
# Out with the OLD!!!
# In with the NEW!!!
## *Progression toward Precision Medicine*

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Assistant Professor

University of Rochester

Department of Pathology and Laboratory Medicine

# My real job!!!

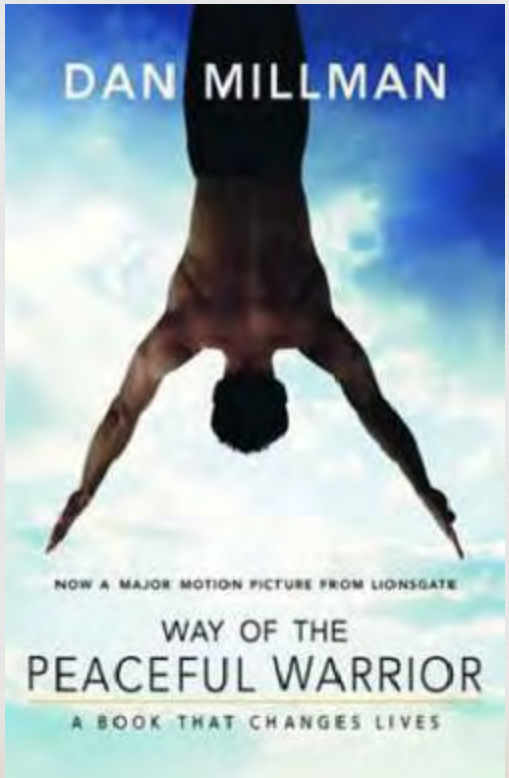
# I have nothing to disclose…… although ……

# Oncotype Dx® year end revenues 2015

| | Year Ended December 31, | | |
|---|---|---|---|
| | 2015 | 2014 | 2013 |
| | (In thousands) | | |
| Product revenues | $ 287,458 | $ 275,706 | $ 259,192 |
| Contract revenues | — | — | 2,403 |
| Total revenues | $ 287,458 | $ 275,706 | $ 261,595 |
| Period over period dollar increase in product revenues | $ 11,752 | $ 16,514 | |
| Period over period percentage increase in product revenues | 4 % | 6 % | |

# Change

- **“ The secret of change is to focus all of your energy not on fighting the old, but building the new”**




- Socrates

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# Pathologic diagnosis, immunohistochemistry, multigene assays and breast cancer treatment: progress toward "precision" cancer therapy

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*Accepted September 18, 2014*

## Abstract

Clinical decisions regarding the suitability of adjuvant systemic therapy for individual patients with breast cancer depends on comprehensive assessment of the underlying biology of each patient's tumor. The previous clinical-pathologic paradigm for treatment, which had been used for decades, now has been augmented by significant advances in molecular analysis of breast tumor tissue samples. Molecular testing has the potential to understand better both tumor biology and clinical behavior, which enables more appropriate therapy choices to be made. We review the rapid evolution in profiling breast cancer tissues, and discuss the current evidence for clinical use of this information and how the emerging molecular paradigm can be integrated into the clinical-pathologic context as we progress toward "precision" therapy for patients with breast cancer and other solid tumors.

**Key words:** breast cancer, ER, HER2, multi-gene assay, PR, prognosis, prediction, rtPCR

# *What is* Precision Medicine?

- .....a medical model that proposes the customization of healthcare, with medical decisions, practices, and/or products being tailored to the ***individual*** patient.

-Wikipedia

# *What is* Precision Medicine?

- .....makes it possible to tailor medical interventions, including combinations, to ***individual*** patients at presentation and potentially throughout the course of their disease as new mutations arise and response to treatment diminishes. However, the concept poses challenges to researchers, patients, regulators and payers.

