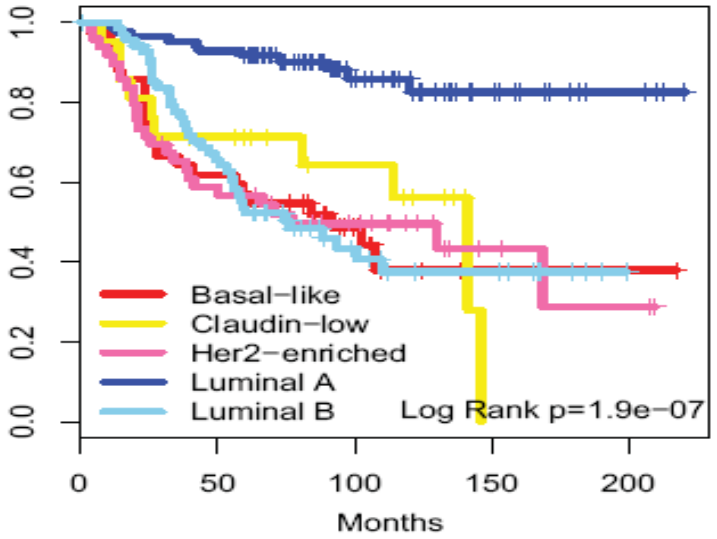
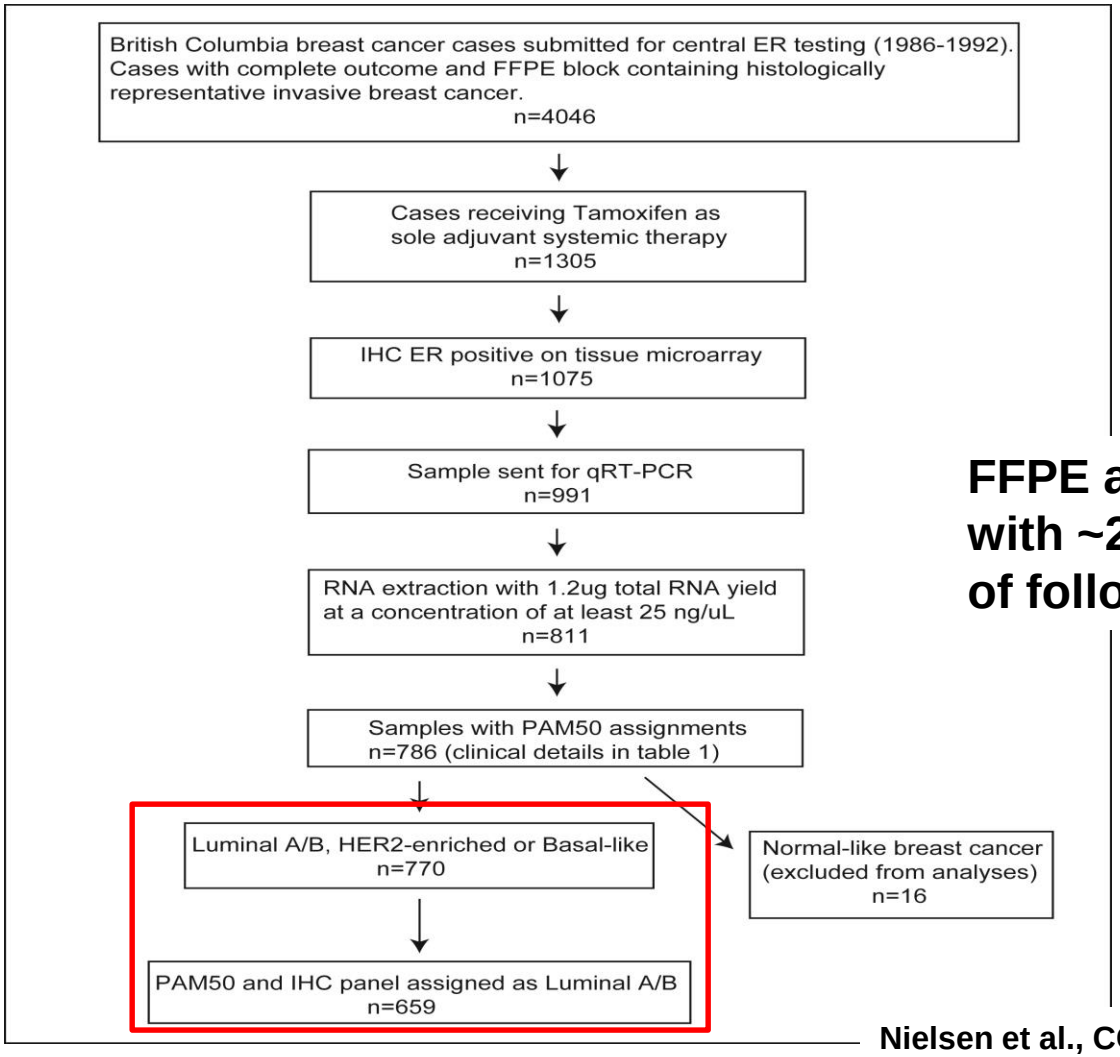


# A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor positive breast cancer

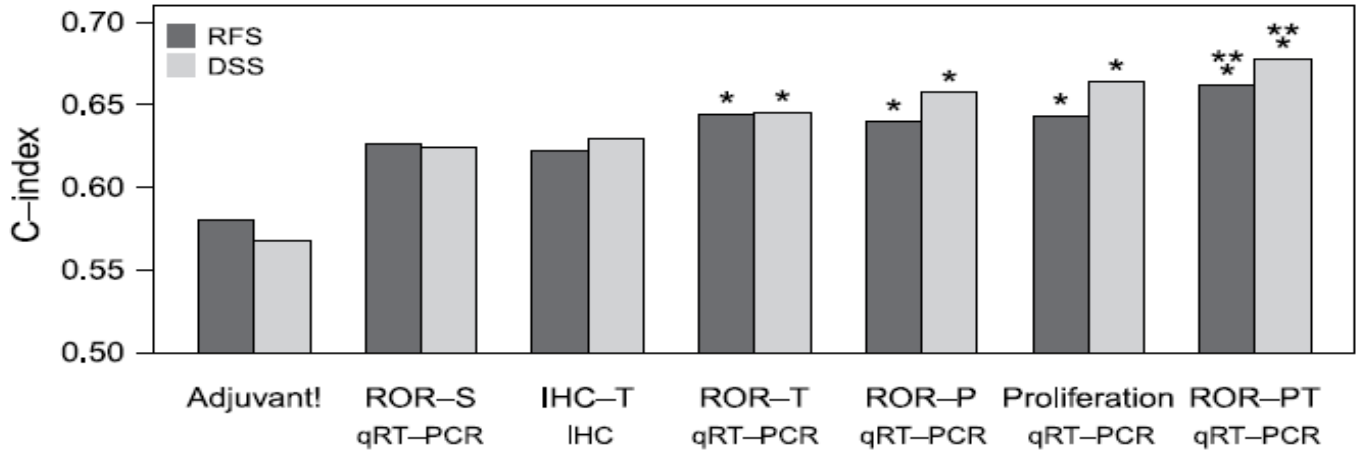
Torsten O. Nielsen, Joel S Parker, Samuel Leung, David Voduc, Mark Ebbert, Tammi Vickery, Sherri R. Davies, Jacqueline Snider, Inge J. Stijleman, Jerry Reed, Maggie C.U. Cheang, Elaine R. Mardis, Charles M. Perou, Philip S. Bernard, Matthew J. Ellis, Clinical Cancer Research 2010

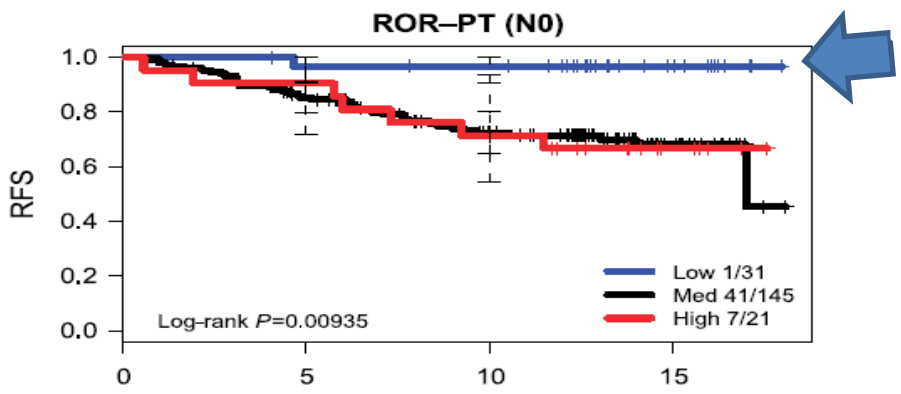


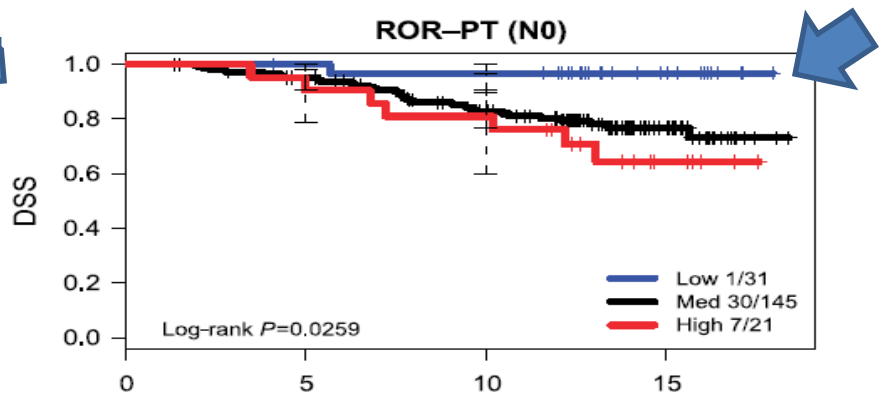

**FFPE archive with ~20 years of follow up**

**Nielsen et al., CCR 2010 (PMID:20837693)**

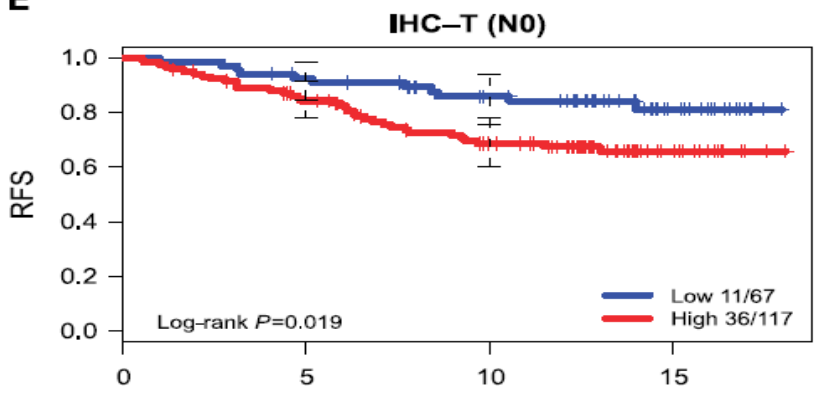
# ER+, tamoxifen treated, node-negative patient subset

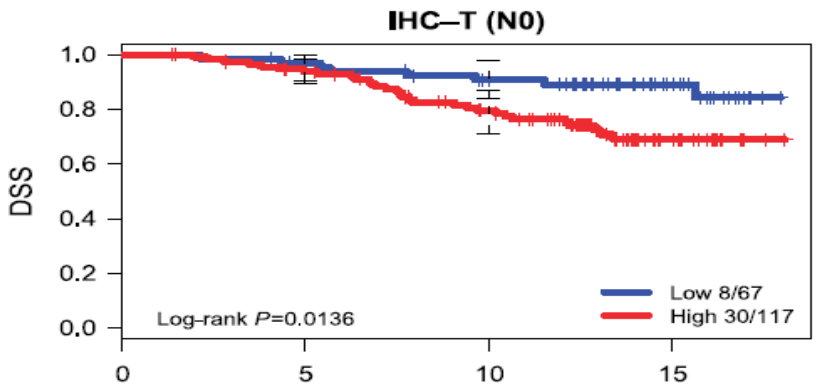



E

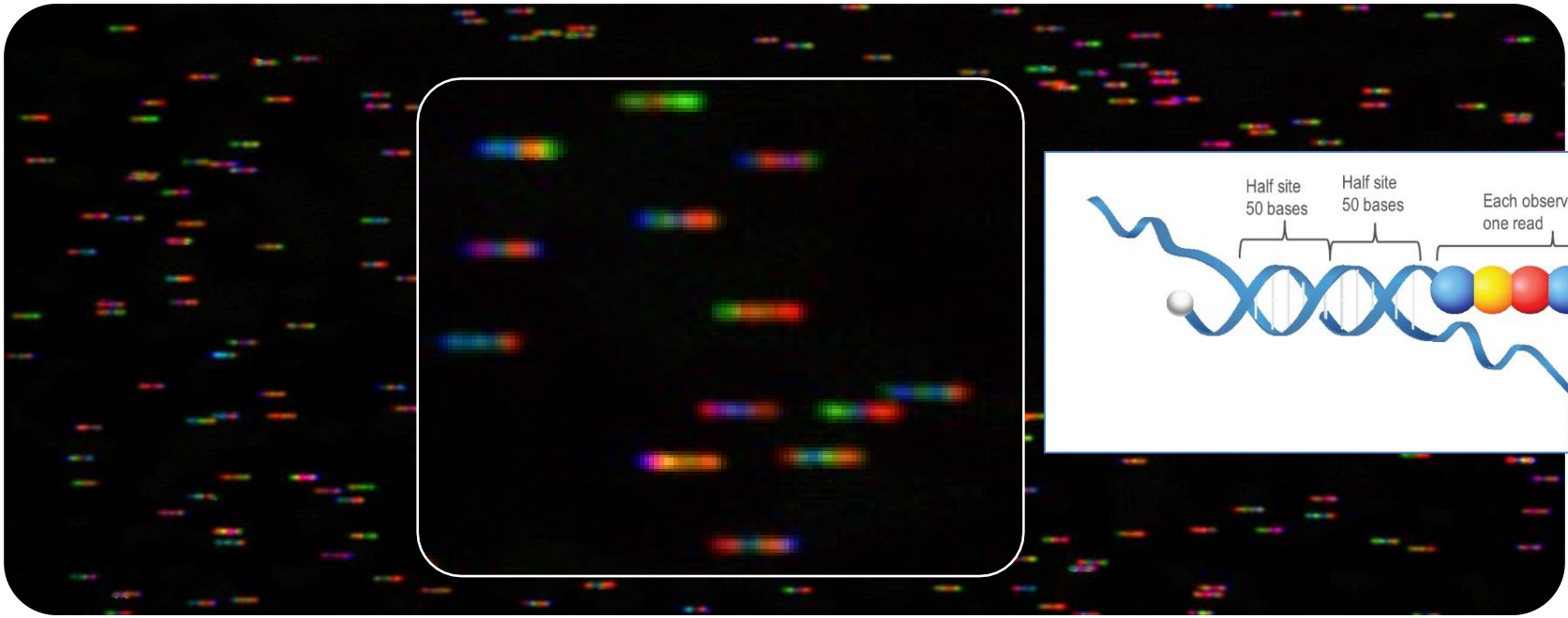



Nielsen et al., CCR 2010 (PMID:20837693)

# Scientific Reasons for PAM50 platform change to NanoString nCounter (~2010)

- **Easier to use than PCR or microarrays**
- **Fast and simple workflow**
- **Compatible with a distributed test and prefabricated kits (IVD)**
- **Compatible with RNA coming from FFPE materials**



# Development and verification of a PAM50-based breast cancer gene signature assay

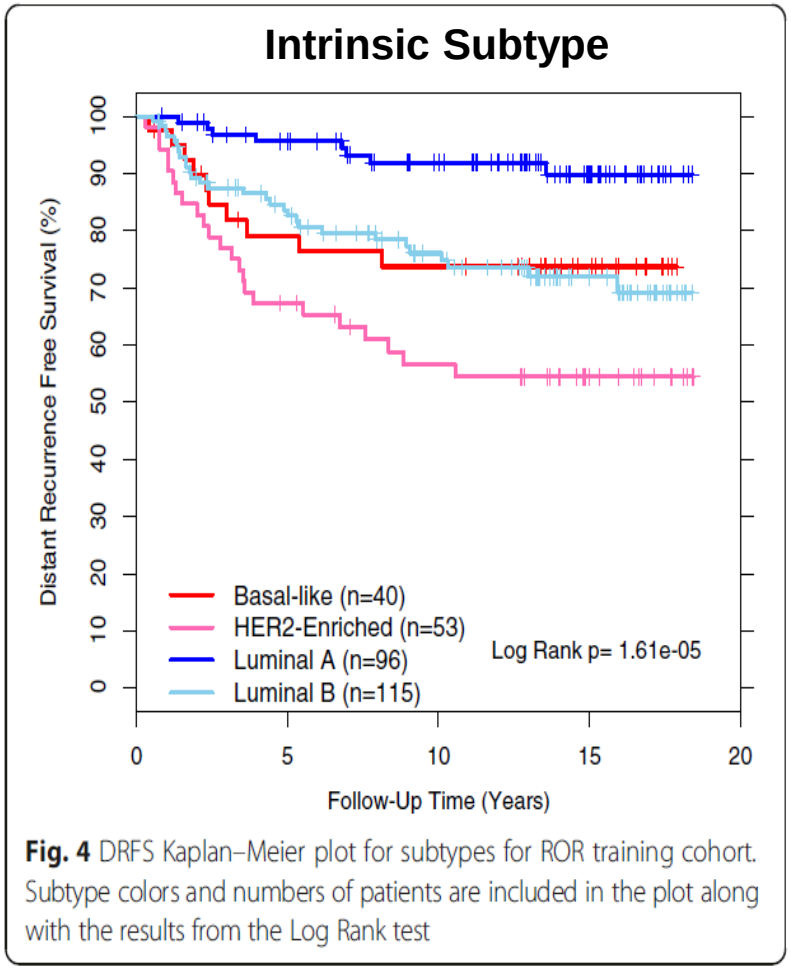



**Fig. 4** DRFS Kaplan–Meier plot for subtypes for ROR training cohort. Subtype colors and numbers of patients are included in the plot along with the results from the Log Rank test

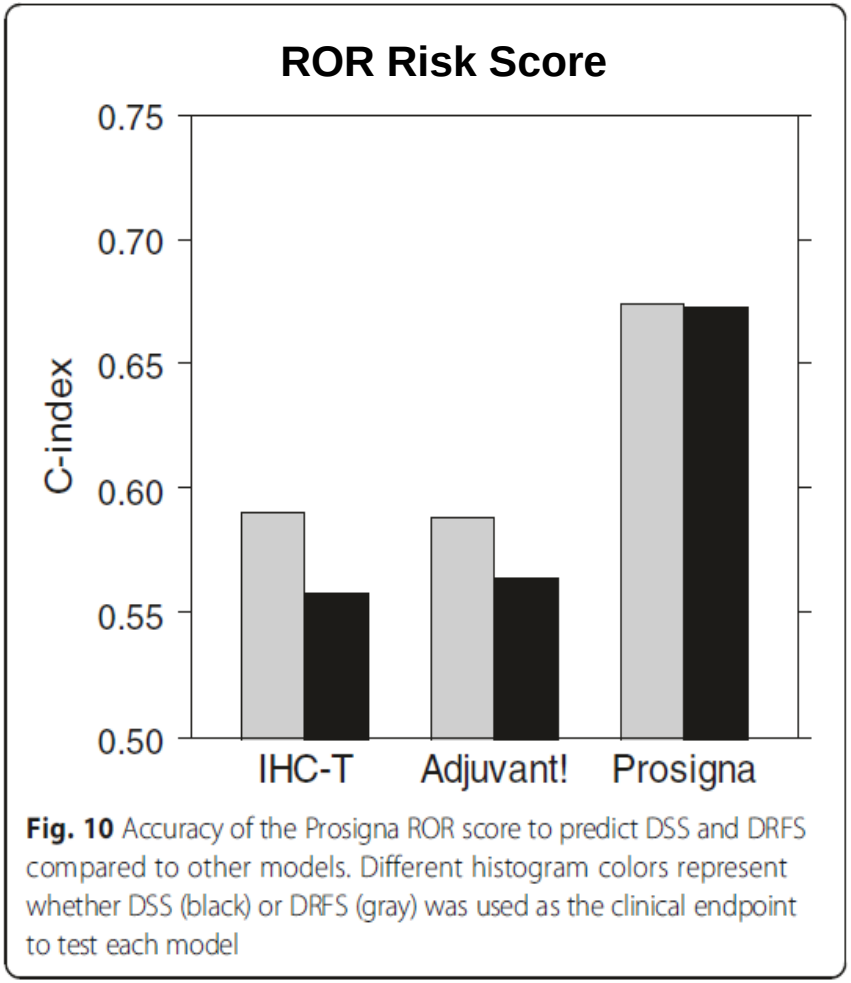



**Fig. 10** Accuracy of the Prosigna ROR score to predict DSS and DRFS compared to other models. Different histogram colors represent whether DSS (black) or DRFS (gray) was used as the clinical endpoint to test each model

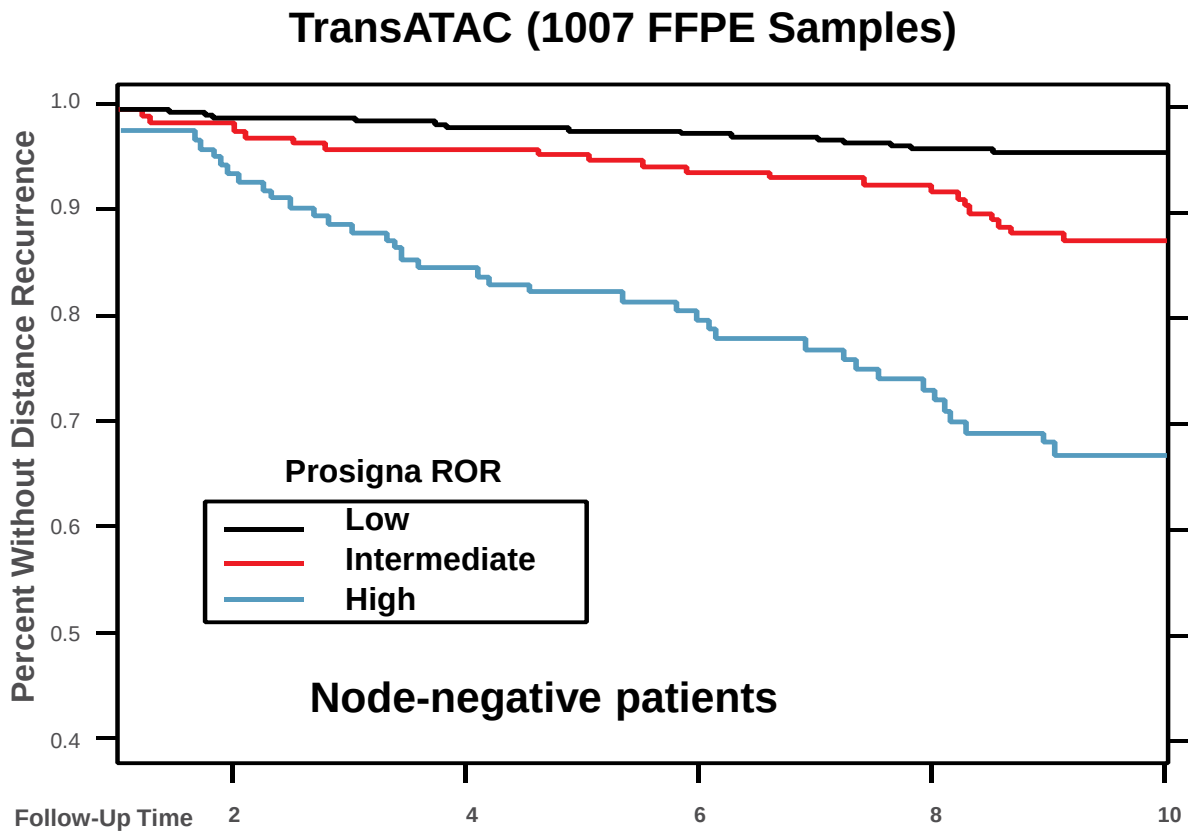
**Wallden et al., BMC Medical Genomics, 2015 (PMID:26297356)**