**Doherty M. et al.** Precision Medicine and Oncology: An Overview of the Opportunities Presented by Next-Generation Sequencing and Big Data and the Challenges Posed to Conventional Drug Development and Regulatory Approval Pathways. Ann Oncol. 2016 Apr 26. [Epub ahead of print]

# The Precision Medicine Initiative Cohort Program – Building a Research Foundation for 21st Century Medicine

Precision Medicine Initiative (PMI) Working Group Report to the Advisory Committee to the Director, NIH

September 17, 2015

# *What is* Precision Medicine?

- "an approach to disease treatment and prevention that seeks to maximize effectiveness by taking into account ***individual*** variability in genes, environment, and lifestyle."

| Table 1.1: Proposed PMI Budget Allocations for FY 2016<br>Department of Health and Human Services | | |
|---|---|---|
| Investment | Agency | Purpose |
| $130 million | National Institutes of Health | To develop a voluntary national research cohort to propel our understanding of health and disease and set the foundation for a new way of doing research. |
| $70 million | NIH National Cancer Institute | To scale up efforts to identify genomic drivers in cancer and develop more effective approaches to cancer treatment. |
| $10 million | Food and Drug Administration | To acquire additional expertise and advance the development of high quality, curated databases to support the regulatory structure needed to advance innovation in precision medicine. |
| $5 million | Office of the National Coordinator | To support the development of interoperability standards and requirements that address privacy and enable secure exchange of data across systems. |

Precision Medicine Initiative (PMI) Working Group Report to the Advisory Committee to the Director, NIH. September 17, 2015

# Precision Medicine Initiative (PMI)

- …to bring us closer to curing diseases like cancer and diabetes
- …to give all of us access to the personalized information we need to keep ourselves and our families healthier
- …to enhance innovation in biomedical research, with the ultimate goal of moving the U.S. into an era where medical treatment can be tailored to each patient.

- NIH's investment in PMI will focus on:
  1. PMI-Oncology, an effort to advance precision oncology:
     - ✓ will test precision therapies for cancer, including targeted agents and immunotherapies, while also developing a national cancer knowledge system
  2. PMI Cohort Program (PMI-CP), the creation of large, voluntary national research cohort:
     - ➢ will lay the foundations for precision medicine approaches more broadly by building a national research cohort of one million or more volunteers who are engaged as partners in a longitudinal long-term effort to:
       - ✓ identify the molecular, environmental and behavioral factors that contribute to diverse diseases
       - ✓ facilitate the development and testing of novel therapies and prevention approaches
       - ✓ pioneering health strategies for improving the efficacy of health care

# Precision medicine

- Focuses on the specific disease
- Targeted treatments
- Individual
- Genetic level

# Traditional approach to cancer

- Location
  - Radiology
- Pathology
  - Histopathology
  - Immunophenotype
- Clinical variables (i.e. age, lymphovascular invasion, lymph node staging, evidence of distant metastasis)
- **Various chemotherapy combinations**

# Precision Approach to cancer

- **ADVANCED GENOMIC TESTING**
  - Gene-mapping analyses
  - Cancer is no longer defined by where it's located, but by its molecular structure.

**Table 1.** Overview of molecular tests with therapeutic implications recommended for routine testing in different tumor types (selection)

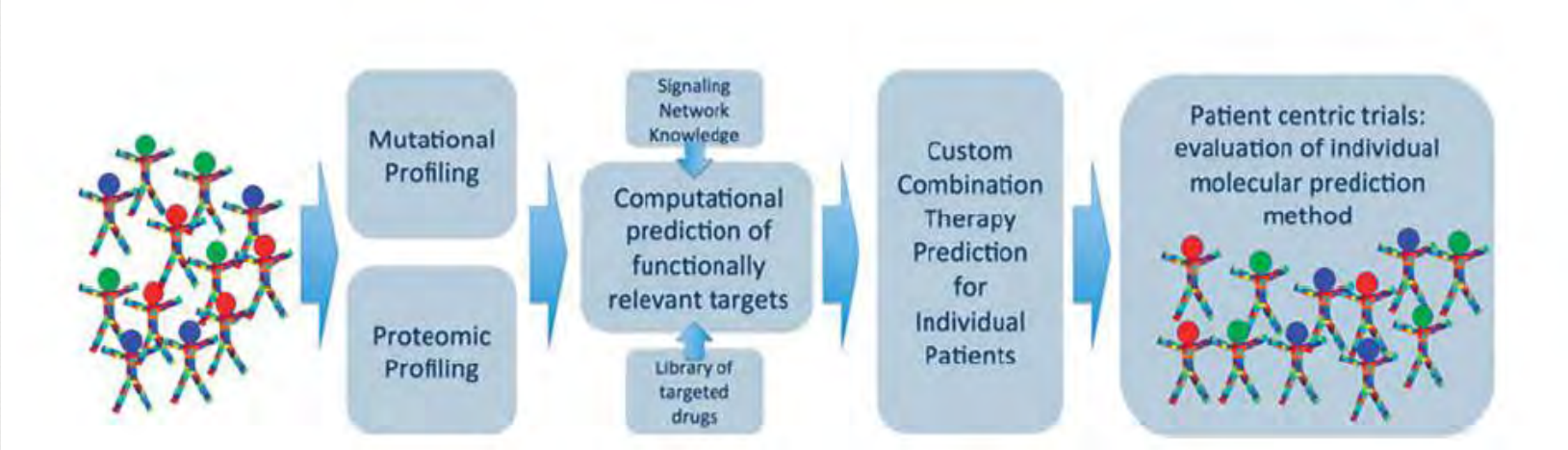
| *Tumor type* | *Affected gene(s)* | *Type of alteration* | *Method for detection* | *Related treatment* |
|---|---|---|---|---|
| Breast cancer | HER2 | Amplification | IHC, ISH, | Herceptin |
| | PIKCA | SNV | Sequencing | Reduced response to anti-HER2 treatment, in particular double blockade (Trastuzumab / Lapatinib) |
| | Gene expression assays (EndoPre-dict, OncotypeDX etc.) | mRNA levels | qRT-PCR | Prognostic assay (endokrine Tx vs Chemoendokrine Tx) |
| Colorectal cancer | RAS (KRAS, NRAS) | SNV | Sequencing | Cetuximab / Panitumumab |
| GIST | KIT | SNV, indel | Sequencing | Imatinib, Sunitinib |
| | PDGFR | SNV | Sequencing | Imatinib, Sunitinib |
| Malignant melanoma | BRAF | SNV | Sequencing | Vemurafenib, Dabrafenib, Trametinib |
| | KIT | SNV, indel | Sequencing | Sunitinib, Dasatinib, Imatinib |
| NSCLC | EGFR | SNV, MNV, indel | Sequencing | Gefitinib, Erlotinib, Afatinib, Dacomitinib |
| | ALK | Translocation | ISH, IHC, Sequencing, | Crizotinib, Ceritinib, Alectinib |
| | ROS1 | Translocation | ISH, Sequencing | Crizotinib |
| | MET | Amplification | ISH, Sequencing | Resistance to EGFR TKIs |
| Serous ovarian cancer | BRCA1/BRCA2 | SNV, MNV, indel | Sequencing | Olaparib |
| Walden-strom´s, ABC-DLBCL | *MYD88* | Mutation L265P and others | Sequencing | IRAK174 inhibitors |
| Walden-strom´s | *CXCR4* | Mutation truncating C-terminal region | Sequencing | CXCR4 inhibitors,(plerixafor), blocking antibodies |
| Classical hairy cell leukemia | *BRAF* | V600E mutation | Sequencing | Vemurafenib |
| Burkitt lymphoma, DLBCL, transformed FL | *MYC* | Translocation, amplification, overexpression | IHC for MYC protein, FISH | BET inhibitors, Protein translation inhibitors |
| DLBCL, FL, MCL, SLL/CLL | *BCL2* | Translocation, amplification, overexpression | IHC for BCL2 protein, FISH | BH3 mimetics |

Abbreviations: ABC, activated B-cell; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; GCB, germinal center B-cell, IHC, Immunohistochemistry, indel-insertion or deletion; ISH, *In situ* hybridization; MCL, mantle cell lymphoma; MNV, multiple nucleotide variation; PCR, Polymerase chain reaction; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia; SNV, single nucleotide variation; TKI, Tyrosine Kinase Inhibitor.

**Dietel M. et al.** A 2015 update on predictive molecular pathology and its role in targeted cancer therapy: a review focussing on clinical relevance. Cancer Gene Ther.. 2015 Sep;22(9):417-30.

# Precision Medicine

- Should molecular testing replace the more traditional paradigms of clinical decision making?




- Can be costly, and poses challenges to researchers, patients, regulators and payers.

**Dietel M. et al.** A 2015 update on predictive molecular pathology and its role in targeted cancer therapy: a review focussing on clinical relevance. Cancer Gene Ther.. 2015 Sep;22(9):417-30.