# Clinical Utility Validation of Prosigna: Data for Registry/Retrospective Studies

| TransATAC Study | ABCSG-8 Study |
| --- | --- |
| • N = 1,007 patients<br>• Published in Dowsett et al., JCO, 2013 (PMID:23816962) | • N = 1,478 patients<br>• Published in Gnant et al., Annals of Oncology, 2014 (PMID:24347518) |

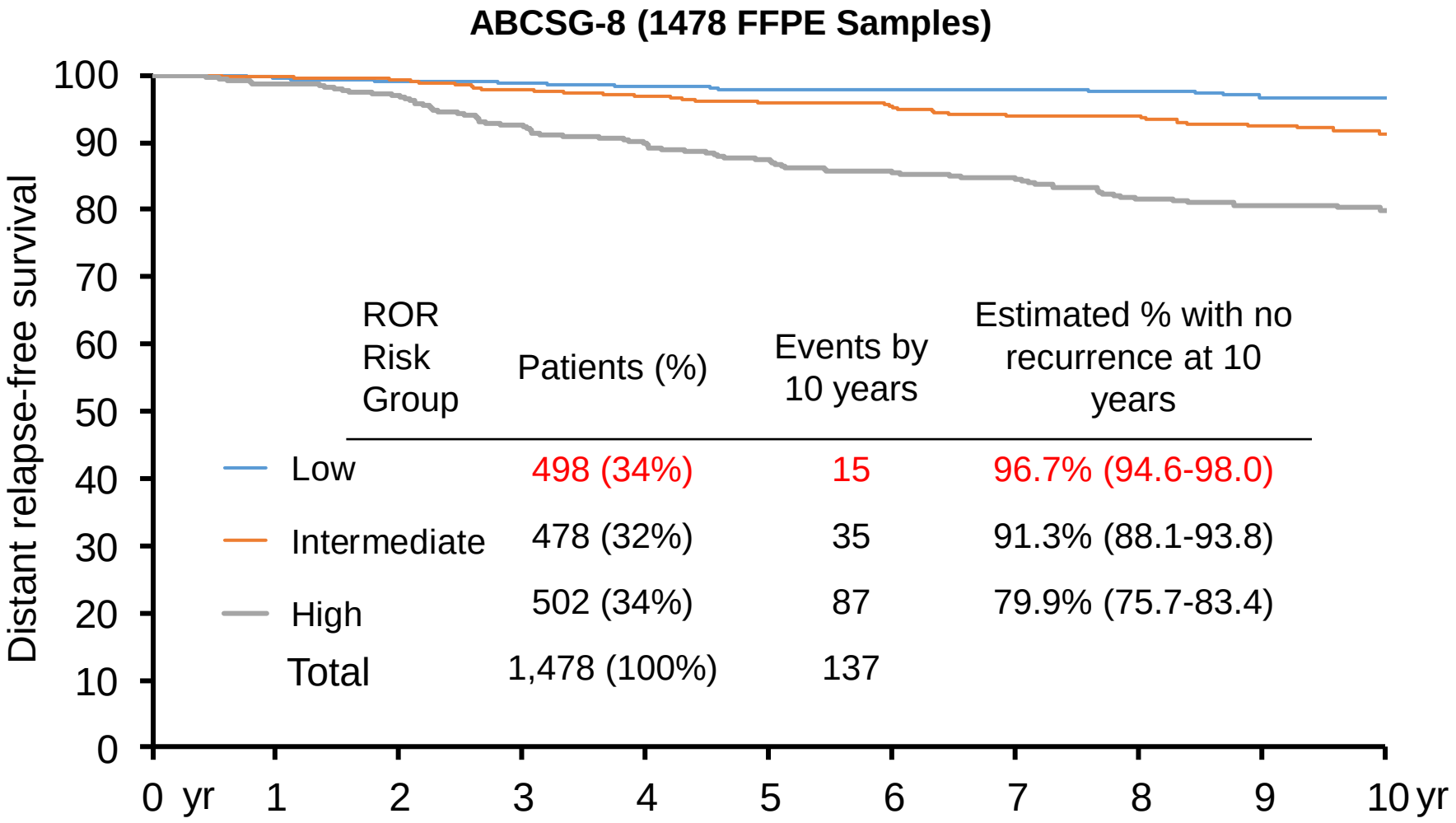
- **Postmenopausal women with Hormone Receptor-positive (ER and/or PR+) breast cancers treated with 5 years of endocrine therapy only; ≥10-yr median f/u**
- **Primary Objective: Validate that Prosigna ROR Score (ROR-PT) is prognostic beyond standard clinico-pathological variables, and can identify a group with >90% 10 yr Distant Recurrence Free Survival**

# Comparison of PAM50 Risk of Recurrence Score with OncotypeDX and IHC4 for Predicting Risk of Distant Recurrence after Endocrine Therapy
## Dowsett et al., JCO, 2013 (PMID:23816962)

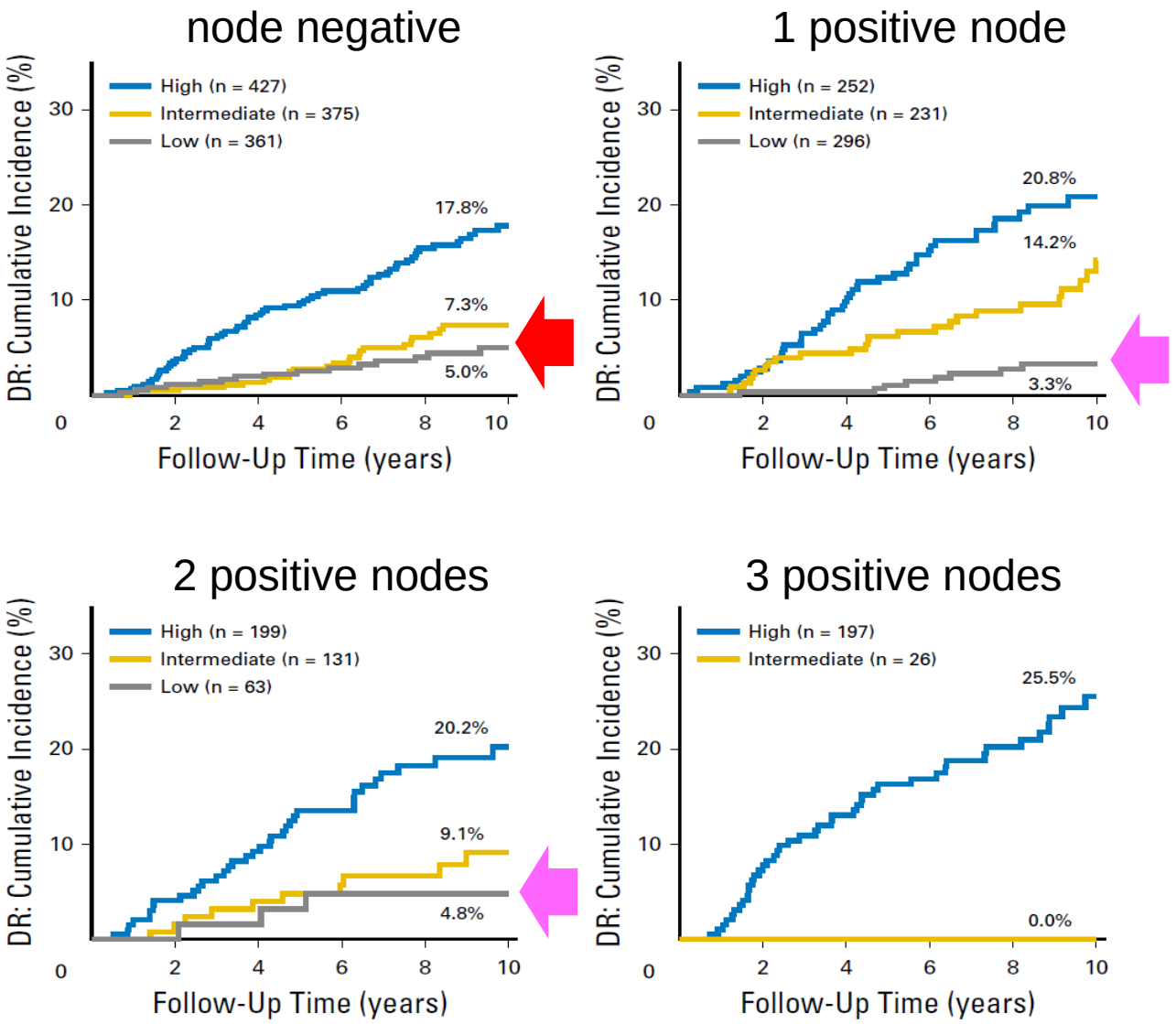



| Risk Group | N (%) | Events | % without recurrence at 10 yr |
|---|---|---|---|
| Low | 431 (58%) | 17 | 96% [94% - 98%] |
| Intermediate | 180 (24%) | 22 | 86% [81% - 92%] |
| High | 128 (17%) | 38 | 67% [59% - 76%] |
| Total | 739 (100%) | 77 | |

**Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy. Gnant et al., Annals of Oncology, 2014 (PMID:24347518)**



# PAM50 Risk of Recurrence Score Predicts 10-Year Distant Recurrence in a Comprehensive Danish Cohort of Postmenopausal Women Allocated to 5 Years of Endocrine Therapy for Hormone Receptor-Positive Early Breast Cancer. Lænkholm et al., JCO 2018 (PMID: 29369732)



**Prediction of late distant recurrence after 5 years of endocrine treatment: a combined analysis of patients from the Austrian breast and colorectal cancer study group 8 and anastrozole, tamoxifen alone or in combination randomized trials using the PAM50 risk of recurrence score.**
**Sestak et al., JCO 2014 (PMID: 25332252)**

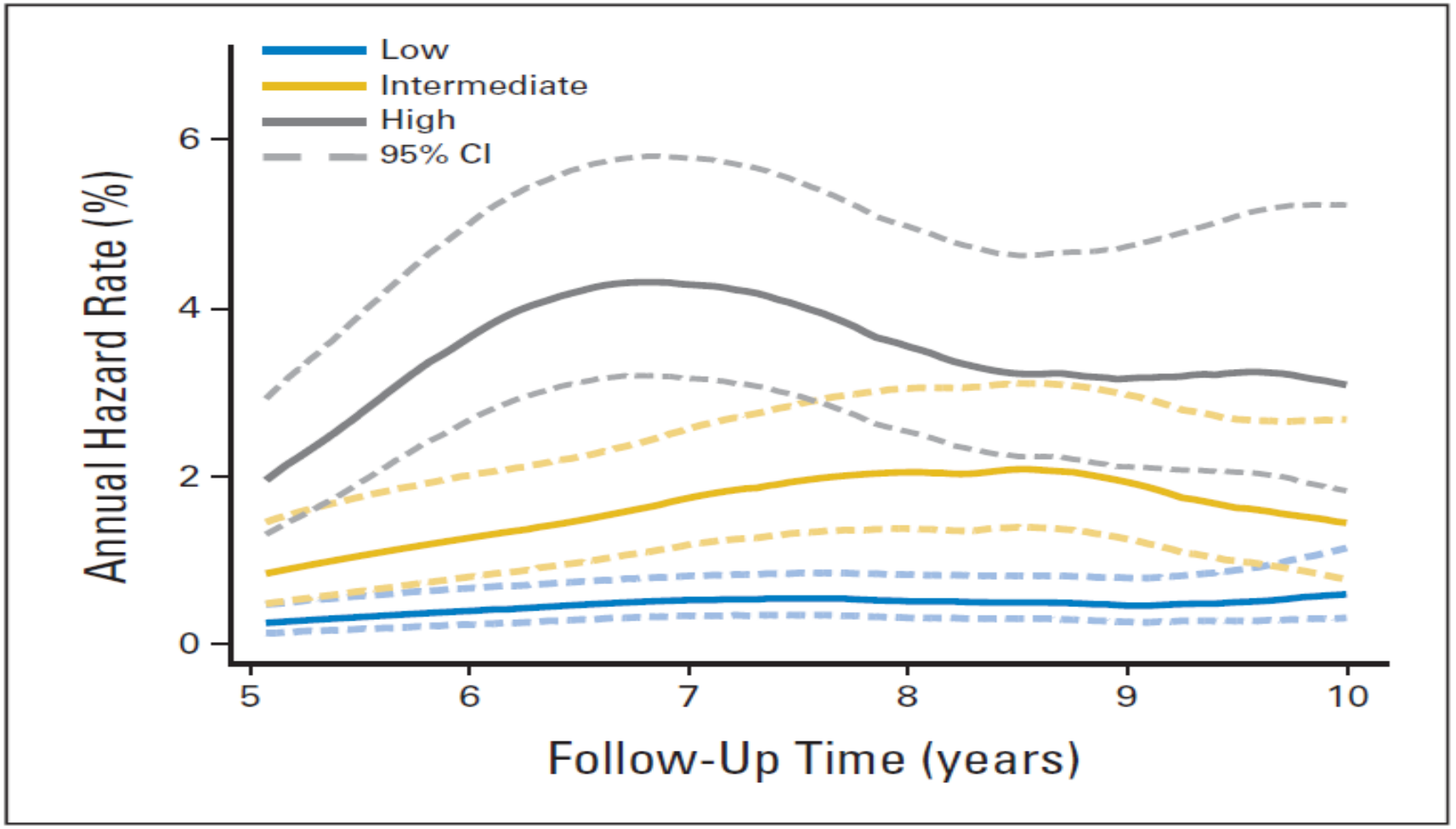



**Fig 2.** Annual hazard rate curve for all patients according to risk of recurrence groups.

# INTERNATIONAL GUIDELINES INCLUDE PROSIGNA®

## AGO Guidelines

AGO Recommendations for Diagnosis and Treatment of Patients with Primary and Metastatic Breast Cancer 2016.
Available from: http://wwwago-onlinede/en/guidelines-mamma/march-2015/ (download January 2016).

- Recognized with level 1B evidence; similar recommendation ("AGO +") as for other gene expression assays
- First guideline to acknowledge **"Low absolute risk implies low absolute benefit"**

## SEOM Guidelines Garcia-Saenz JA, et al. Clin Transl Oncol 2015 Oct 26.

- Recognized with IB evidence for the prediction of the risk of recurrence at 10 years

## St. Gallen Guidelines 2015 Coates AS, et al. Ann Oncol 2015 May 4.

- Highest support among all tests for prognosis in years 1-5
- Greatest level of support among all tests for late recurrence (years 5-10)
- Subtypes are recognized as predictive

## NCCN Guidelines www.nccn.org

- NCCN added Prosigna in v1.2016
- Prosigna® has been clinically validated for prediction of prognosis in discussion
- Other prognostic multigene assays may be considered to help assess risk of recurrence but have not been validated to predict response to chemotherapy – 2A Evidence rating (comparable to other assays)

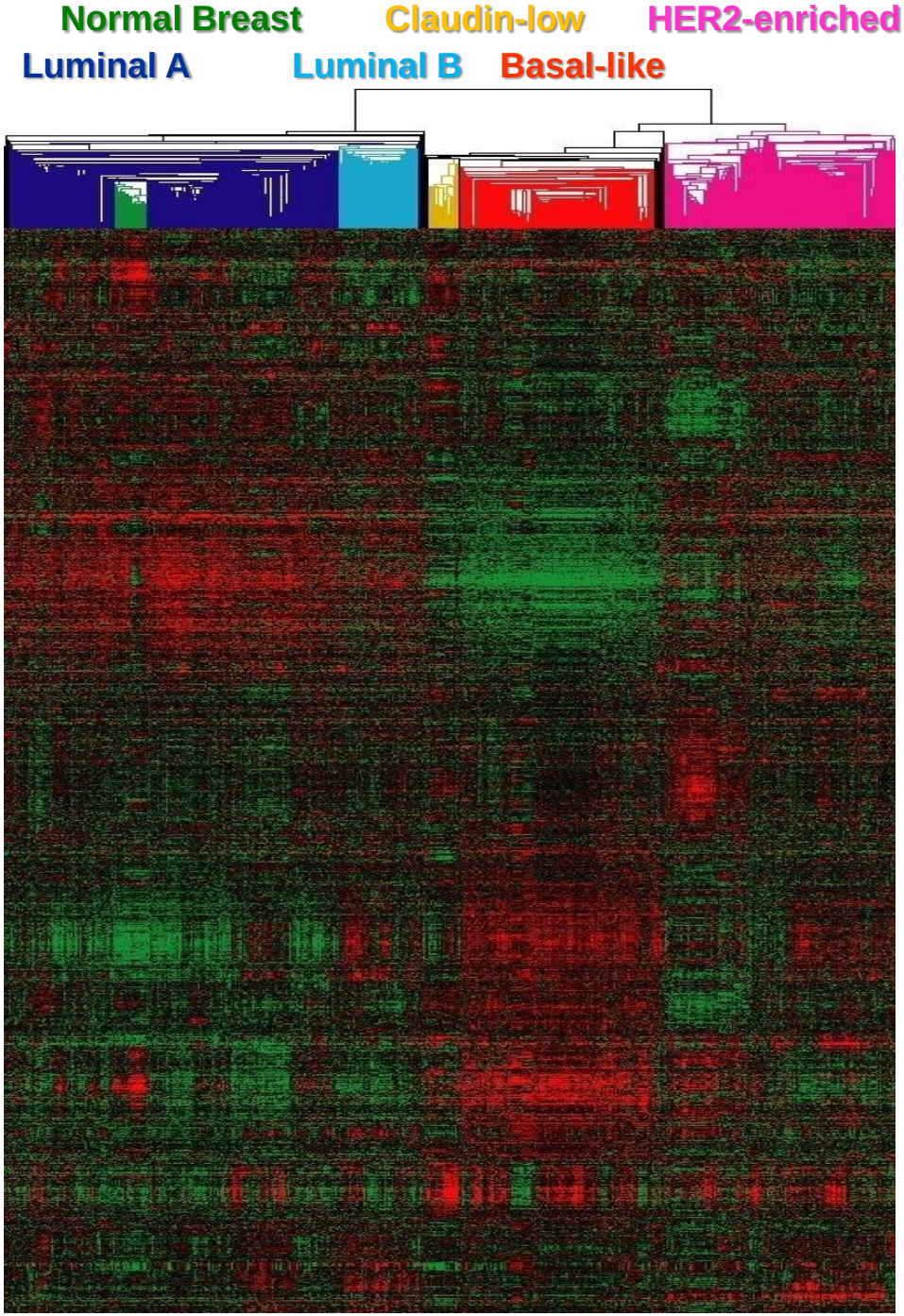
## ESMO Guidelines Senkus E, et al. Ann Oncol 2015 September 1, 2015;26(suppl 5):v8-v30.