# Our goals today

- Review the current evidence of how the emerging molecular paradigm can be integrated with the more traditional clinical-pathologic paradigms as we progress toward “precision” therapy for patients

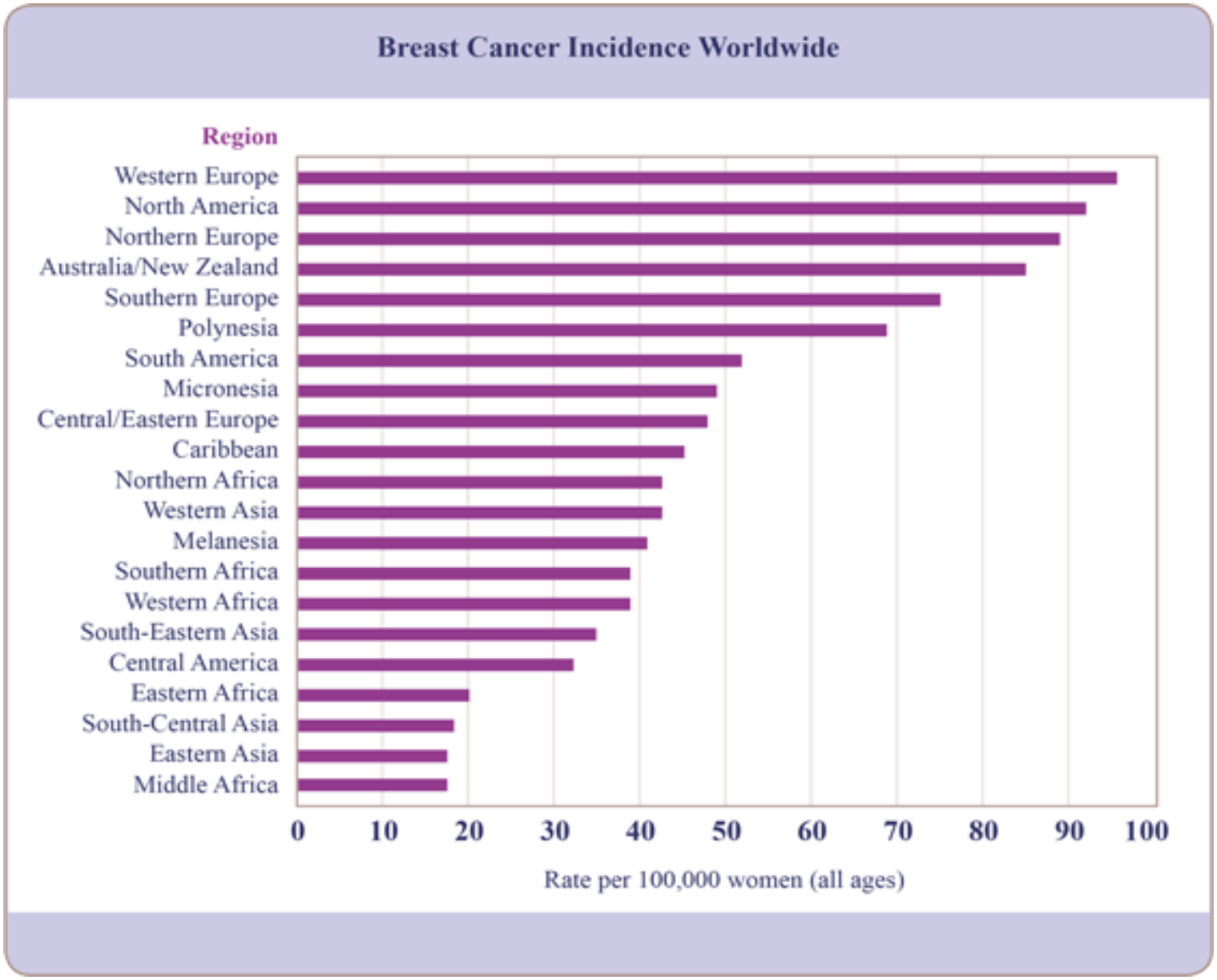
- Discussion will center around breast cancer

- Many of these concepts can be applied to other tumor types

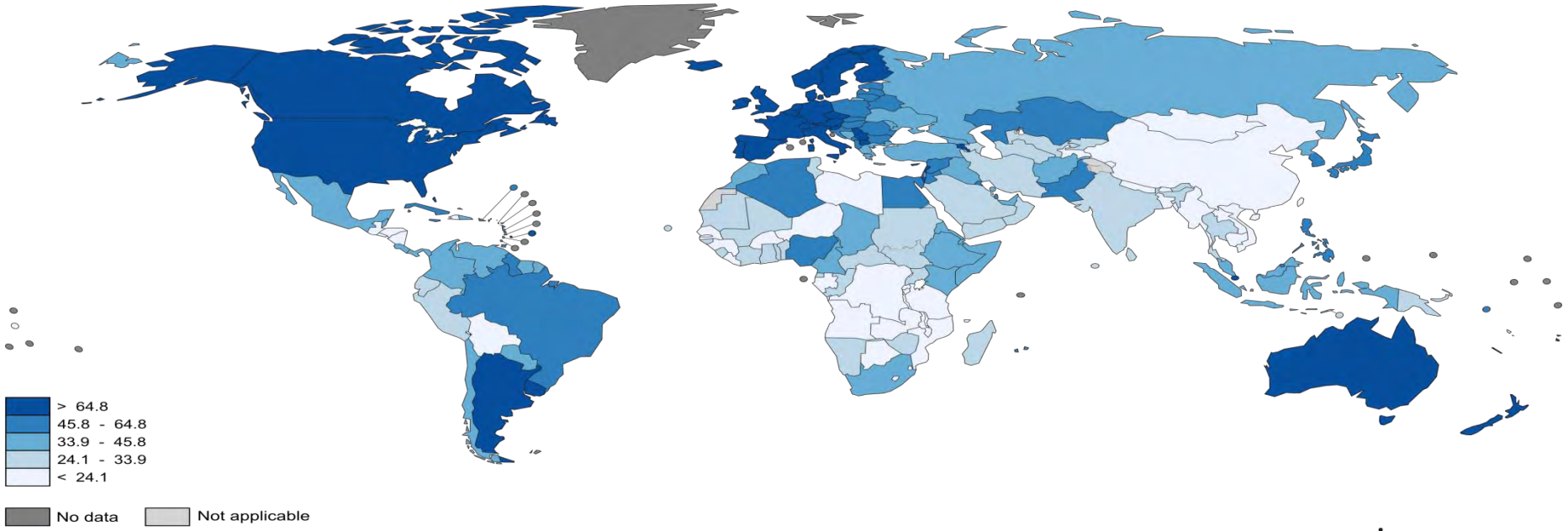
# The problem

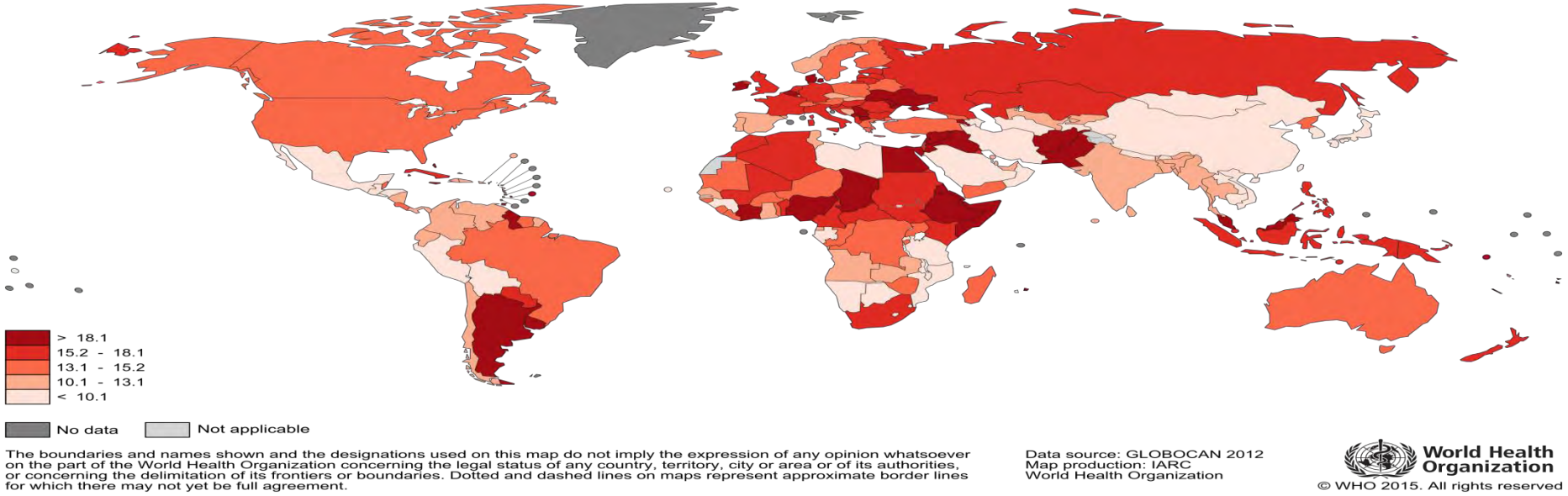

- ****MAJOR CHALLENGE****
  - identifying patients who are more likely to develop recurrence of the disease

- **CANCER DIVERSITY**
  - **TUMOR BIOLOGY**
  - Tumor microenvironment
  - Individual
    - ✓ Age
    - ✓ Menopausal status
    - ✓ Lifestyles
    - ✓ Individual environment




International Agency for Research on Cancer (IARC) and World Health Organization (WHO). GLOBOCAN 2012: Estimated cancer incidence, mortality and prevalence worldwide in 2012. **http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx, 2016.**