- Recognized with level IB evidence, at parity with other established gene expression assays
- Recommended for use to predict the benefit of chemotherapy

## ASCO 2015 Guidelines Harris LN, et al. J Clin Oncol 2016 Feb 8.

- Recommends clinician may use the PAM50/Prosigna in conjunction with other clinicopathologic variables, to guide decisions on adjuvant systemic therapy for women with Node neg. ESBC.
- Evidence quality: high; Strength of recommendation: strong

Slide courtesy Prof. Nadia Harbeck

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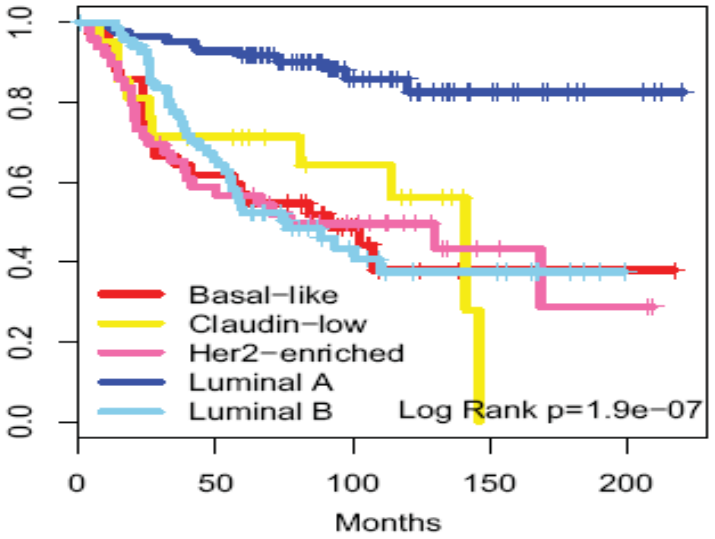

# Neoadjuvant pathological complete response (pCR) to anthracycline/taxane-based (TFAC) regimen according to PAM50 subtype, and clinical markers (n=441[1], no trastuzumab)

Usary et al., Clinical Cancer Research 2013 (PMID:23780888)

| | Classification | pCR rate | # of patients | MVA[2] OR (95% C.I.) | p-value |
|---|---|---|---|---|---|
| | All Patients | 84 (19%) | 441 | | |
| PAM50 subtype | Luminal A | 4 (3%) | 136 | 1 | - |
| | Luminal B | 9 (14%) | 63 | 4.13(1.22-16.4) | 0.028 |
| | Normal-like | 4 (15%) | 27 | 4.41(0.927-21) | 0.055 |
| | HER2-Enriched | 4 (17%) | 23 | 4.54(0.927-22.2) | 0.055 |
| | Basal-like | 48 (38%) | 125 | 10.5(3.27-41.4) | <0.001 |
| | Claudin-low | 15 (22%) | 67 | 5.84(1.69-23.8) | 0.008 |
| ER | ER+ | 27 (10%) | 266 | 1 | - |
| | ER- | 57 (33%) | 175 | 1.27(0.596-2.74) | 0.543 |
| PR | PR- | 62 (27%) | 227 | 1 | - |
| | PR+ | 22 (10%) | 214 | 1.07(0.507-2.3) | 0.854 |

[1]Microarray and Clinical Neoadjuvant Data taken from Hatzis et al., JAMA, 2011

[2]MVA model also included grade, clinical T status, and nodal status measured at baseline

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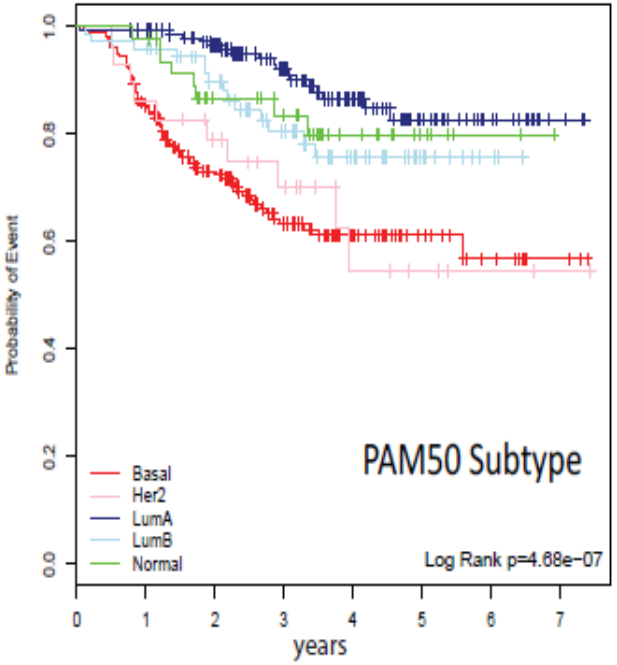
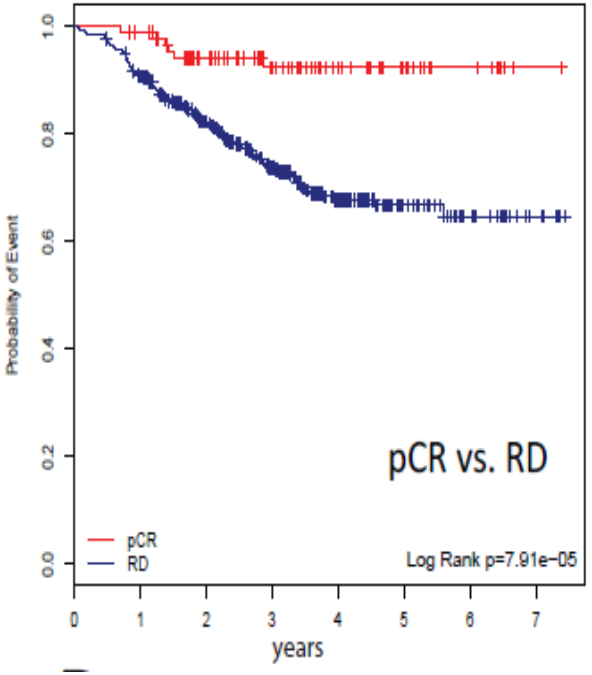
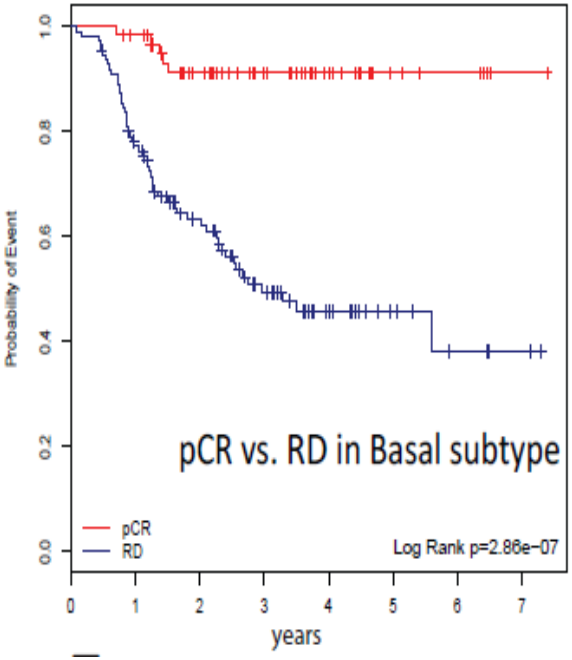


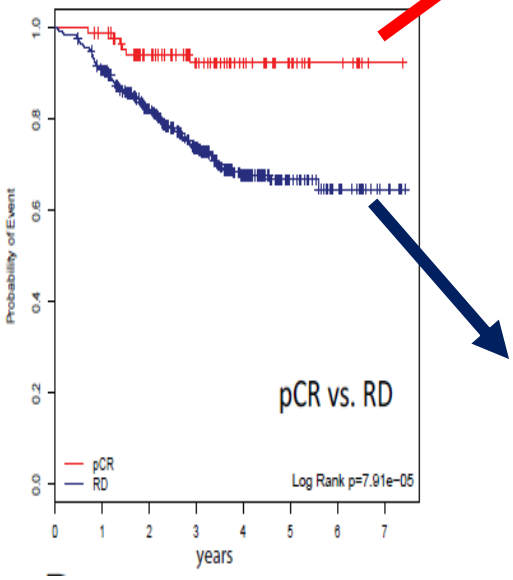

[1]Microarray and Clinical Neoadjuvant Data taken from Hatzis et al., JAMA, 2011

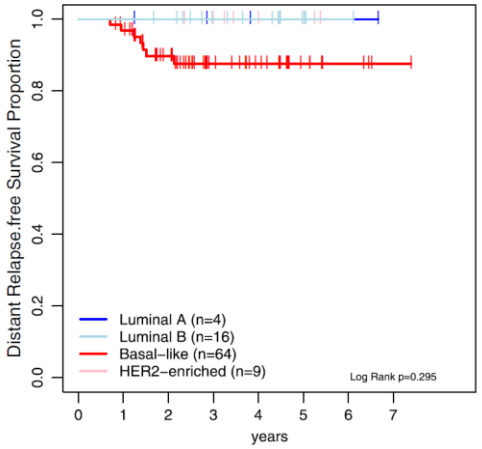
# Response and survival of breast cancer intrinsic subtypes following multi-agent neoadjuvant chemotherapy, Prat et al., BMC Medicine, 2015 (PMID:26684470)

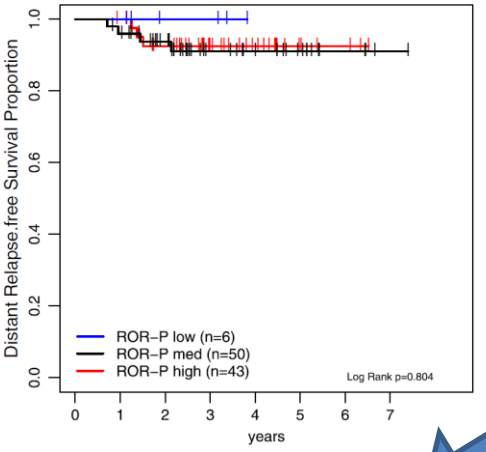
**Methods:** Gene expression and clinical-pathological data were evaluated in a combined dataset of 957 breast cancer patients, including 350 with TNBC, treated with sequential anthracycline and anti-microtubule-based neoadjuvant regimens. Intrinsic subtype, risk of relapse score based on subtype and proliferation (ROR-P), the Claudin-low subtype and the 7-TNBCtype subtype classification were evaluated. Logistic regression models for pathological complete response (pCR) and Cox models for distant relapse-free survival (DRFS) were used.

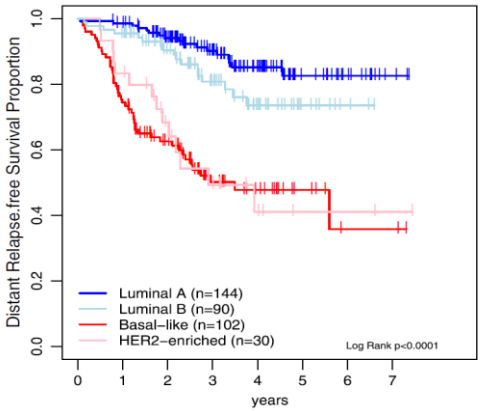
PAM50 subtype

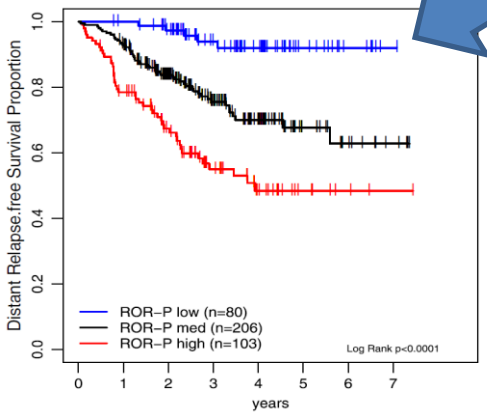
PAM50 ROR-PT Score



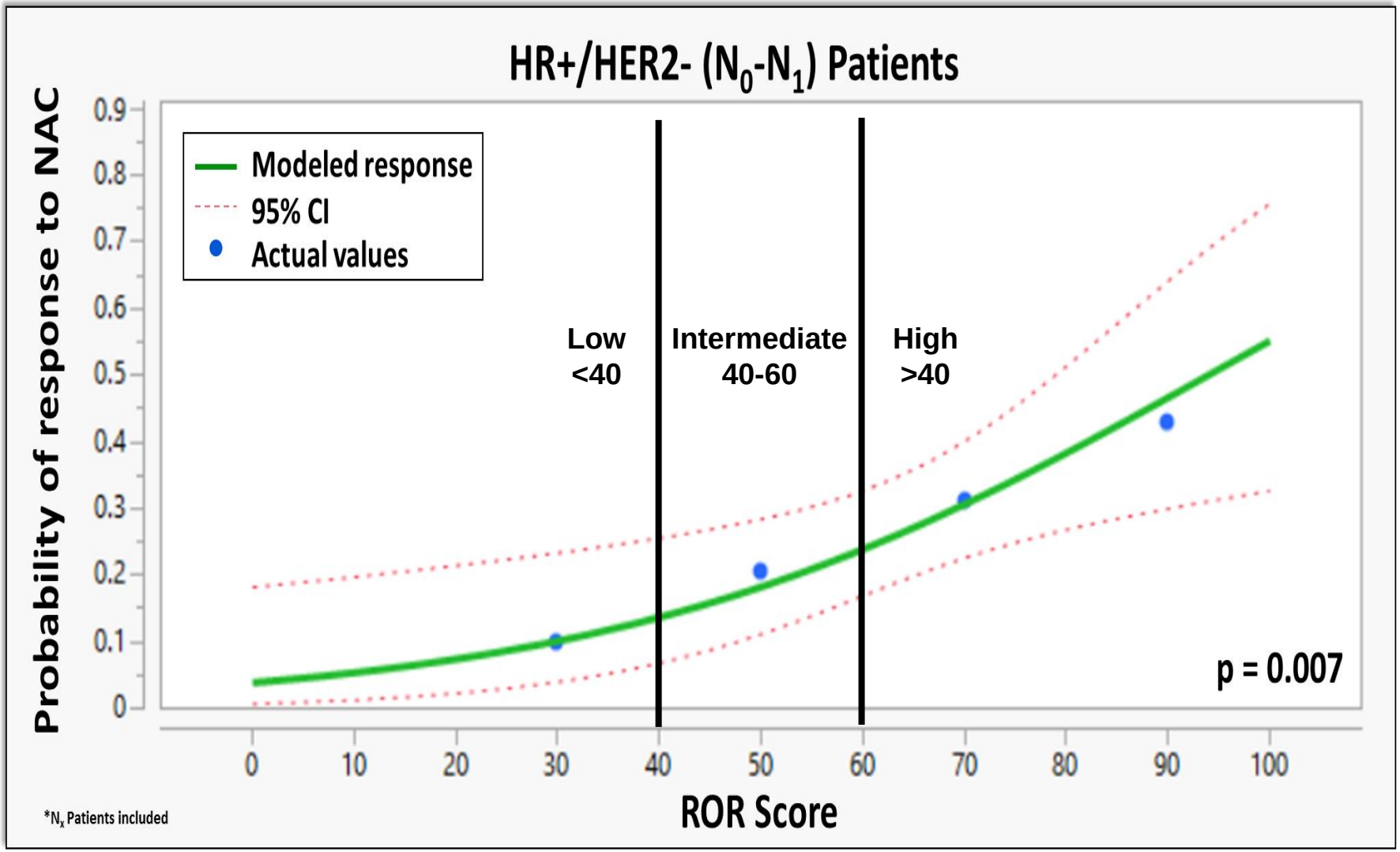



# Prediction of Response to NeoAdjuvant Chemotherapy (NAC) Using Core Needle Biopsy Samples with the Prosigna Assay, Prat et al., Clinical Cancer Research 2015. (PMID:26152740)

| | Categorical Odds Ratio for Response (RCB0,1 vs 2,3) to NAC | | | |
|---|---|---|---|---|
| | HR+/HER2- ($N_0$-$N_1$) Responders | | HR+/HER2- ($N_0$-$N_3$) Responders | |
| Intrinsic Subtype* | N | % | N | % |
| Luminal A | 43 | 11.6 | 54 | 9.3 |
| Luminal B | 69 | 30.4 | 105 | 20.0 |
| HER2 Enriched | 4 | 25.0 | 7 | 14.3 |
| Basal | 12 | 58.3 | 14 | 50.0 |
| Intrinsic Subtype* | Odds Ratio (95% CI) | P-value | Odds Ratio (95% CI) | P-value |
| LumA vs Non-LumA | 0.25 (0.08-0.67) | 0.009 | 0.34 (0.11-0.87) | 0.037 |
| Basal vs Non-Basal | 4.62 (1.37-16.73) | 0.014 | 5.15 (1.64-16.23) | 0.004 |

# Prediction of Response to Neoadjuvant Chemotherapy (NAC) Using Core Needle Biopsy Samples with the Prosigna Assay, Prat et al., Clinical Cancer Research 2015. (PMID:26152740)



# Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women with Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guidelines. Harris et al., JCO 2016 (PMID: 26858339)

## Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline

*Lyndsay N. Harris, Nofisat Ismaila, Lisa M. McShane, Fabrice Andre, Deborah E. Collyar, Ana M. Gonzalez-Angulo, Elizabeth H. Hammond, Nicole M. Kuderer, Minetta C. Liu, Robert G. Mennel, Cathy van Poznak, Robert C. Bast, and Daniel F. Hayes*

Author affiliations appear at the end of this article.

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Clinical Practice Guideline Committee approval: September 21, 2015.

Editor's note: This American Society of Clinical Oncology clinical practice guideline provides recommendations based on the comprehensive review and analyses of the relevant literature for each recommendation. Additional information, which may include a data supplement with additional evidence tables, a methodology supplement, slide sets, clinical tools and resources, and links to patient information at www.cancer.net, is available at: www.asco.org/guidelines and www.asco.org/guidelineswiki.

Authors' disclosures of potential conflicts of interest are found in the article online at www.jco.org. Author contributions are found at the end of this article.

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0732-183X/16/3499-1/$20.00

DOI: 10.1200/JCO.2015.65.2289

A B S T R A C T

**Purpose**
To provide recommendations on appropriate use of breast tumor biomarker assay results to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer.

**Methods**
A literature search and prospectively defined study selection sought systematic reviews, meta-analyses, randomized controlled trials, prospective-retrospective studies, and prospective comparative observational studies published from 2006 through 2014. Outcomes of interest included overall survival and disease-free or recurrence-free survival. Expert panel members used informal consensus to develop evidence-based guideline recommendations.

**Results**
The literature search identified 50 relevant studies. One randomized clinical trial and 18 prospective-retrospective studies were found to have evaluated the clinical utility, as defined by the guideline, of specific biomarkers for guiding decisions on the need for adjuvant systemic therapy. No studies that met guideline criteria for clinical utility were found to guide choice of specific treatments or regimens.

**Recommendations**
In addition to estrogen and progesterone receptors and human epidermal growth factor receptor 2, the panel found sufficient evidence of clinical utility for the biomarker assays Oncotype DX, EndoPredict, PAM50, Breast Cancer Index, and urokinase plasminogen activator and plasminogen activator inhibitor type 1 in specific subgroups of breast cancer. No biomarker except for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 was found to guide choices of specific treatment regimens. Treatment decisions should also consider disease stage, comorbidities, and patient preferences.

*J Clin Oncol 34. © 2016 by American Society of Clinical Oncology*

### INTRODUCTION

This guideline provides evidence-based recommendations to practicing oncologists and other stakeholders on the appropriate use of breast tumor biomarker assay results to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer with known hormone receptor (estrogen and progesterone receptors [ER/PgRs]) and human epidermal growth factor receptor 2 [HER2]) status. In an era of great interest in personalized, precision medicine, the role of tumor biomarker assays in guiding clinical care has taken on even greater importance than in the past.

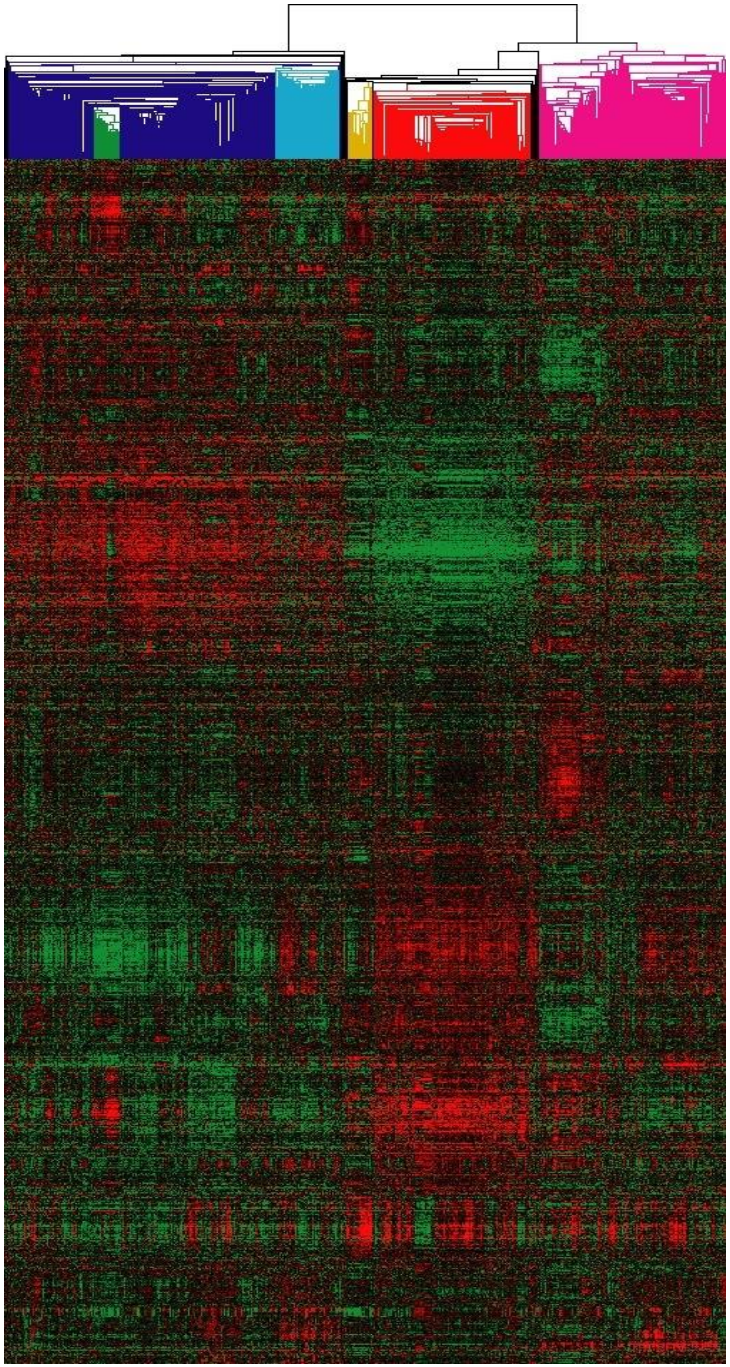
A prior update of the ASCO guideline on the use of tumor markers in breast cancer[1] considered all indications or uses for biomarker assay results, which include screening, diagnosis, prognosis, and monitoring for recurrence or progression. Subsequently, ASCO collaborated with the College of American Pathologists (CAP) to publish and update guidelines on testing for HER2[2,3] and ER/PgR.[4] Thus, to facilitate future updates in a rapidly developing field, the ASCO Breast Cancer

**Table 1.** Requirements for a Marker-Based Test to Reach Level IB Evidence of Clinical Utility on the Basis of Prospective-Retrospective Studies

| Requirements |
|---|
| 1. Adequate amounts of archived specimen must be available from enough patients from a prospective trial (which for predictive factors should generally be a randomized design) for analyses to have adequate statistical power and for the patients included in the evaluation to be clearly representative of the patients in the trial. |
| 2. The marker-based test should be analytically and preanalytically validated for use with archived specimens. |
| 3. The plan for marker evaluation should be completely specified in writing before the performance of marker assays on archived specimens and should be focused on the evaluation of a completely defined marker-based test. |
| 4. The results from archived specimens should be validated by using specimens from one or more similar, but separate studies. |

NOTE. Adapted from Simon et al.[9]

**"If a patient has ER/PgR-positive, HER2-negative (node-negative) …… Chemotherapy should be considered for patients in the PAM50 high-risk group, but is not indicated for patients in the low-risk group. Future studies are needed to inform recommendations about adjuvant chemotherapy in patients with an intermediate PAM50-ROR."**