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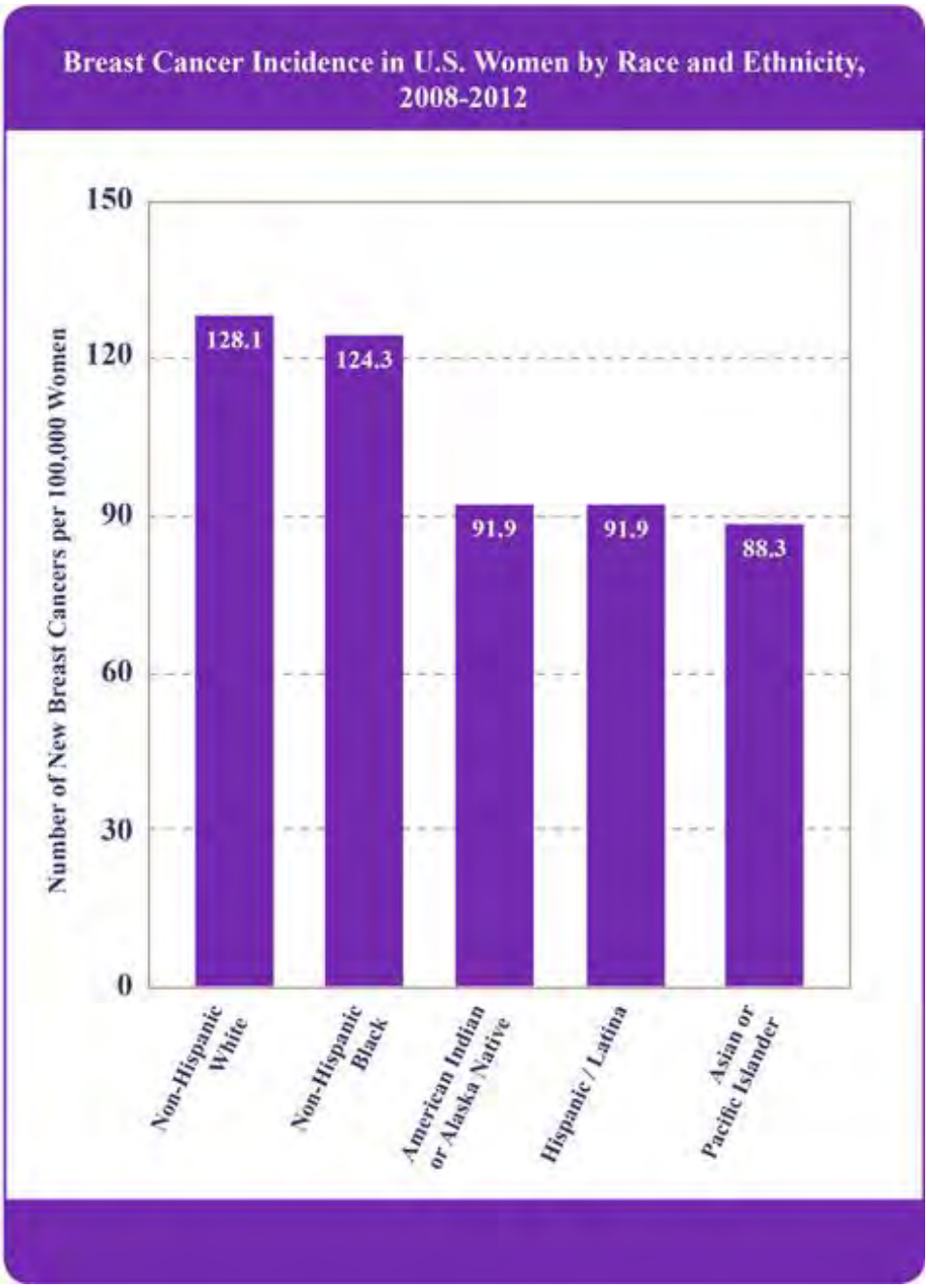
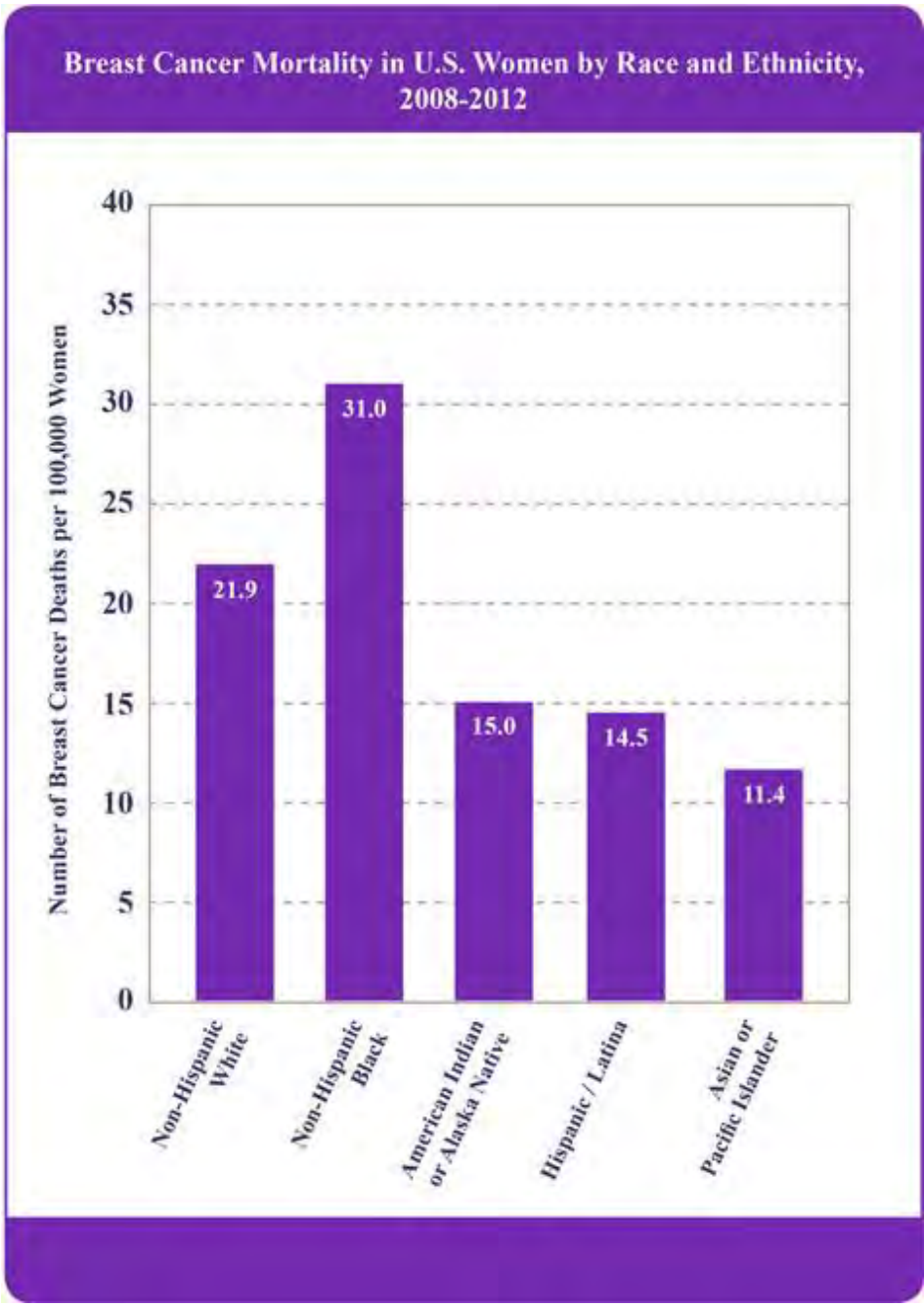