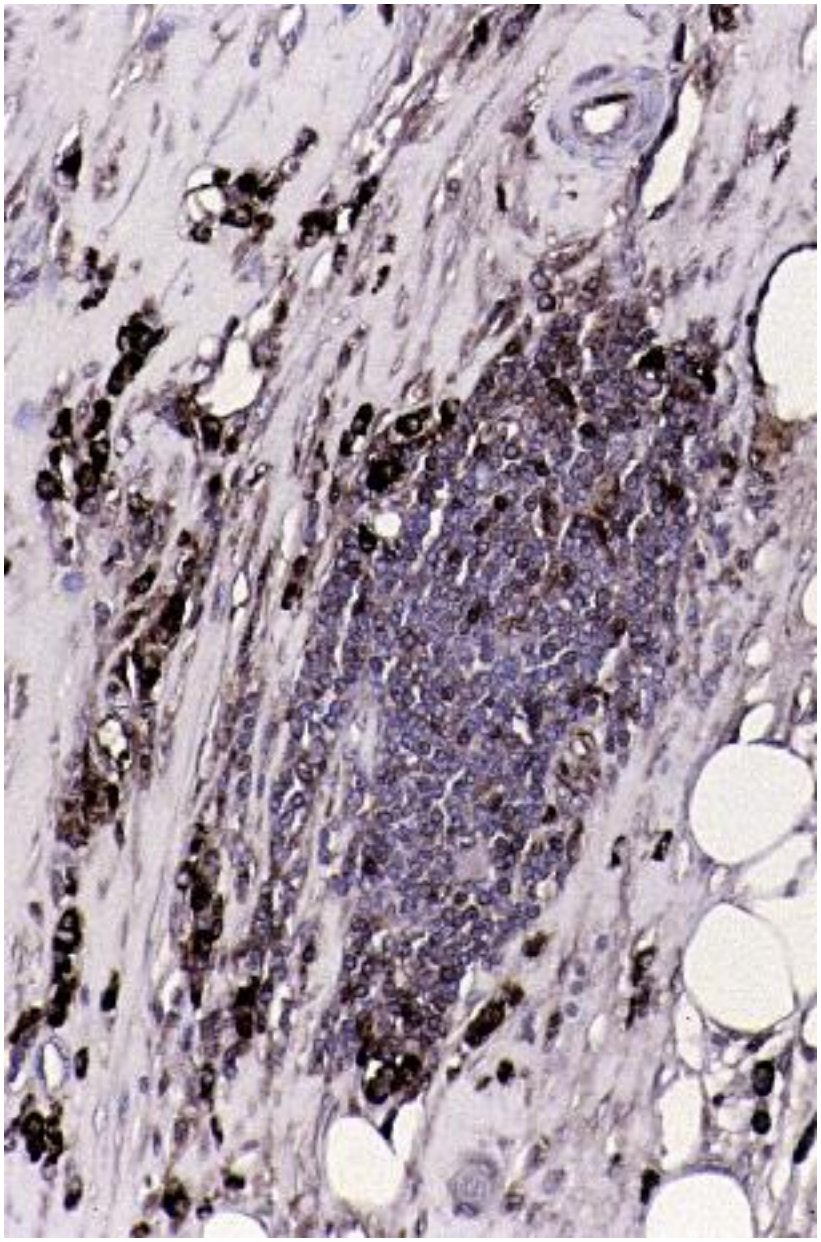
# ER+/HER2- Patient Gene Expression Test Summary

1. Several gene expression tests (Prosigna, EndoPredict, Mammaprint, OncotypeDX) show Level 1 evidence in ER+/HER2- patients for prognosis on endocrine therapy

2. The Low Risk patients by Prosigna and EndoPredict (but not OncotypeDX) may not need extended adjuvant endocrine therapy beyond 5 years due to their low risk of relapse between years 5-10 when given 5 years of endocrine therapy alone

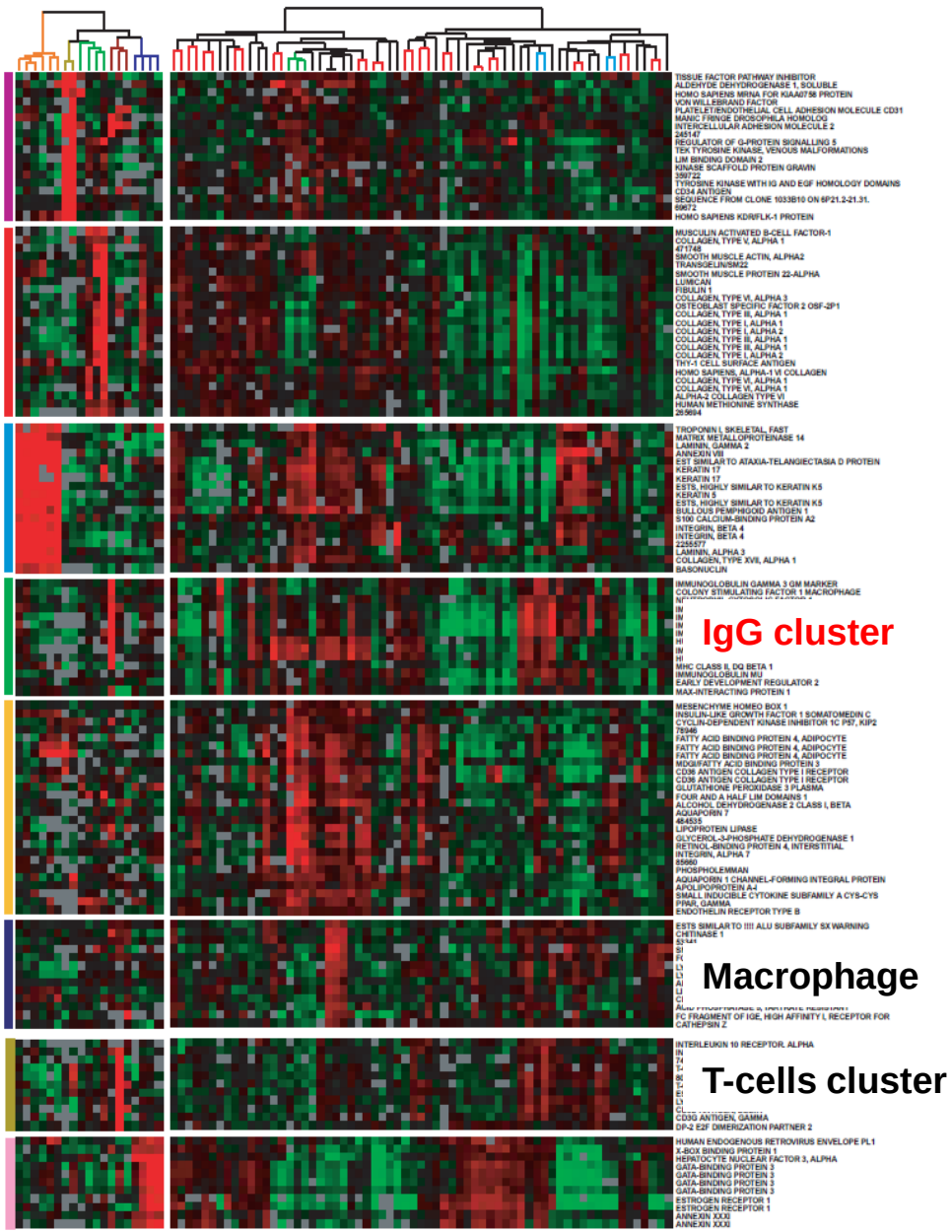
3. The Low Risk patients by these assays likely do not need adjuvant chemotherapy due to their low risk of distant relapse

4. The High Risk patients by these assays may benefit from adjuvant chemotherapy, however, some guidelines do not advocate the use of expression tests for this purpose.

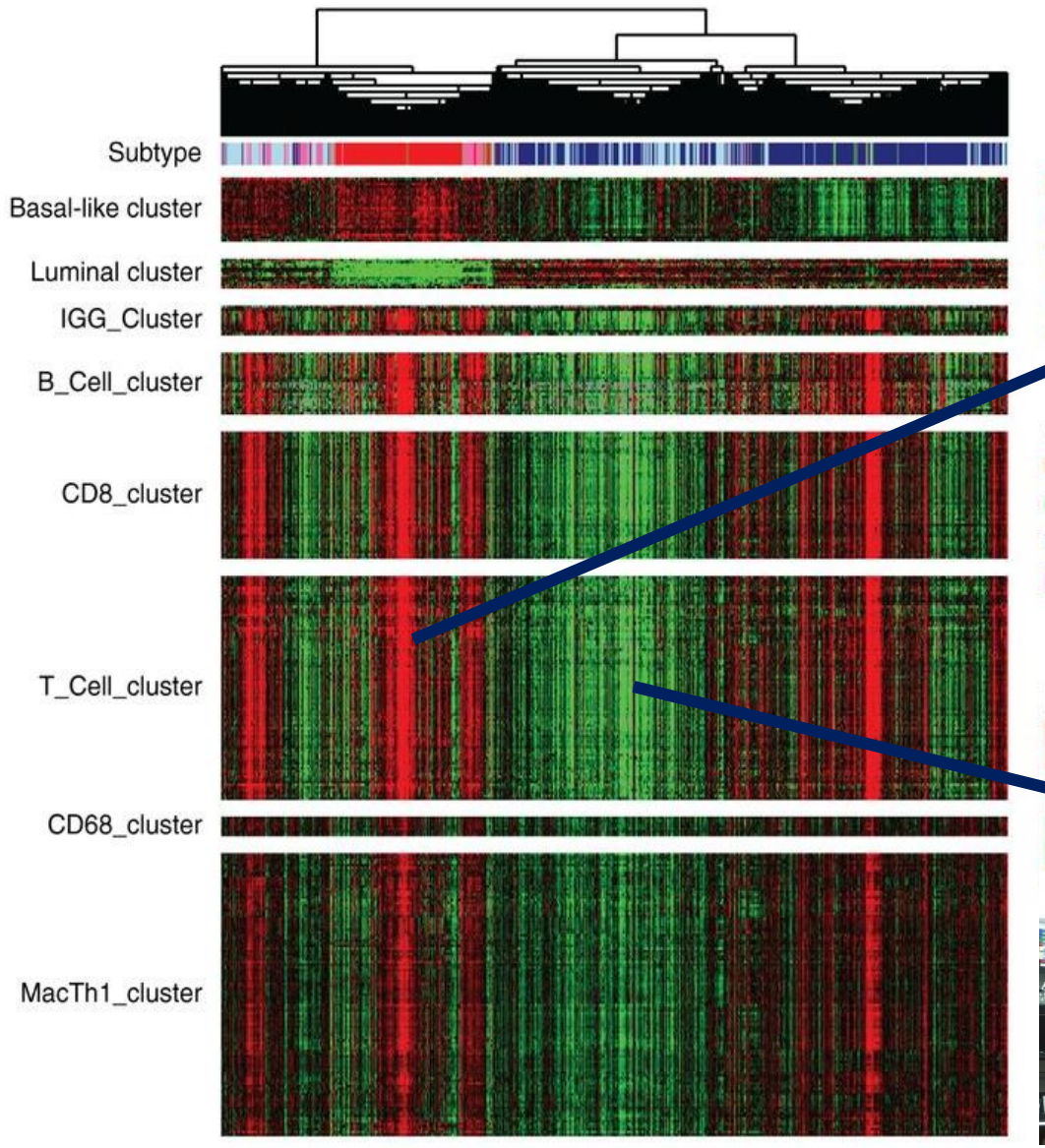


Breast tumor stained with antibodies against IgG lambda light chain

Perou et al., Nature, 2000, PMID:10963602



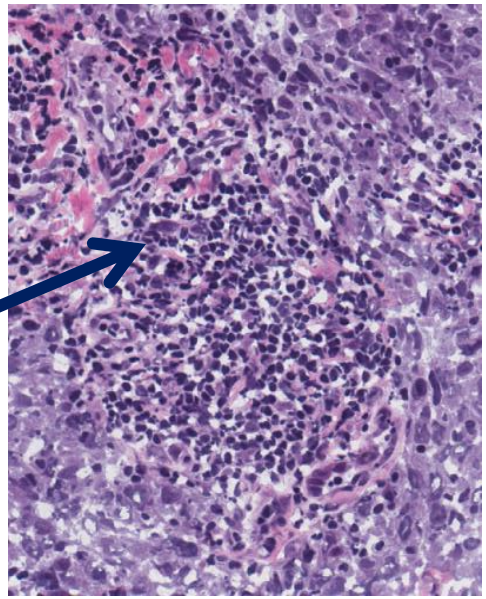
**Prognostic B-cell signatures using mRNA-seq in patients with subtype-specific Breast and Ovarian Cancer. Iglesia et al., Clinical Cancer Research 2014. PMID:24916698**

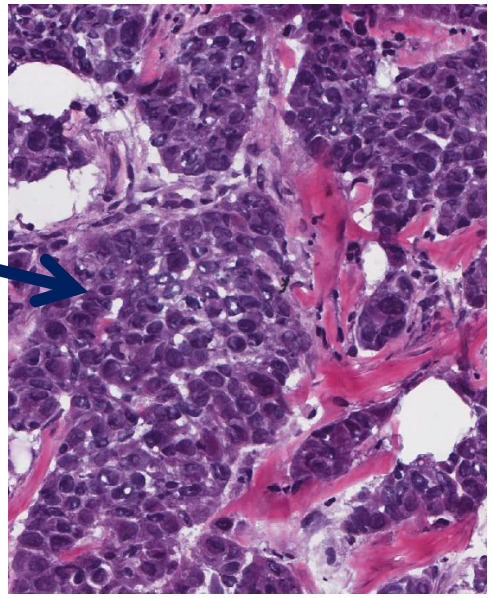



**Breast subtype**
- Basal-like
- HER2-enriched
- Luminal A
- Luminal B
- Normal-like

**Ovarian subtype**
- Differentiated
- Immunoreactive
- Mesenchymal
- Proliferative

**Expression**
3.00
2.00
1.00
−1.00
−2.00

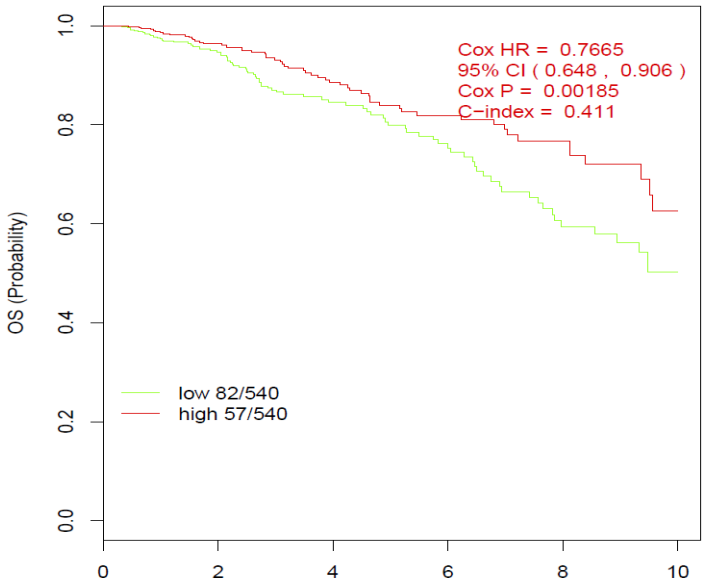




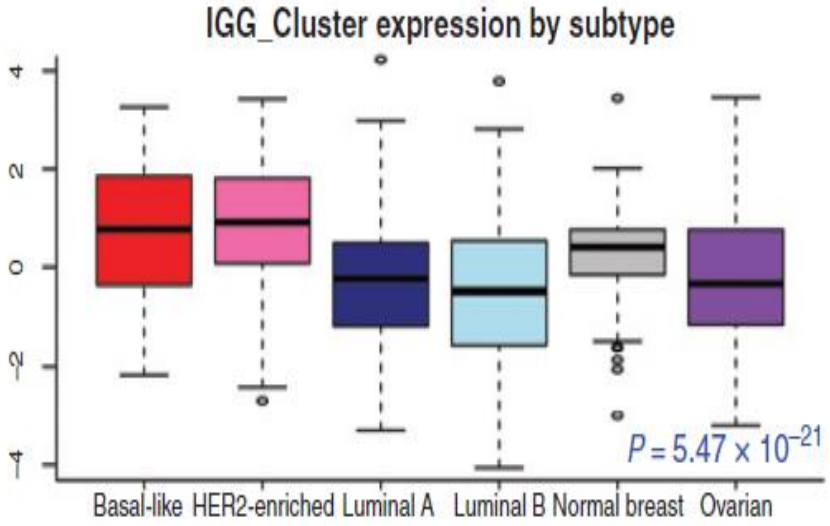
**Michael Iglesia**

**Prognostic B-cell signatures using mRNA-seq in patients with subtype-specific Breast and Ovarian Cancer. Iglesia et al., Clinical Cancer Research 2014. PMID:24916698**

| | | IGG_Cluster[a] | | TNBC_B-Cell[b] | | B_Cell[a] | | T_Cell_cluster[d] | | CD8_cluster[d] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | HR | *P* | HR | *P* | HR | *P* | HR | *P* | HR | *P* |
| Breast | | | | | | | | | | | |
| All breast | 855 | 0.847 | 6.61E−04 | 0.901 | 1.56E−03 | 0.585 | 1.52E−02 | 0.858 | 2.27E−02 | 0.865 | 1.81E−02 |
| Luminal A | 243 | | NS | | NS | | NS | | NS | | NS |
| Luminal B | 162 | | NS | | NS | | NS | | NS | | NS |
| HER2-enriched | 144 | 0.755 | 4.67E−03 | 0.827 | 1.12E−02 | 0.323 | 9.16E−03 | 0.687 | 5.03E−03 | 0.705 | 4.15E−03 |
| Basal-like | 140 | 0.599 | 1.20E−04 | 0.686 | 4.24E−05 | 0.17 | 2.39E−03 | 0.496 | 1.29E−04 | 0.548 | 2.35E−04 |
| Claudin-low | 90 | | NS | | NS | | NS | | NS | | NS |




IGG Cluster



# Monitoring immune checkpoint blockade: response evaluation and biomarker development
# Nishino et al., Nat Rev Clin Oncol. 2017 (PMID:28653677)

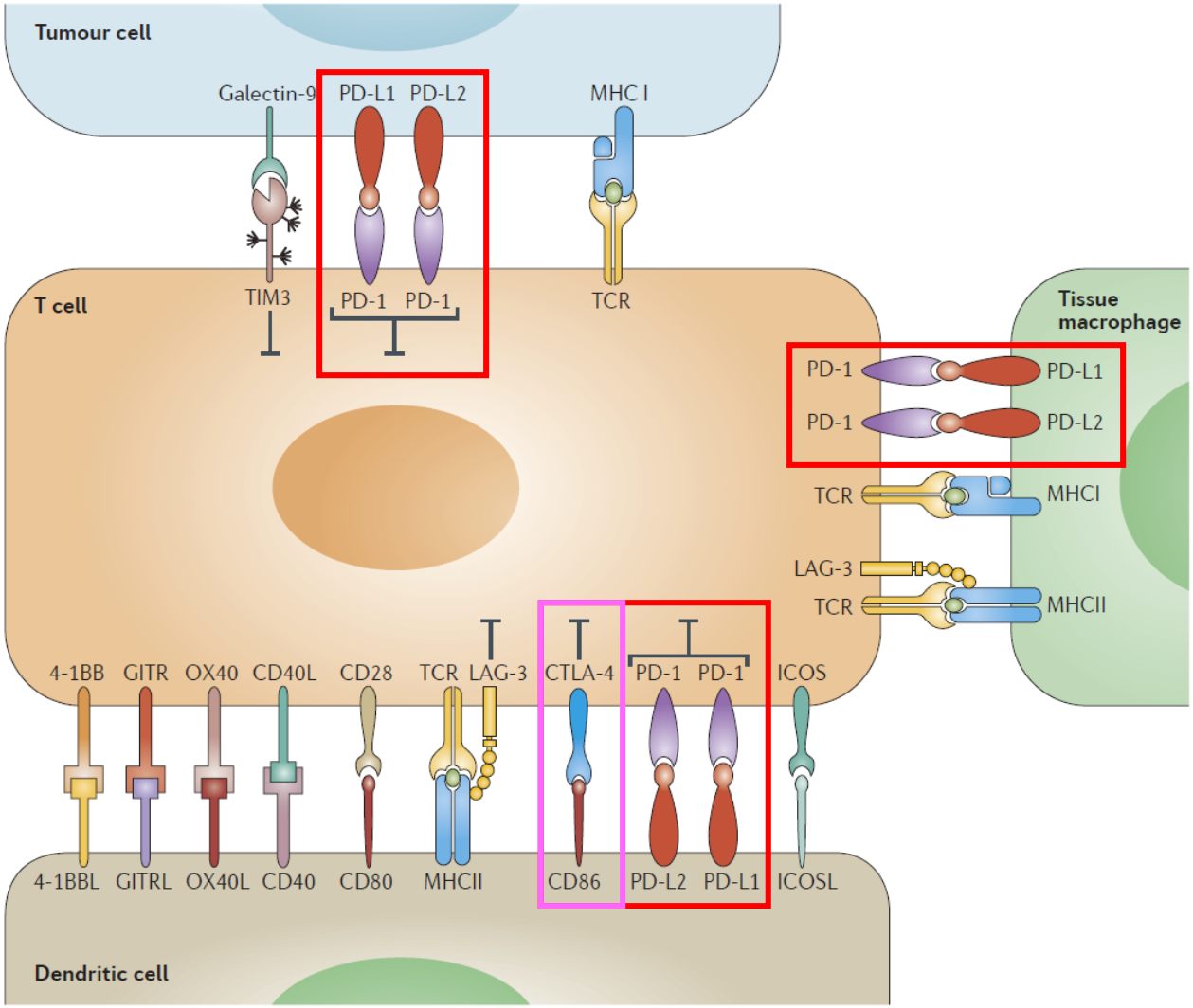
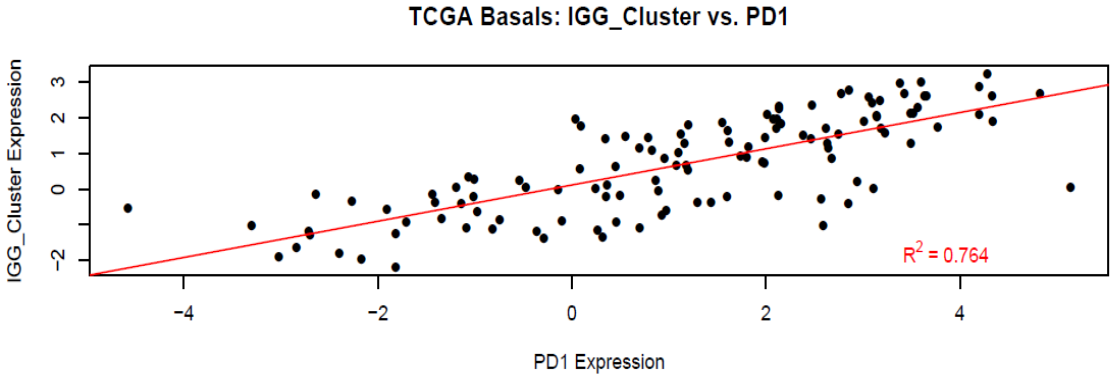
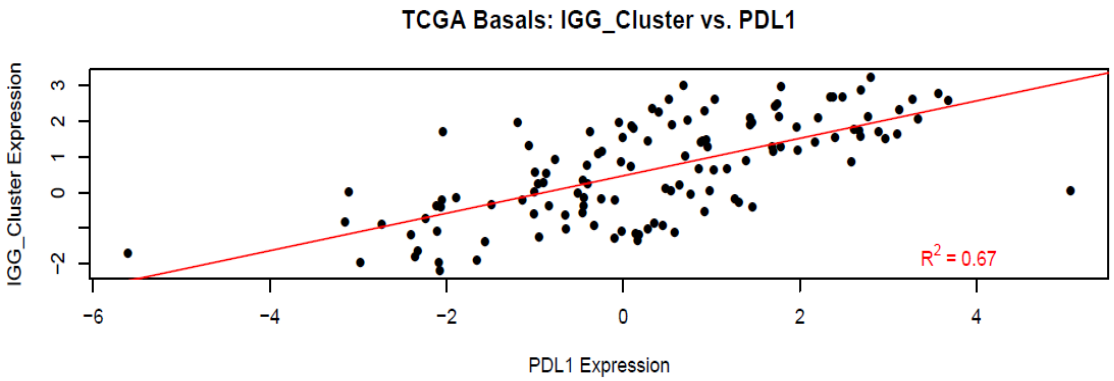
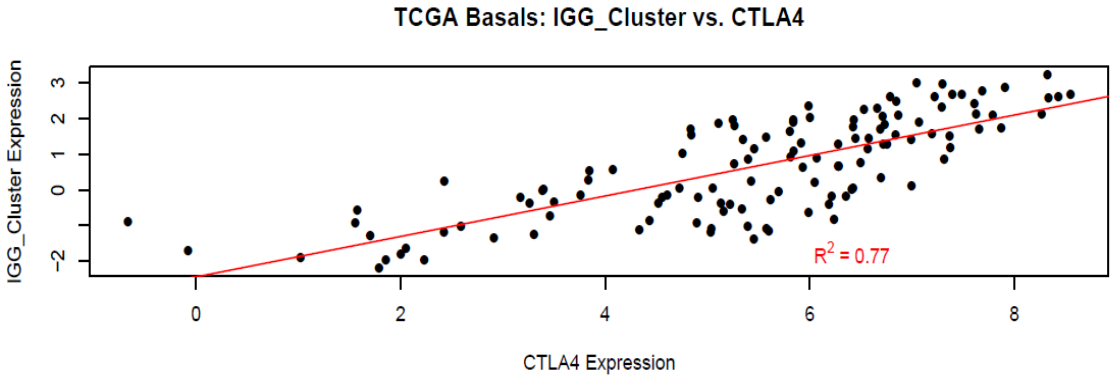


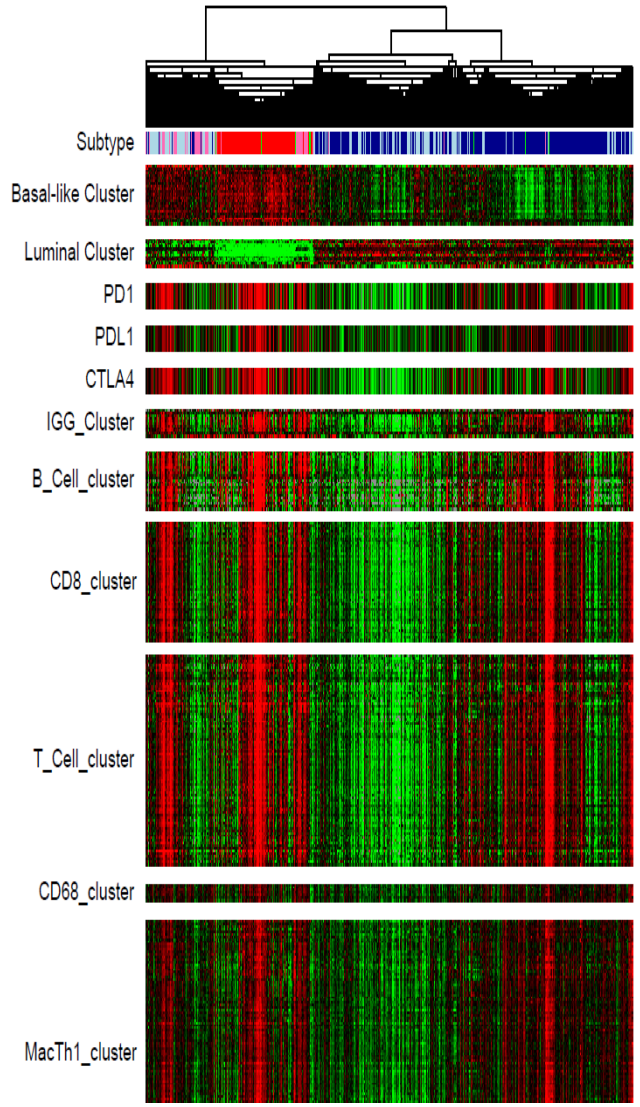

Figure 1 | **Ligand–receptor interactions between tumour cells and immune cells in the tumour microenvironment.** An overview of the immune-checkpoint molecules involved in the regulation of the antitumour immune response.

# Prognostic B-cell signatures using mRNA-seq in patients with subtype-specific Breast and Ovarian Cancer. Iglesia et al., Clinical Cancer Research 2014. PMID:24916698

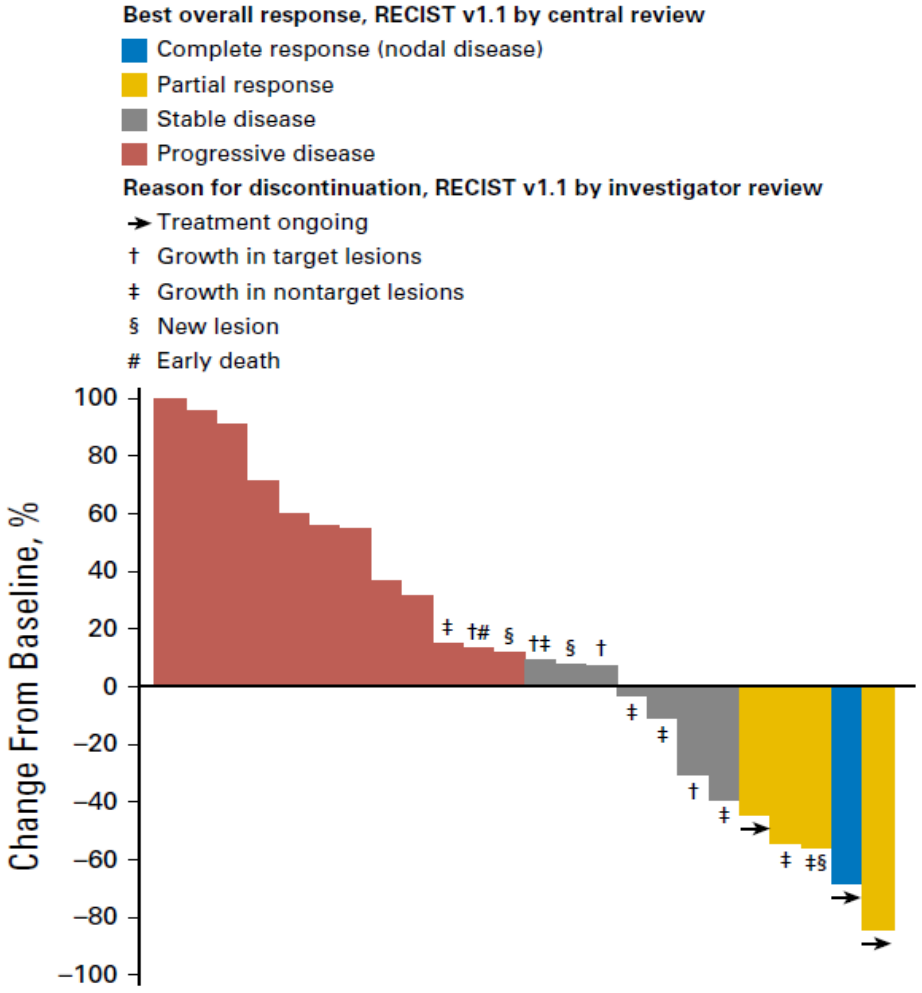


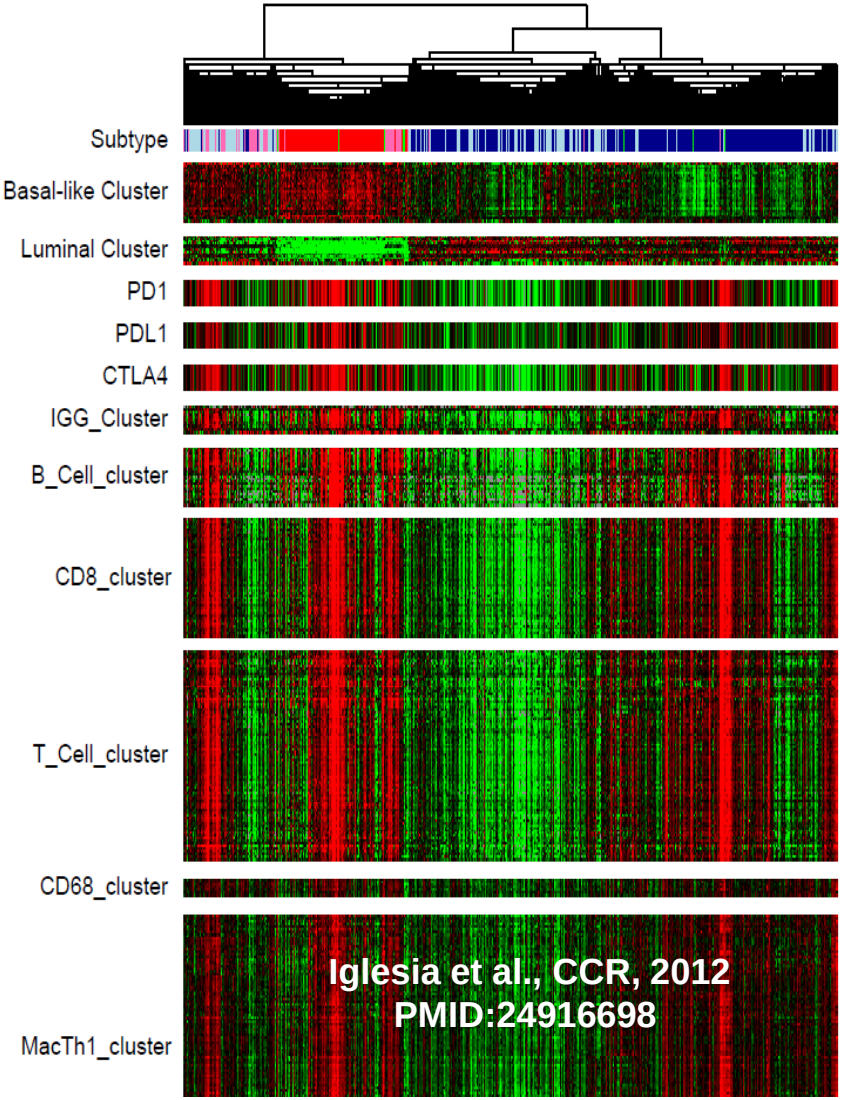



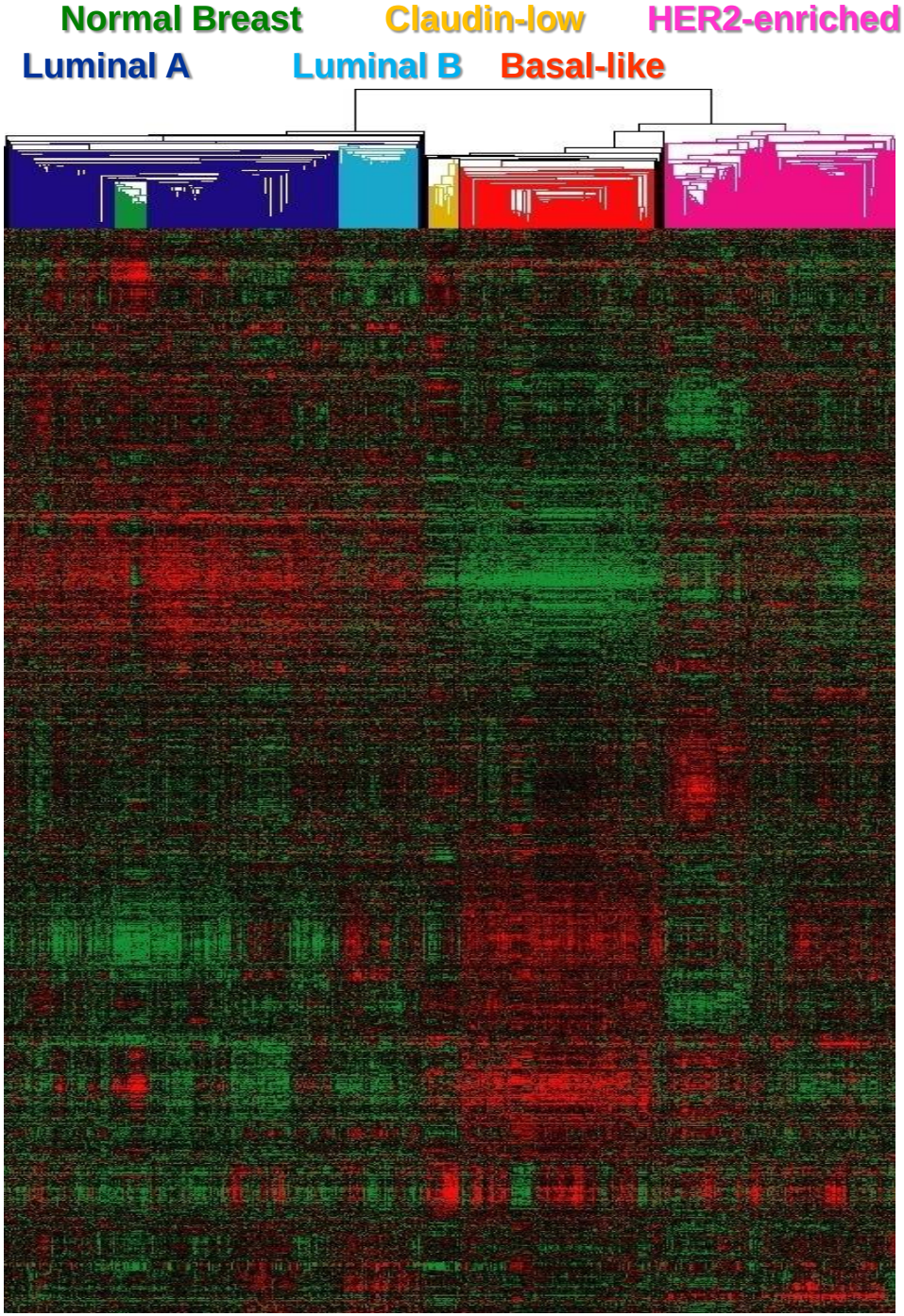



# Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study, Nanda et al., JCO 2016 (PMID:27138582)

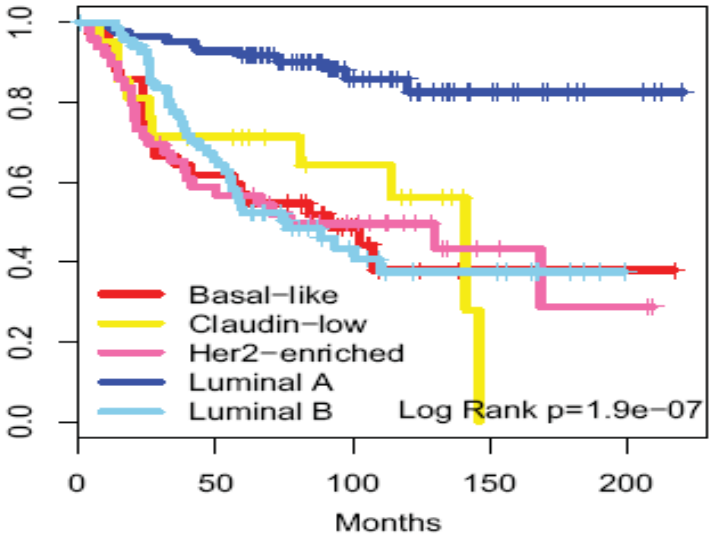





# Intrinsic Subtypes and the ROR Score provide valuable information for:

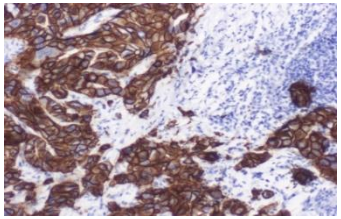
1. The biology of breast cancer
2. Baseline prognosis
3. Prognosis/prediction for endocrine therapy treated patients
4. Prediction of response to chemotherapy
5. Prediction of response to HER2-targeting



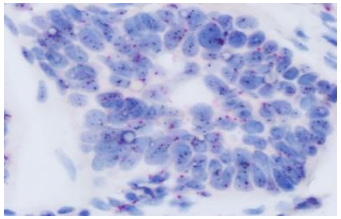
# HER2+ Clinical Subtype Background

- **HER2-positive (HER2+) breast cancer is clinically and biologically heterogeneous (~20% of all tumors).**
- **Based on gene expression, HER2+ breast cancer is composed of 4 intrinsic molecular subtypes (Luminal A, Luminal B, HER2-enriched [HER2-E] and Basal-like) and a Normal-like group)**
- **These intrinsic subtypes are not fully recapitulated by hormone receptor status.**

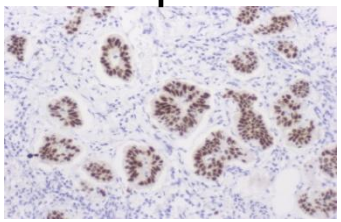
HER2 +3



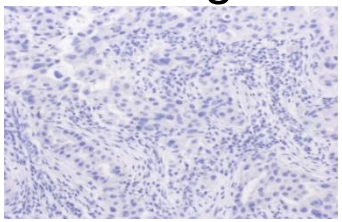
HER2 ISH+



ER-positive

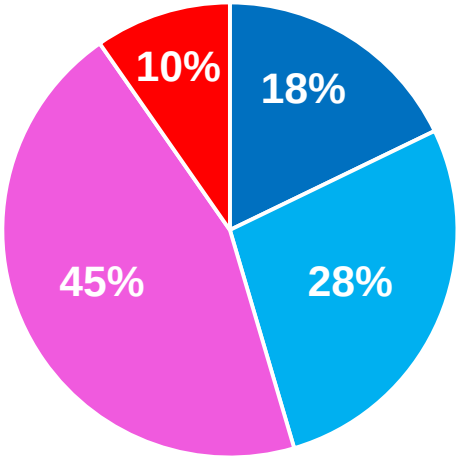


ER-negative



Courtesy of Dr. Pedro Fernández

N=1,217 HER2+ breast cancers

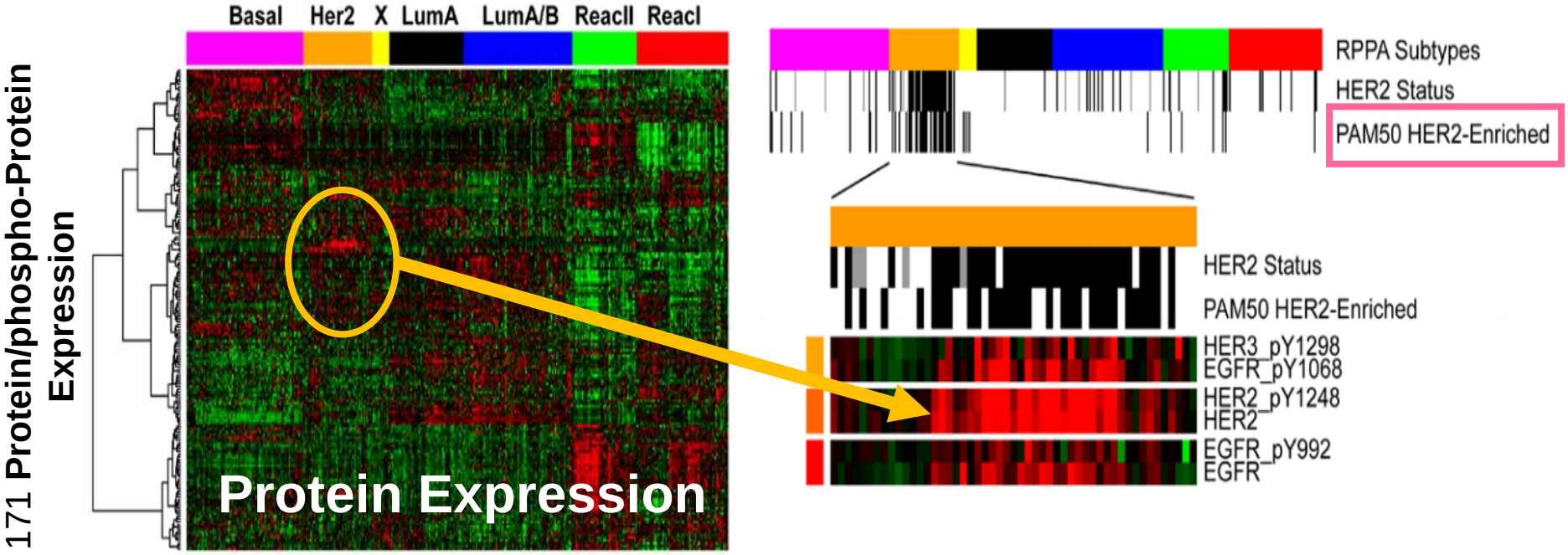




TCGA Nature 2012; Prat CCR 2014; Prat JNCI 2014;
Ferrari Nat Com 2015; Carey JCO 2016; Fumagalli JAMA Oncol 2016

# HER2+ Clinical Subtype Background

- **Among the different subtypes, the HER2-E is characterized by the highest expression of HER2/EGFR proteins and phospho(p)-HER2/p-EGFR.**
- **Thus, clinical HER2+/HER2-E disease is likely to have the highest activation of HER2 pathway.**




**TCGA Nature 2012; N=403**
**Groups based on <u>Protein Expression</u>**

**Aleix Prat,**
**Hospital Clínic de Barcelona,**
**Universitat de Barcelona,**
**Barcelona, Spain**

Pamela

# PAMELA trial schema

**N=150**

**HER2+**
**Breast Cancer stage I-IIIA**

**18 weeks**

**Trastuzumab 6 mg/kg every 3 weeks**
**+ Lapatinib 1000 mg/day**
**+ Letrozole or Tamoxifen if HR+**

**SURGERY**

Adjuvant systemic treatment was at the discretion of the treating physician

**Baseline**
PAM50

**Week 2**
PAM50

**Week 6**
Ultrasound

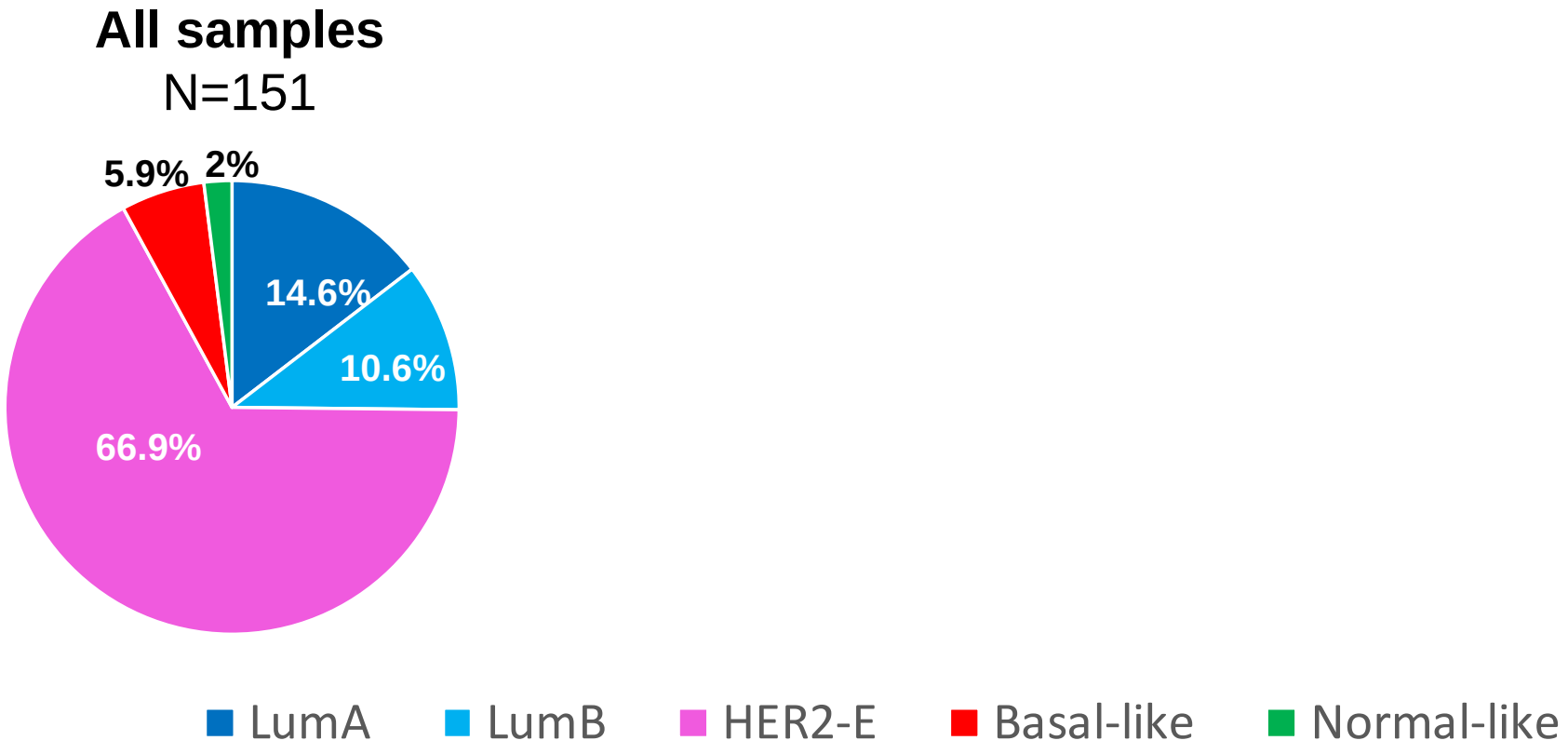
**PD*** → **Trastuzumab 6 mg/kg every 3 weeks**
**Lapatinib** 750 mg/day
**Paclitaxel** 80 mg/m$^2$ weekly x 12

*, defined as any increase in tumor size.

Prat et al., Lancet Oncology, 2017 (PMID: 28238593)

SOLTI

# Intrinsic subtype distribution at baseline



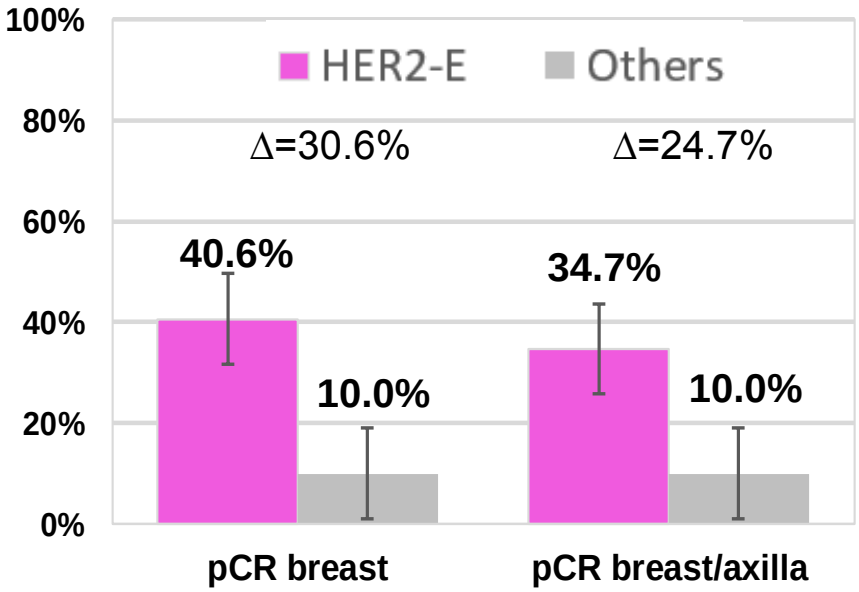

Prat et al., Lancet Oncology, 2017 (PMID: 28238593)

SOLTI

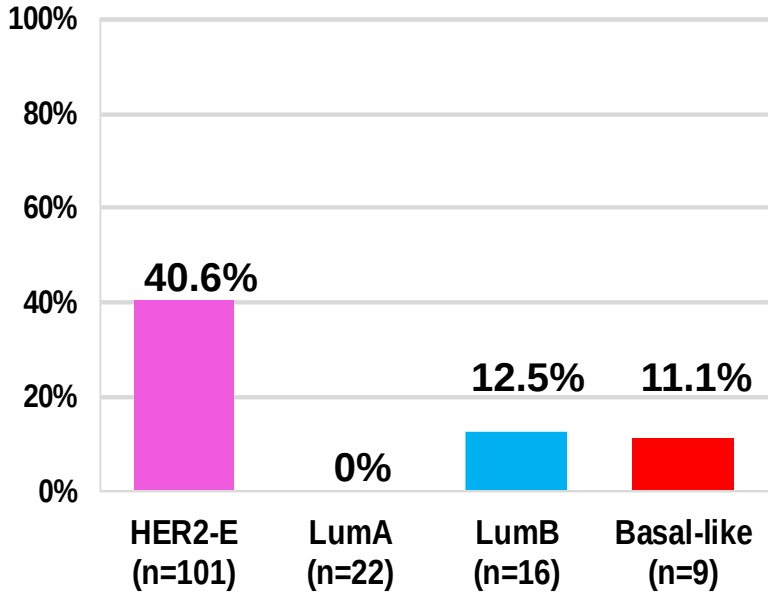
# Intrinsic subtype at baseline vs. pCR in the breast

**Baseline samples (N=151)**

**Predefined primary endpoint was pCR rate in HER2-Enriched subtype**




**pCR rates**




**pCR rates in breast**

Prat et al., Lancet Oncology, 2017 (PMID: 28238593)

SOLTI

# Molecular Heterogeneity and Response to Neoadjuvant HER2 Targeting in CALGB 40601, a Randomized Phase III Trial of Paclitaxel Plus Trastuzumab With or Without Lapatinib.

Carey et al., JCO 2015 (PMID:26527775)

Lisa Carey

**Stage II-III HER2+**

**N=300**

- **Paclitaxel + Lapatinib x 16 weeks** → **pCR Rate 32%**
- **Paclitaxel + Trastuzumab x 16 weeks** → **pCR Rate 46%**
- **Paclitaxel + Trastuzumab + Lapatinib x 16 weeks** → **pCR Rate 56%**

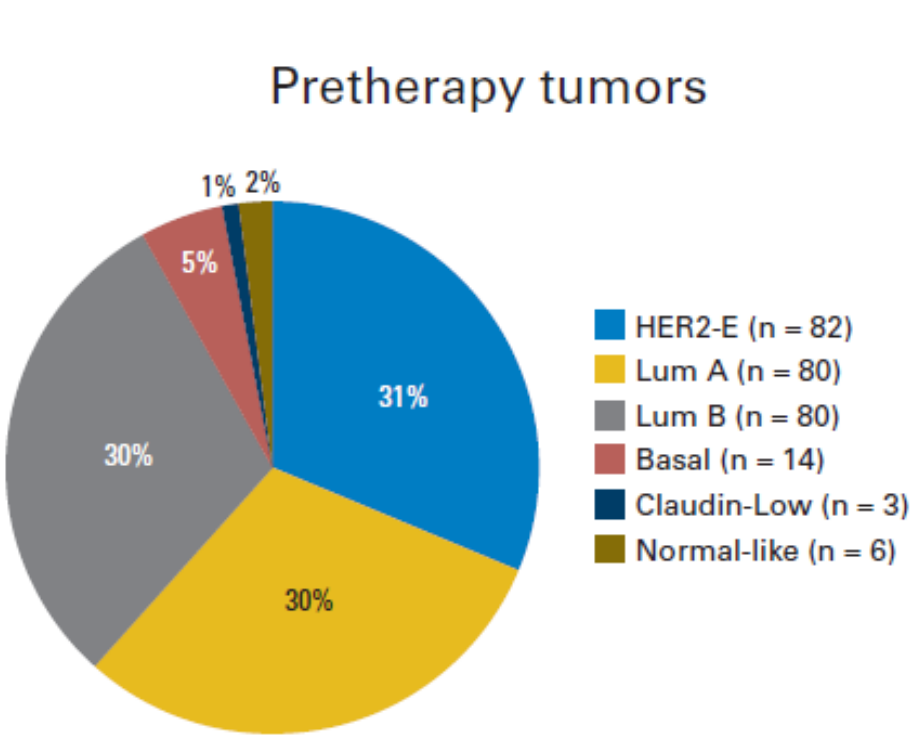
**SURGERY**

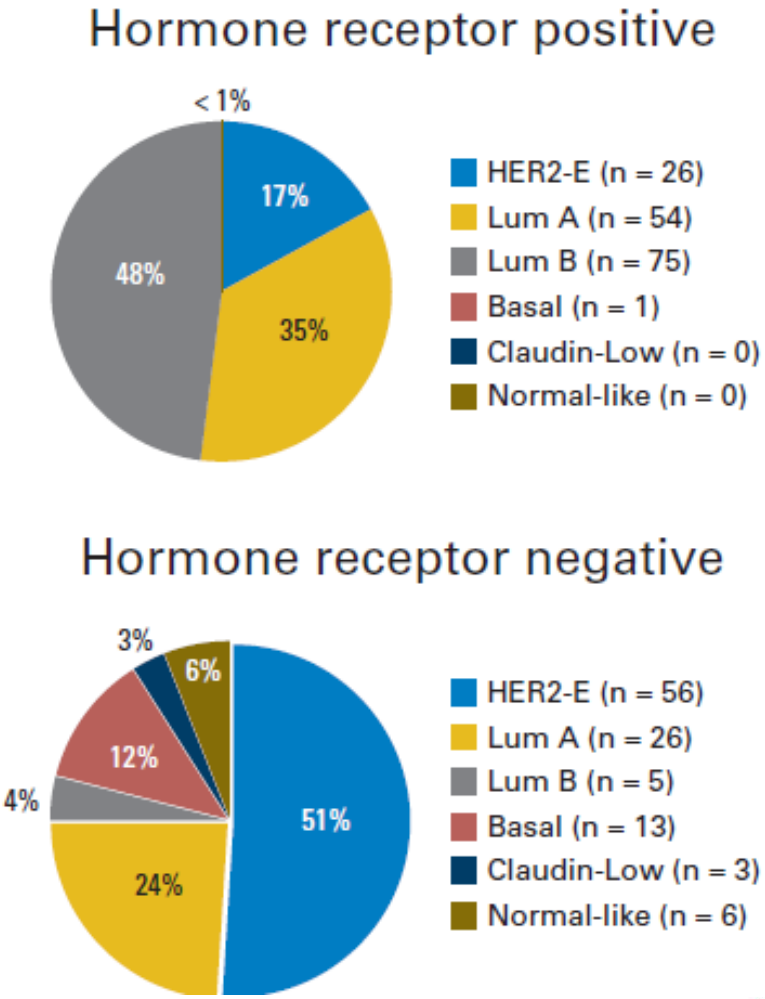
**Pretreatment Research biopsy**

**Post-treatment Research biopsy**

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

# Intrinsic Subtype Frequencies in CALGB 40601 According to Hormone Receptor Status

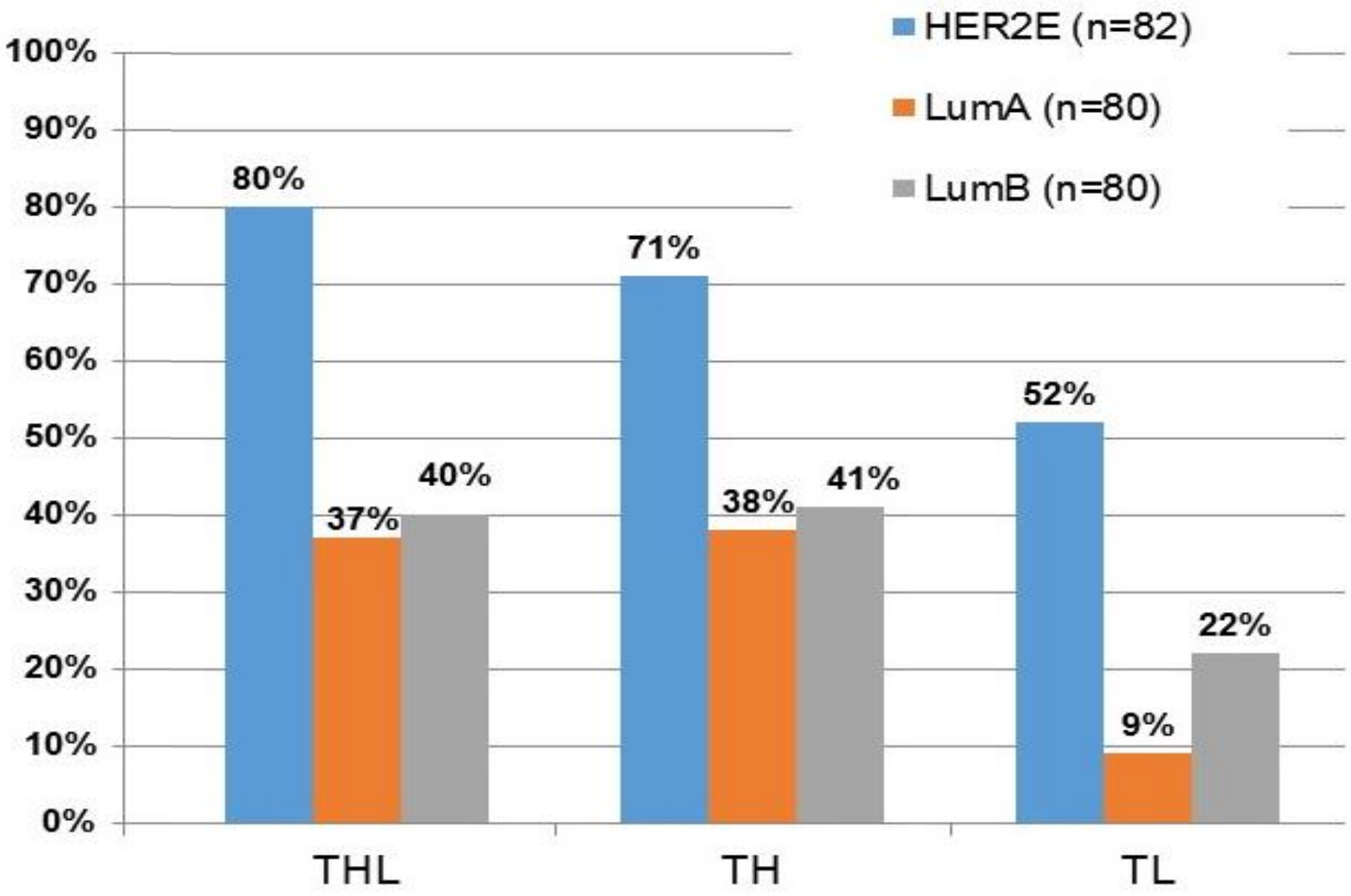



Carey et al., JCO 2015 (PMID:26527775)

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

# pCR Rate in CALGB 40601 According to Intrinsic Subtypes




**Other subtypes: 3 Claudin-low (0 pCR), 14 basal-like (36% pCR), 6 Normal-like Excluded**

Carey et al., JCO 2015 (PMID:26527775)

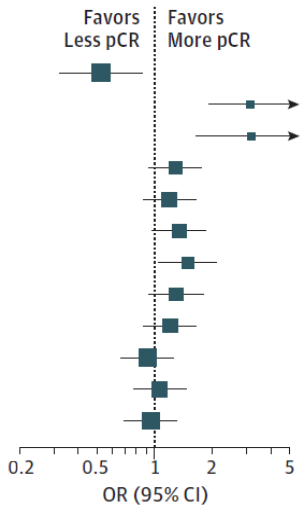
ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

# Genomic and Clinical Predictors of pCR in CALGB 40601
# Carey et al., JCO 2015 (PMID:26527775)

| | Univariable Model | | | Multivariable Model | | |
|---|---|---|---|---|---|---|
| Variable | OR | 95% CI | *P* | OR | 95% CI | *P*‡ |
| Treatment arm | | | .0392 | | | .0077 |
| THL *v* TH | 1.39 | 0.81 to 2.41 | | 1.43 | 0.76 to 2.71 | |
| TL *v* TH | 0.59 | 0.30 to 1.15 | | 0.43 | 0.19 to 0.93 | |
| Hormone receptor§ | 2.17 | 1.33 to 3.59 | < .001 | | NC | |
| Clinical stage II *v* III | 0.67 | 0.40 to 1.13 | .6548 | | NC | |
| Intrinsic subtype‖ | | | < .001 | | | .0264 |
| Luminal A *v* HER2-E | 0.22 | 0.11 to 0.43 | | 0.61 | 0.22 to 1.66 | |
| Basal *v* HER2-E | 0.24 | 0.07 to 0.78 | | 0.24 | 0.06 to 0.90 | |
| Luminal B *v* HER2-E | 0.25 | 0.13 to 0.48 | | 0.39 | 0.18 to 0.81 | |
| Normal *v* HER2-E | 0.44 | 0.08 to 2.51 | | 1.66 | 0.21 to 14.02 | |
| Gene expression signature | | | | | | |
| *p53* mutation | 2.40 | 1.69 to 3.50 | < .001 | 2.06 | 1.17 to 3.70 | .0119 |
| IgG | 1.65 | 1.30 to 2.12 | < .001 | 1.54 | 1.16 to 2.05 | .0024 |
| *HER2* amplicon | 1.54 | 1.23 to 1.93 | < .001 | 1.35 | 1.04 to 1.77 | .0252 |
| HER2-E correlation | 1.98 | | | | | |
| ER signaling | 0.47 | | | | | |
| B cell | 1.49 | | | | | |
| PI3K signaling | 1.72 | | | | | |
| T cell | 1.39 | | | | | |
| HER1 | 1.50 | | | | | |
| CD8 | 1.37 | | | | | |
| Proliferation | 1.43 | | | | | |
| Immune cell | 1.34 | | | | | |
| Hypoxia/VEGF | 1.26 | | | | | |
| Fibroblast | 0.84 | | | | | |
| *KRAS* amplicon | 1.11 | | | | | |

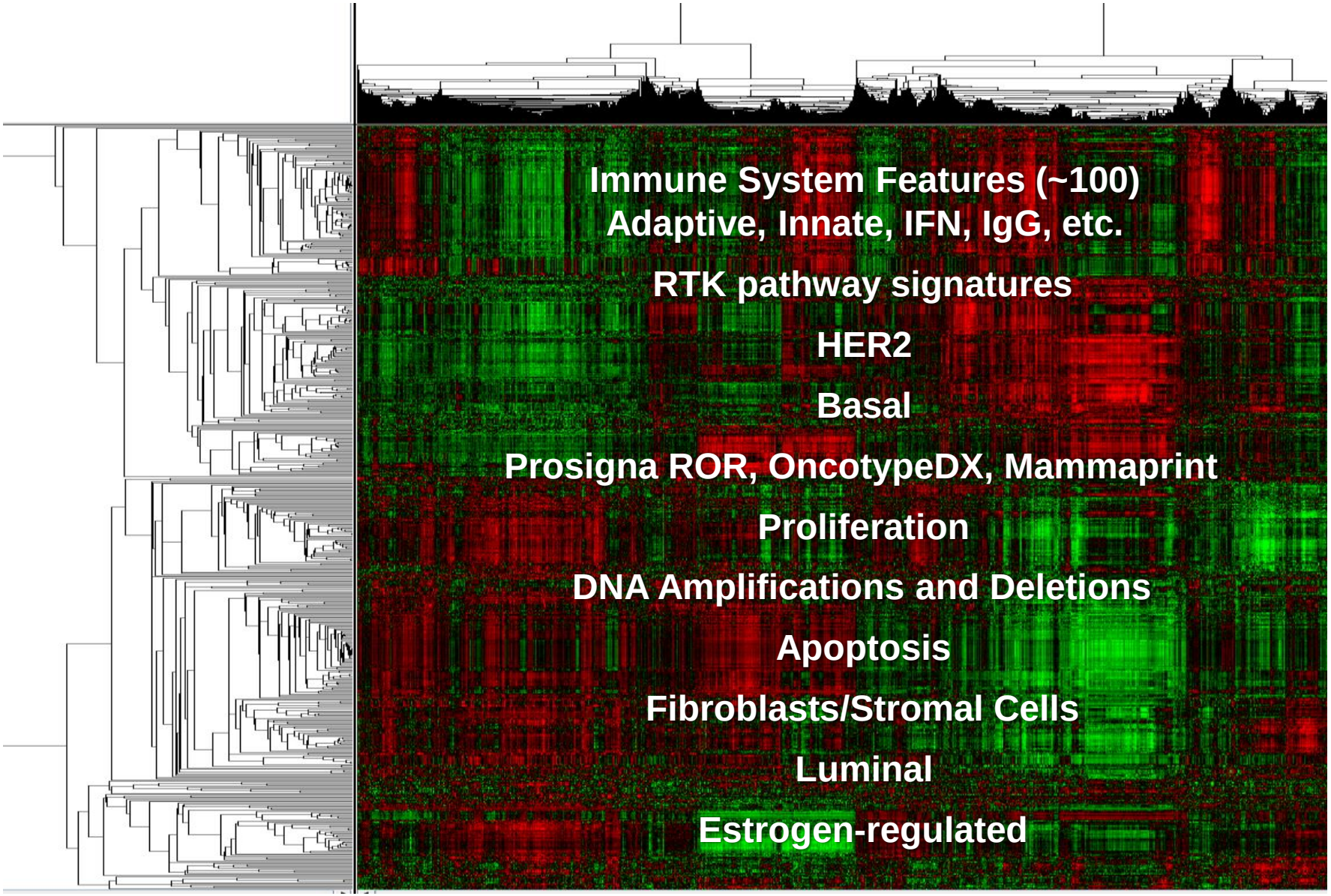
B Adjusted for clinicopathological parameters and treatment arm

| Parameter | OR (95% CI) | FDR | *P* Value |
|---|---|---|---|
| *ESR1* | 0.53 (0.33-0.86) | 0.016 | .008 |
| *ERBB2/HER2* | 3.1 (1.9-5.1) | $2.1 \times 10^{-7}$ | <.001 |
| HER2 enriched (PAM50) | 3.2 (1.7-6.0) | $9.9 \times 10^{-4}$ | <.001 |
| Immune1 | 1.3 (0.95-1.8) | 0.085 | .10 |
| Immune2 | 1.2 (0.89-1.6) | 0.16 | .24 |
| Immune3 | 1.3 (0.99-1.8) | 0.065 | .05 |
| Genomic Grade Index | 1.5 (1.1-2.1) | 0.021 | .01 |
| Aurka | 1.3 (0.95-1.8) | 0.085 | .10 |
| AKT/mTOR | 1.2 (0.89-1.6) | 0.16 | .21 |
| Stroma1 | 0.92 (0.68-1.2) | 0.36 | .60 |
| Stroma2 | 1.1 (0.79-1.5) | 0.36 | .66 |
| *AR* | 0.96 (0.70-1.3) | 0.39 | .77 |




**NeoALTTO (Fumagalli et al., 2016, PMID:27684533)**

# Cluster of >600 Gene Expression Signatures using 1100 Breast Cancer Patients (300 signatures from Perou Lab and 332 from publications)



# Multivariate Computational Predictors built using Elastic Net

**Package 'glmnet'**

August 29, 2013

**Type** Package

**Title** Lasso and elastic-net regularized generalized linear models

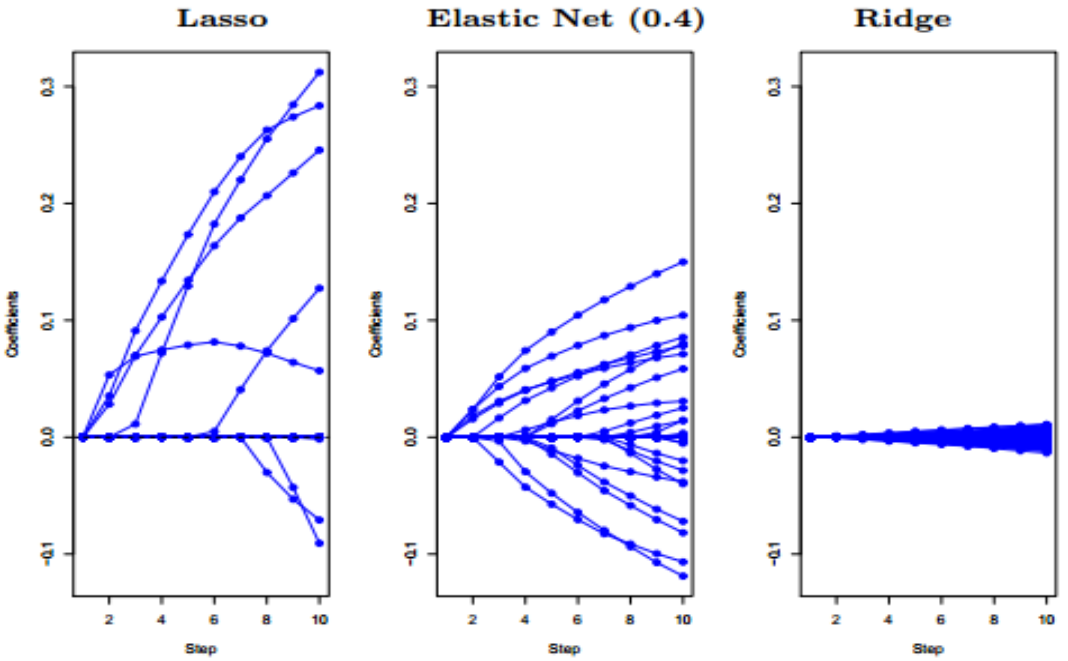
**Version** 1.9-5

**Date** 2013-8-1

**Author** Jerome Friedman, Trevor Hastie, Rob Tibshirani

$$P_\alpha = \sum_{i=1}^{p} \left[ \frac{1}{2}(1-\alpha) b_j^2 + \alpha |b_j| \right]$$

- α = 0 ---> Ridge regression
- α = 1 ---> LASSO
- 0 < α < 1 ---> Elastic Net




- ELASTIC NET is a modeling approach that can be used to perform both feature selection (from multiple data types) and parameter estimation. It is a hybrid of Ridge Regression and Least Absolute Shrinkage and Selection Operator (LASSO) Regression. Like the LASSO, ELASTIC NET performs automatic feature selection and shrinkage to produce sparse models with high prediction accuracy.

- LASSO sometimes fails to do grouped feature selection, and it tends to select one feature from a group of correlated features and ignore the others. ELASTIC NET does not have this limitation, and seems to strike a good balance between selecting just one correlated feature versus selecting all correlated features (Hastie, http://www-stat.stanford.edu/~hastie/TALKS/glmnet.pdf )

# Integrated analysis of multidimensional genomic data on CALGB 40601 (Alliance), a randomized neoadjuvant phase III trial of weekly paclitaxel (T) and trastuzumab (H) with or without lapatinib (L) for HER2-positive breast cancer

## SABCS Oral Presentation 2016, and manuscript submitted

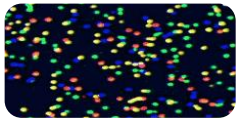
Clinical Stage II-III HER2+

Paclitaxel + Trastuzumab x 16wk

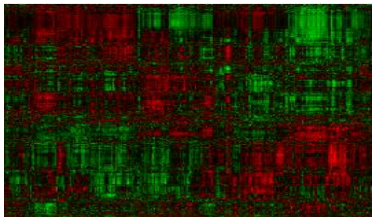
Paclitaxel + Trastuzumab + Lapatinib x 16wk

SURGERY

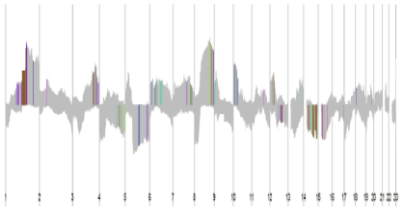
Pretreatment Research Biopsy



Illumina HiSeq sequencing
For RNAseq + DNA Exomes



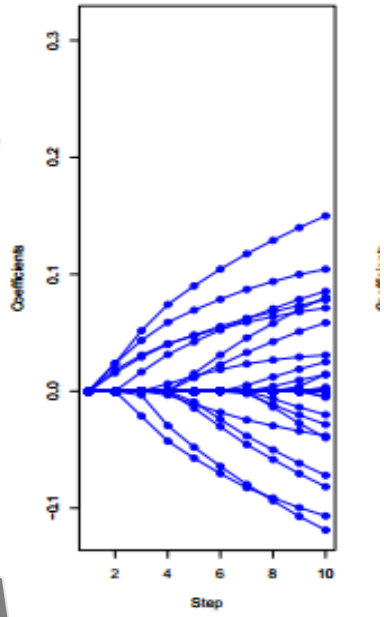
**mRNA Gene Expression:**
**>600 gene signatures**



**DNA Copy Number (CN)**
**515 DNA segment-level features**


**Somatic Mutations:**
**12 genes with mutations in more than 10 patients (>6%)**

**Elastic Net Model(s)**

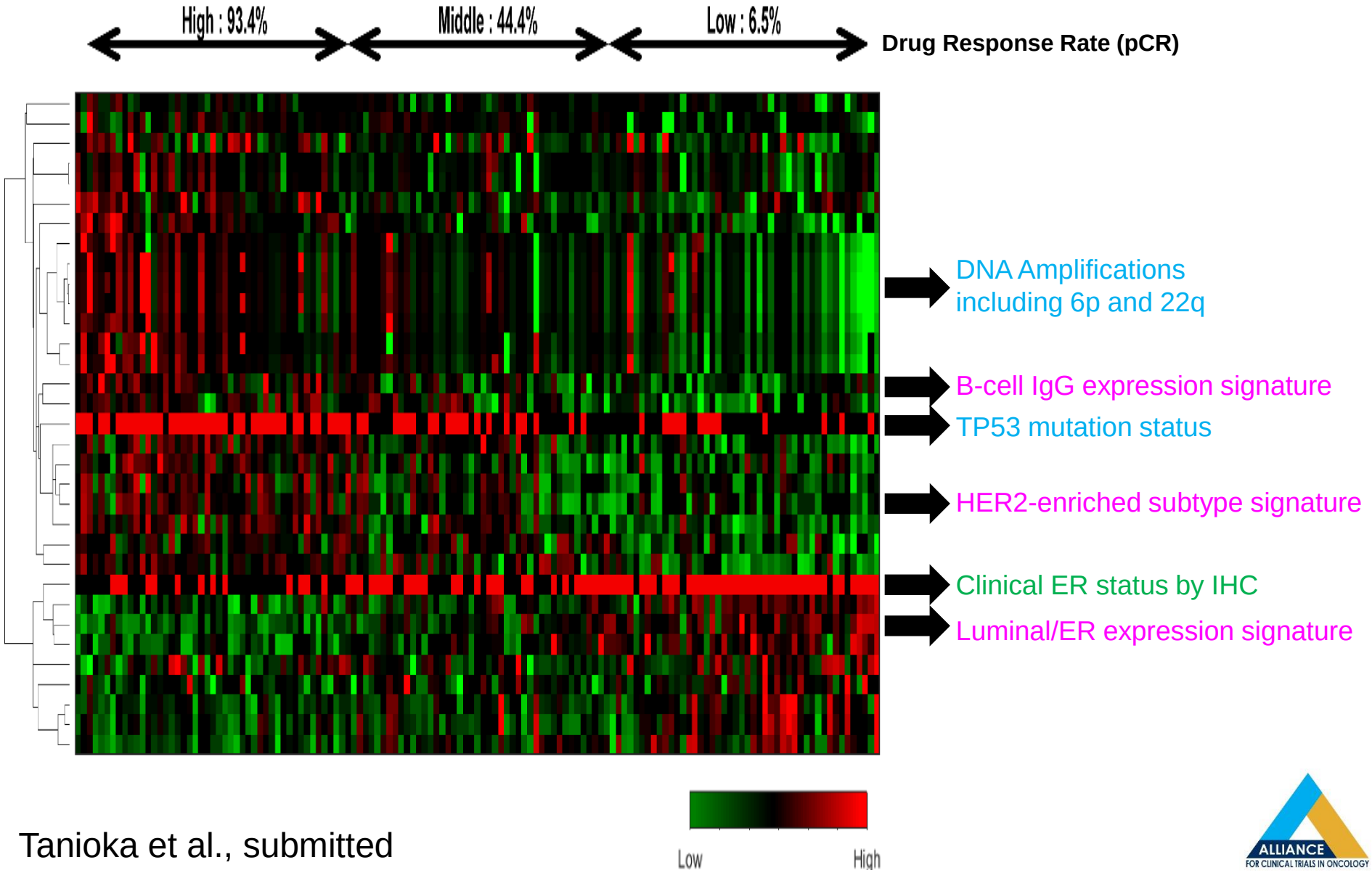



**Maki Tanioka,**
**University of North Carolina, Chapel Hill, NC**

**Now at National Cancer Center Hospital Tokyo, Japan**

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

# Supervised Clustering using 35 Elastic Net selected pCR Features and 161 patients from CALGB 40601 receiving TH or THL




Tanioka et al., submitted

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

San Antonio Breast Cancer Symposium, December 5-9, 2017

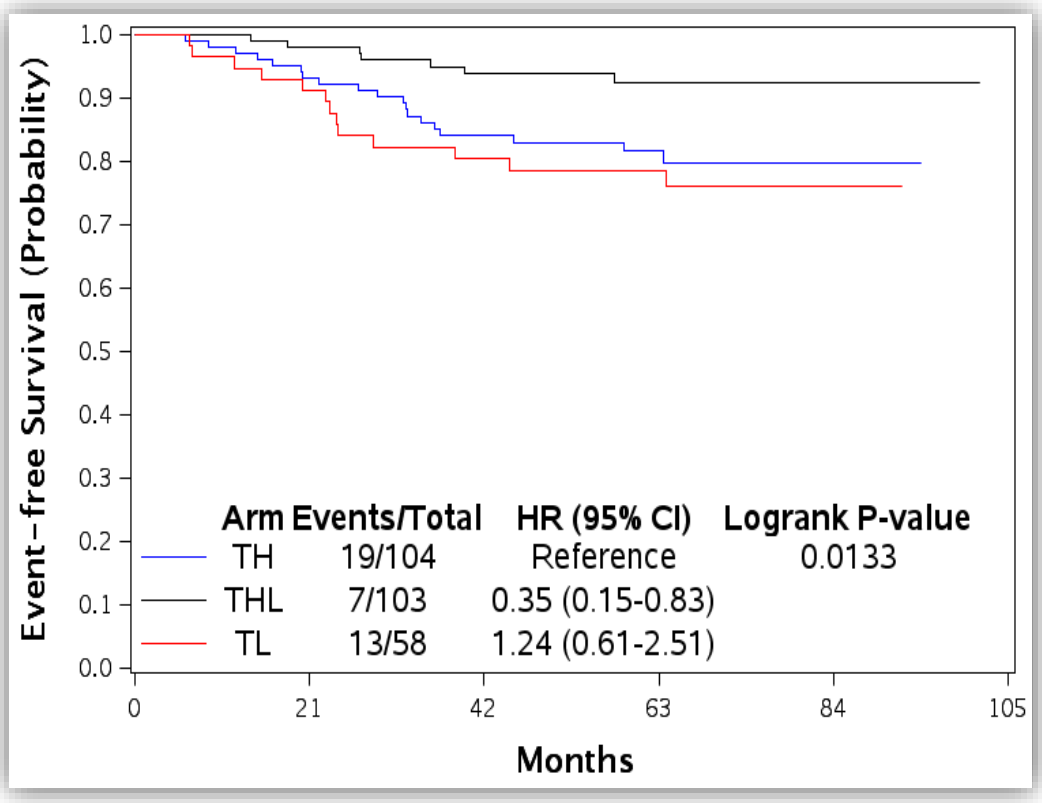


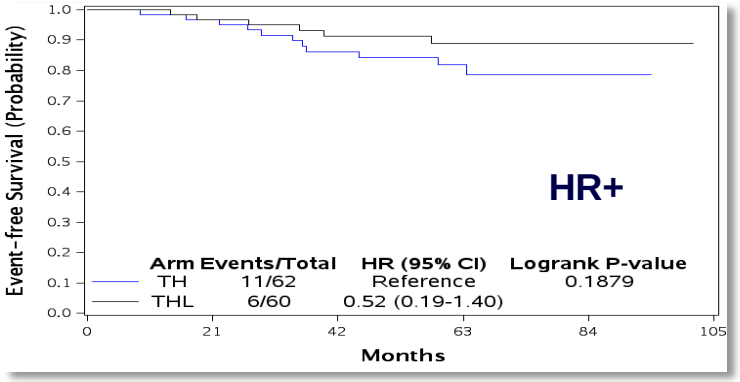
# Event-free survival and gene expression signatures in CALGB (ALLIANCE) 40601

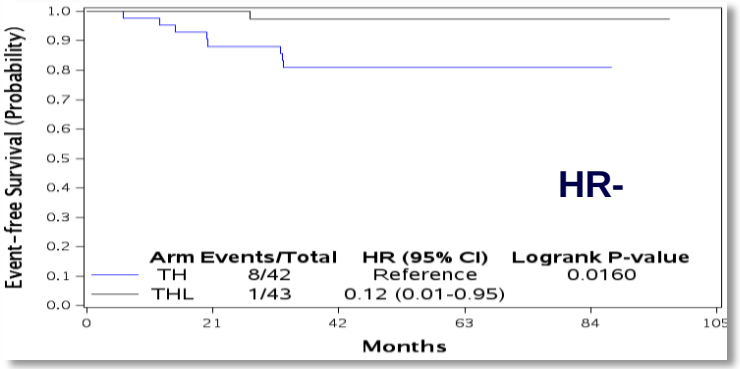
Ian E. Krop, David Hillman, Mei Polley, Maki Tanioka, Joel S. Parker, Lucas Huebner, N. Lynn Henry, Sara Tolaney, Chau Dang, Lyndsay Harris, Donald A. Berry, Charles M. Perou, Ann Partridge, Eric P. Winer, and Lisa A. Carey

on behalf of the Alliance for Clinical Trials in Oncology

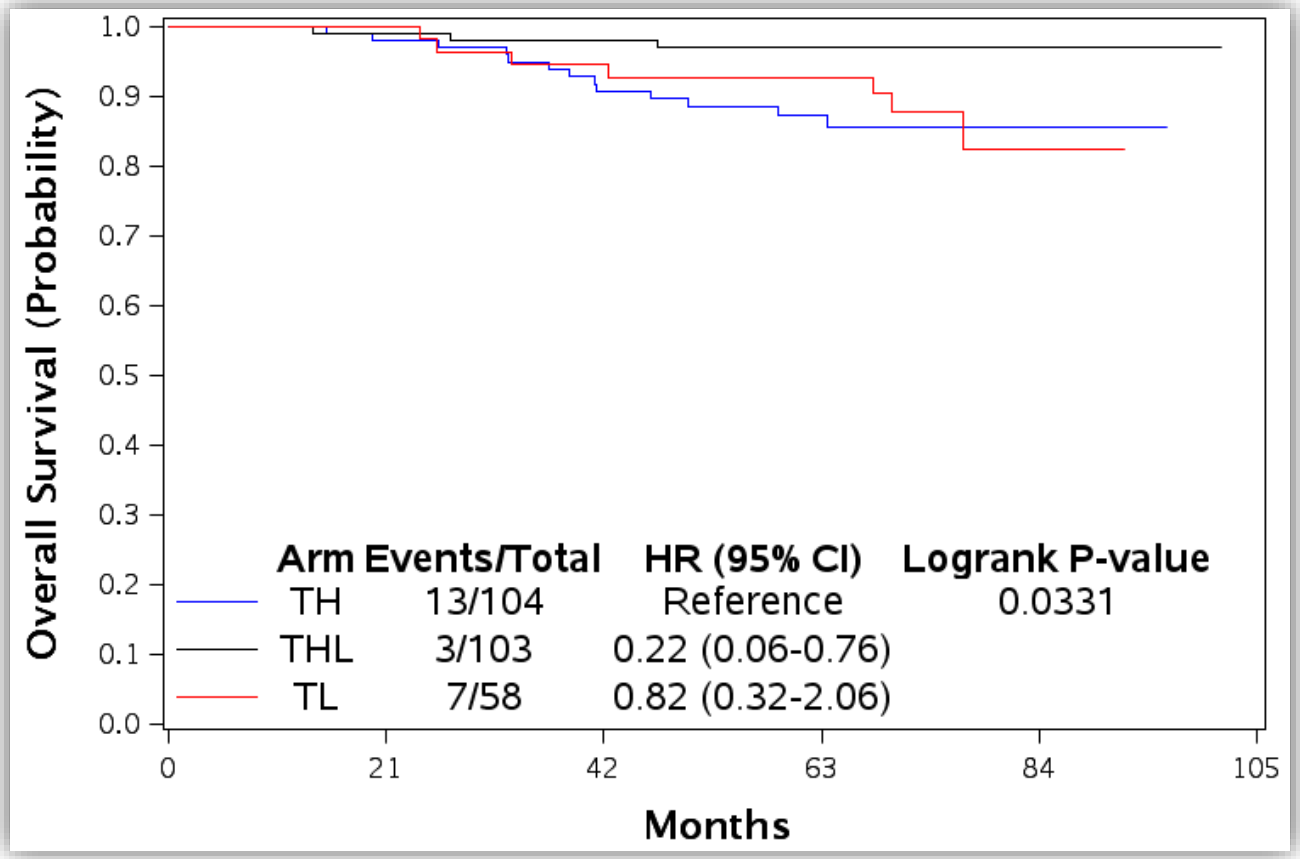
# EFS by Treatment Arm



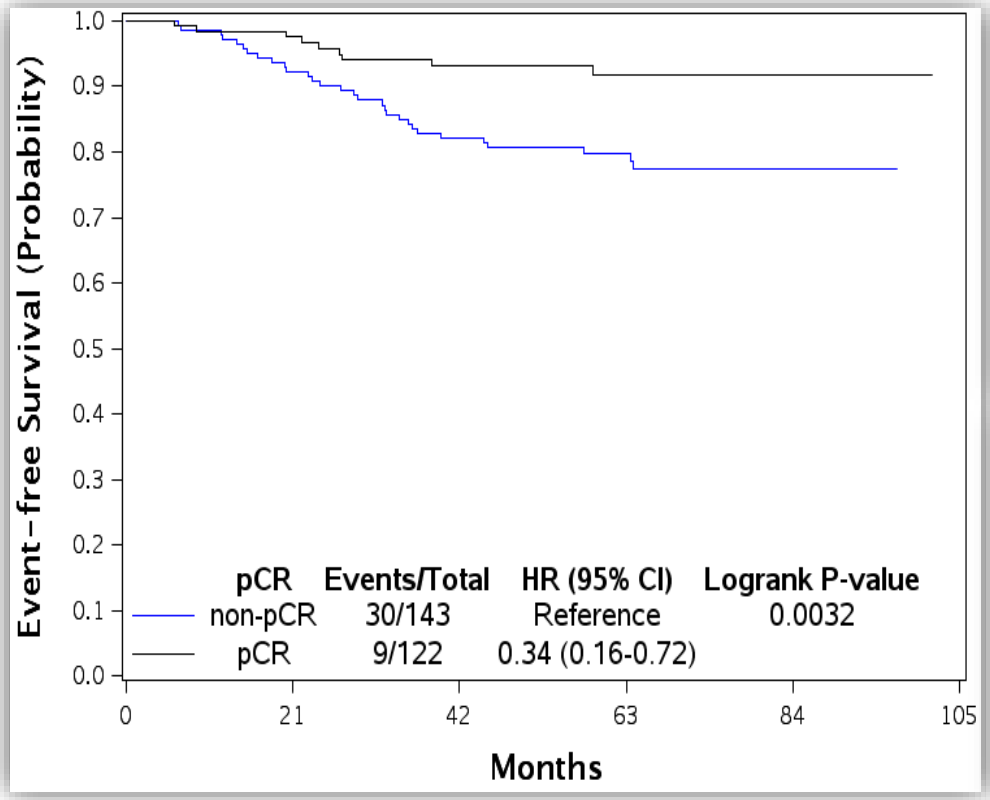



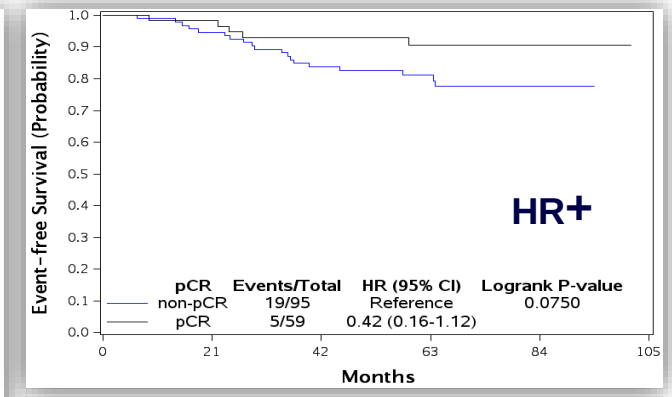



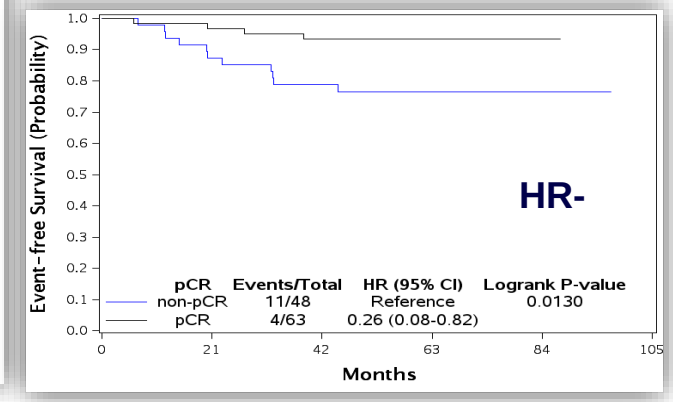
# OS by Treatment Arm



# EFS by pCR Status

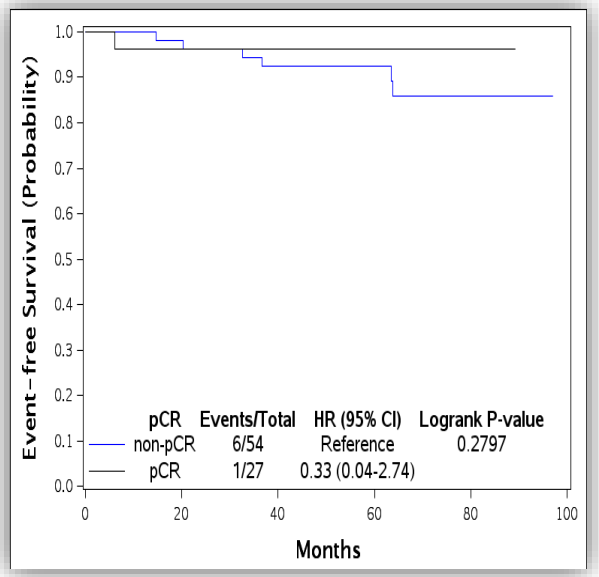


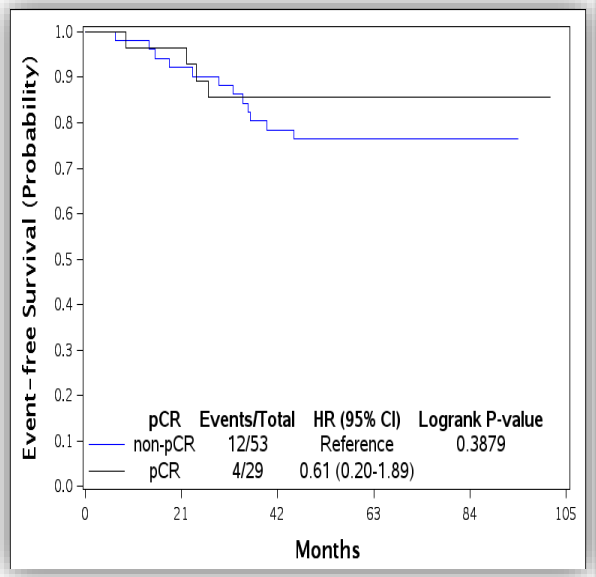



# EFS: Impact of pCR by subtype

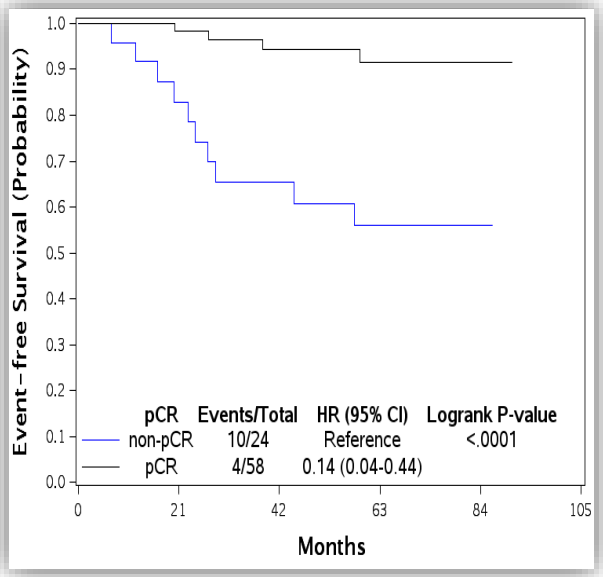
## LumA




## LumB

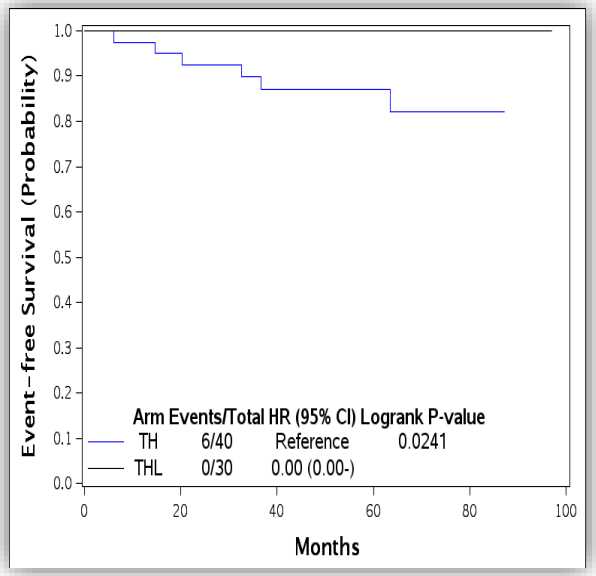



## HER2-Enriched

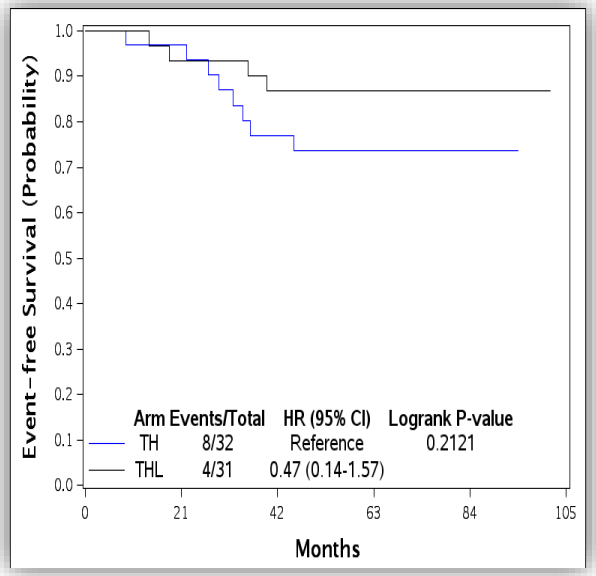


# EFS: Single vs Dual HER2-targeting effect by Intrinsic Subtype

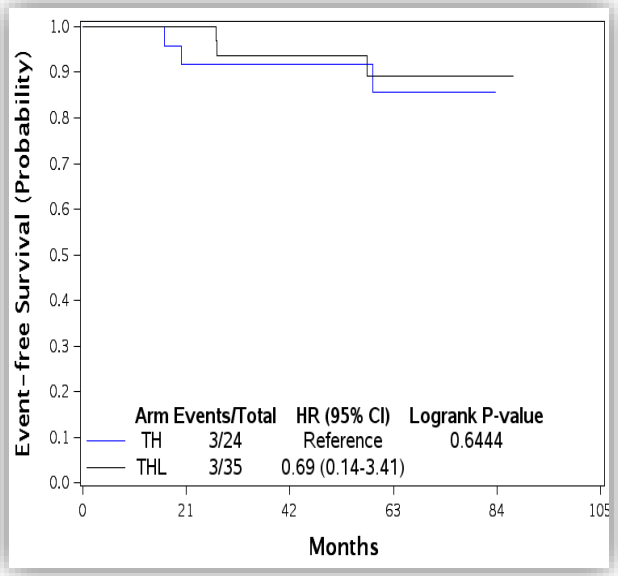
## LUM A



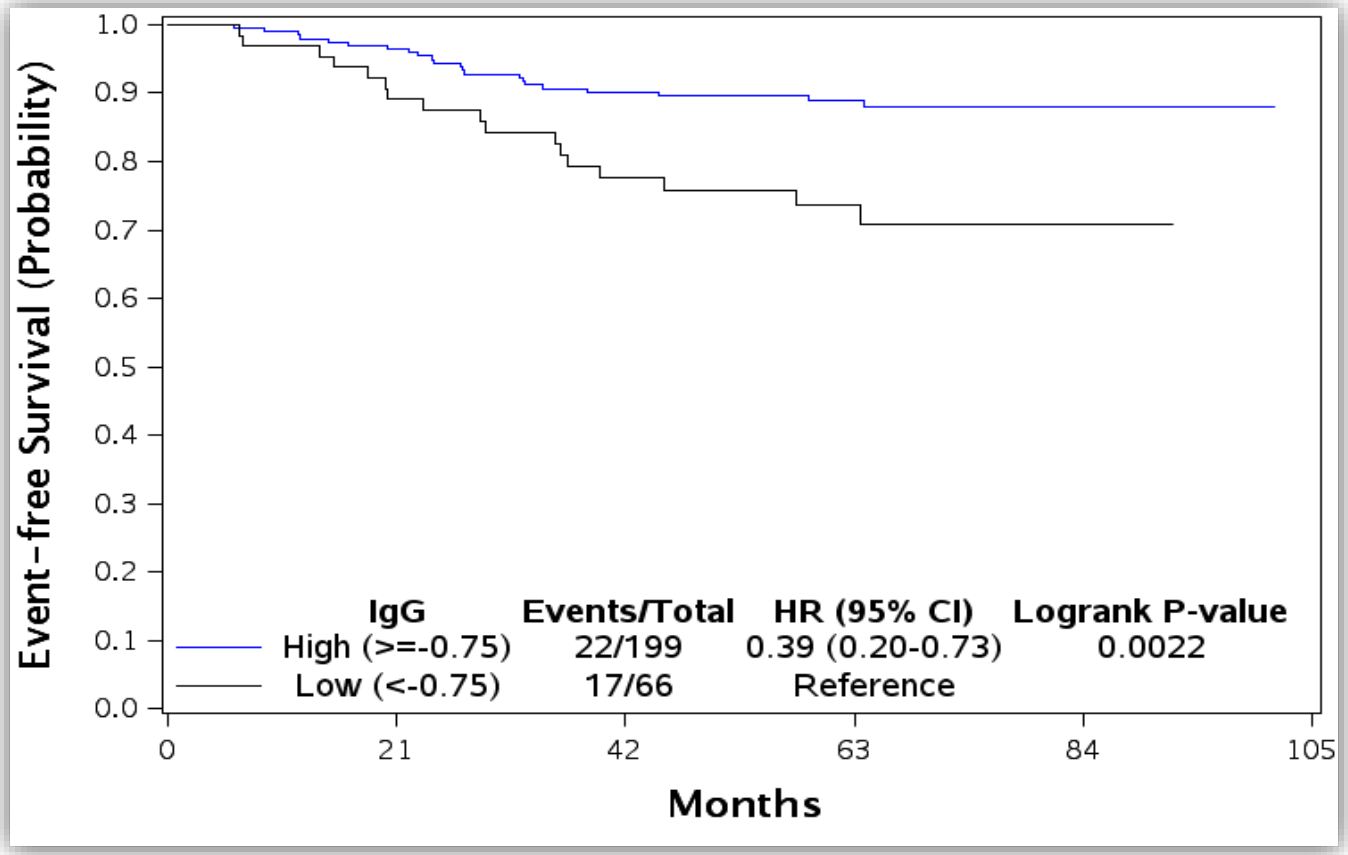

## LUM B




## HER2-Enriched



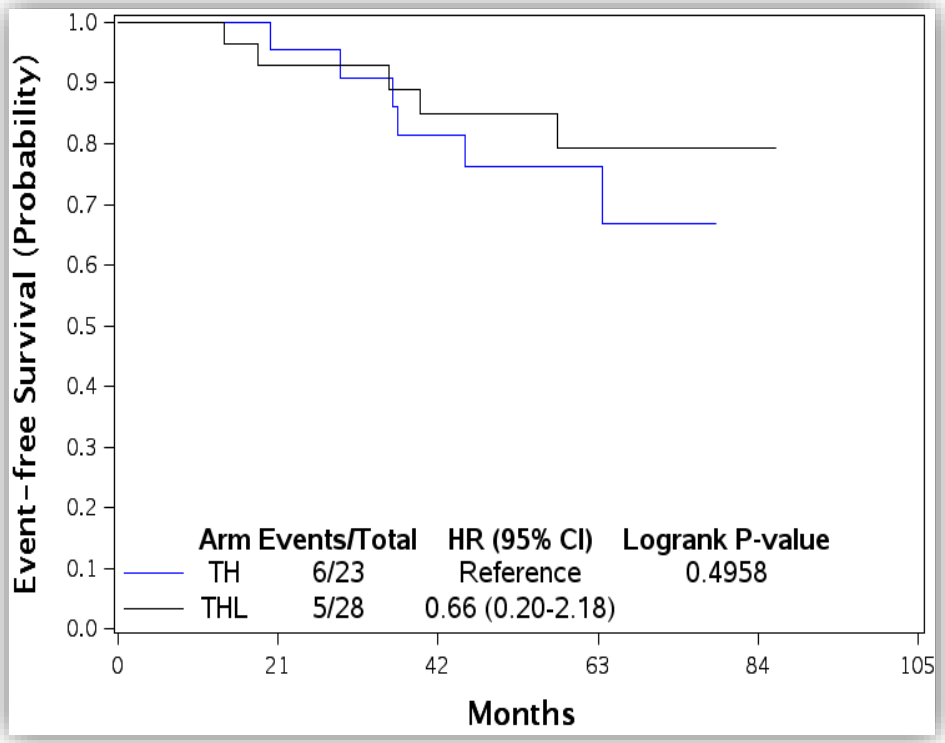
# EFS by IgG Signature



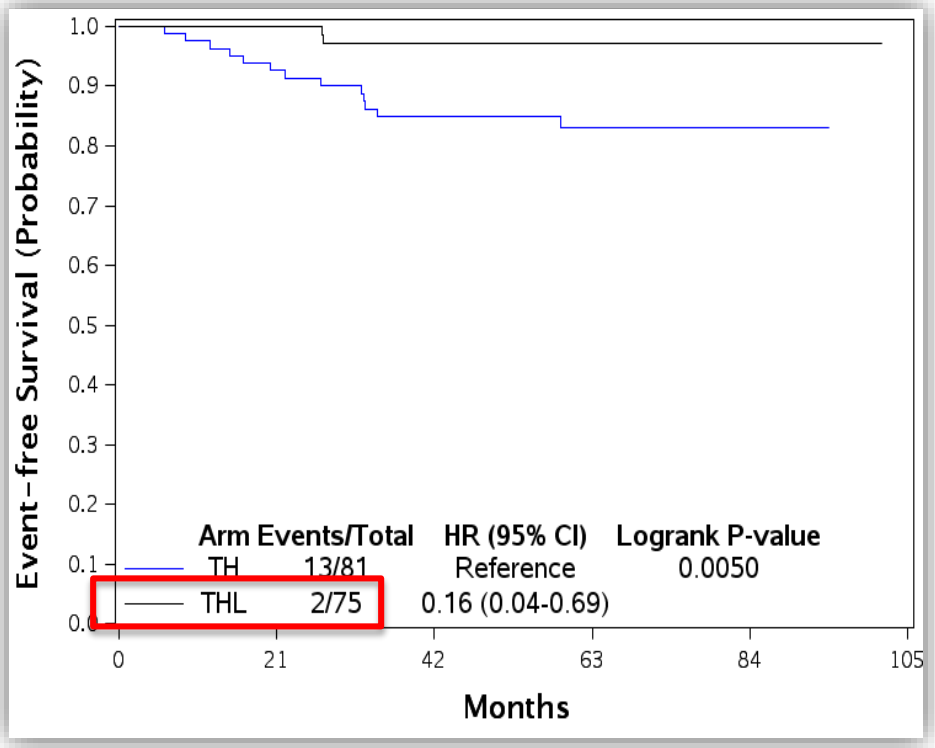

*Lower quartile vs upper 3 quartiles

# EFS: Single vs Dual HER2-targeting effect by IgG Signature

## IgG Low

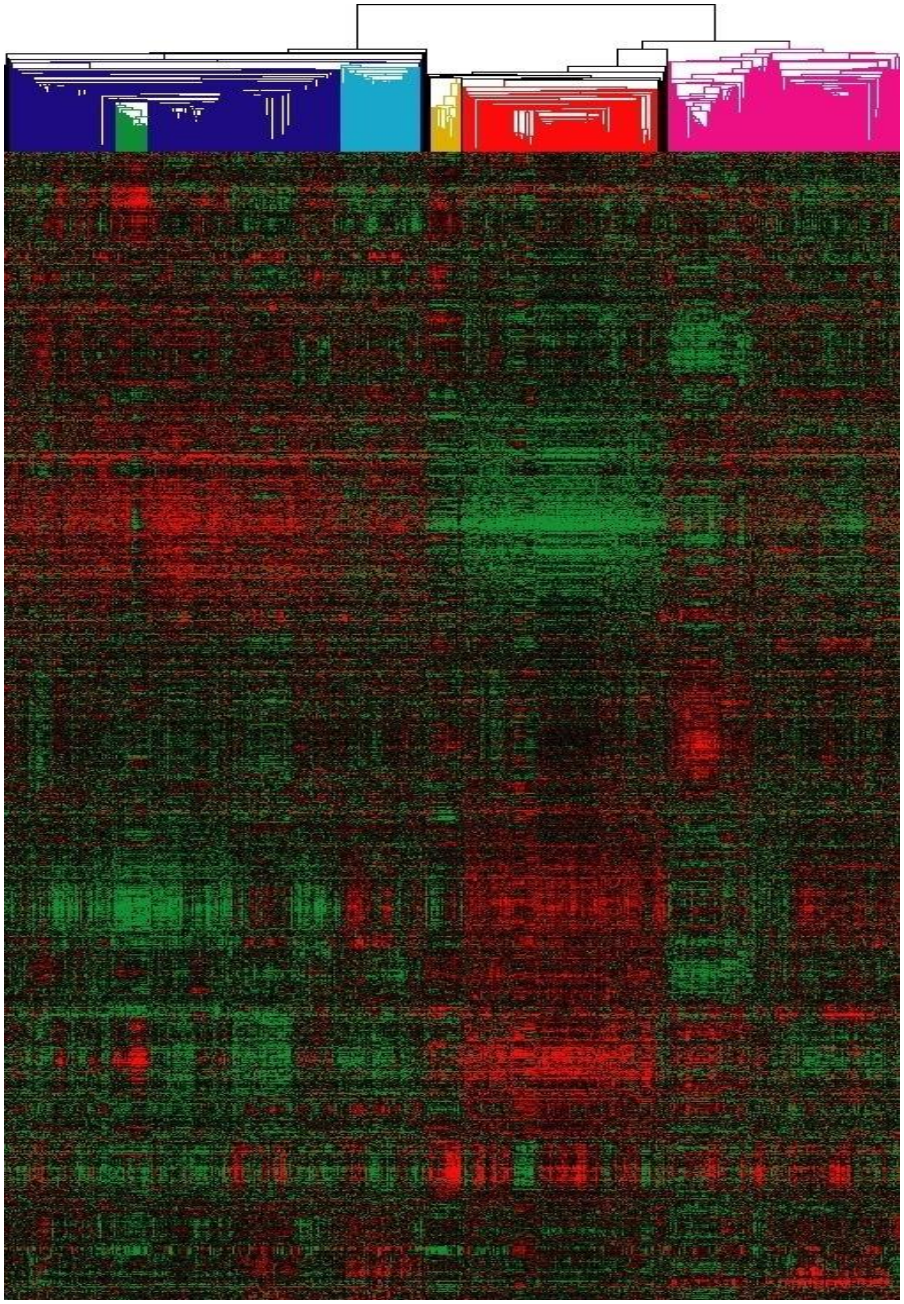



## IgG High



# Multivariate Model for EFS

| Variable | HR | 95% CI | p-value |
|---|---|---|---|
| **Treatment Arm** | | | |
| THL | 0.29 | (0.12, 0.71) | 0.007 |
| TL | 1.06 | (0.51, 2.23) | 0.87 |
| TH | Ref | Ref | |
| **Gene signatures** | | | |
| IgG immune active | 0.70 | (0.50, 0.98) | 0.04 |
| HER2E Correlation | 1.79 | (1.24, 2.57) | 0.002 |
| **PCR** | | | |
| Yes | 0.28 | (0.12, 0.66) | 0.003 |
| No | Ref | Ref | |
| **Clinical Stage** | | | |
| II | Ref | Ref | |
| III | 2.19 | (1.13, 4.24) | 0.02 |



# HER2+ Targeting Summary

**1. The addition of lapatinib to trastuzumab/taxane regimen was associated with a significant improvement in Event Free Survival and Overall Survival**

**2. Immune activation assayed by a RNA expression signature (IgG signature) was an independent predictor of favorable pCR and EFS**

**3. Dual HER2-targeting for pCR benefit was primarily observed in HER2-Enriched tumors, including the chemo-free regimen in PAMELA**

**4. Dual HER2-targeting for EFS benefit was primarily observed in Luminal A tumors, and IgG signature high tumors**

**5. Molecular studies with pertuzumab + trastuzumab dual HER2-targeting are needed**

**6. Elastic Net Regression (i.e. machine learning) is able to objectively identify and link together multiple features to create an integrated predictor that is better than any single data type predictor**

## Perou Lab Current Members

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Joe Garay
Susana Recio Garcia
Dan Hollern
Aatish Thennavan
Koby Amankwah
Youli Xia
Jonathan Shepherd
Lynn Chollet Hinton
Marni Siegel
Aranzazu Fernandez Martinez

## Collaborating Past Perou Lab Members

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Michael Iglesia (Washington University)
Katie Hoadley (Department of Genetics, UNC)
Jason Herschkowitz (University at Albany-SUNY)
Michael Gatza (Rutgers University)
J. Chuck Harrell (Virginia Commonwealth University)
Maggie Cheang (The Institute for Cancer Research, Sutton, UK)
Aleix Prat (Hospital Clínic de Barcelona, Universitat de Barcelona, Spain)
Melissa Troester (Department of Epidemiology, UNC)

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Gary Johnson (Pharmacology)
Steve Marron, Andrew Nobel (Statistics)
Danyu Lin, Michael Kosorok (Biostatistics)
Corbin Jones and members of the High Throughput Sequencing Facility (HTSF)
Joel Parker, Chris Fan, Sai Balu and members of the LCCC Bioinformatics Group
Lisa Carey, Carey Anders, Neil Hayes, Jon Serody, Ben Vincent, Hy Muss (Oncology)

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Matthew Ellis Lab

University of British Columbia
Torsten Nielsen Lab

University of Utah
Phil Bernard Lab

Nationwide Children's Hospital
Elaine Mardis Lab

Salk Institute
Geoff Wahl Lab

NYU Langone Medical Center
Kwok Wong Lab

## Clinical Trial Groups

ALLIANCE for
Clinical Trials in Oncology

Translational Breast Cancer
Research Consortium (TBCRC)

GEICAM

SOLTI

All Individuals who
donate their tissues
to medical research