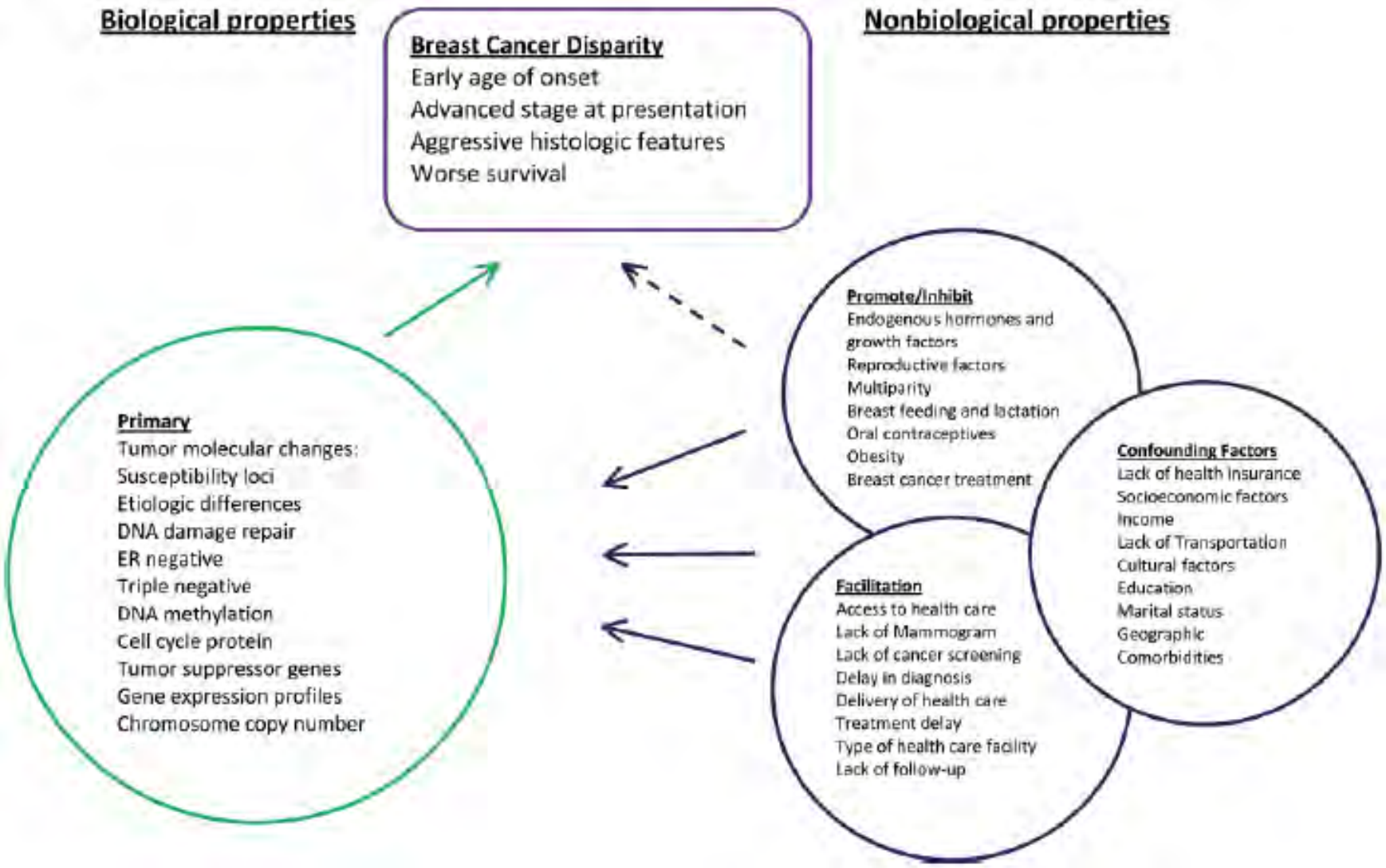


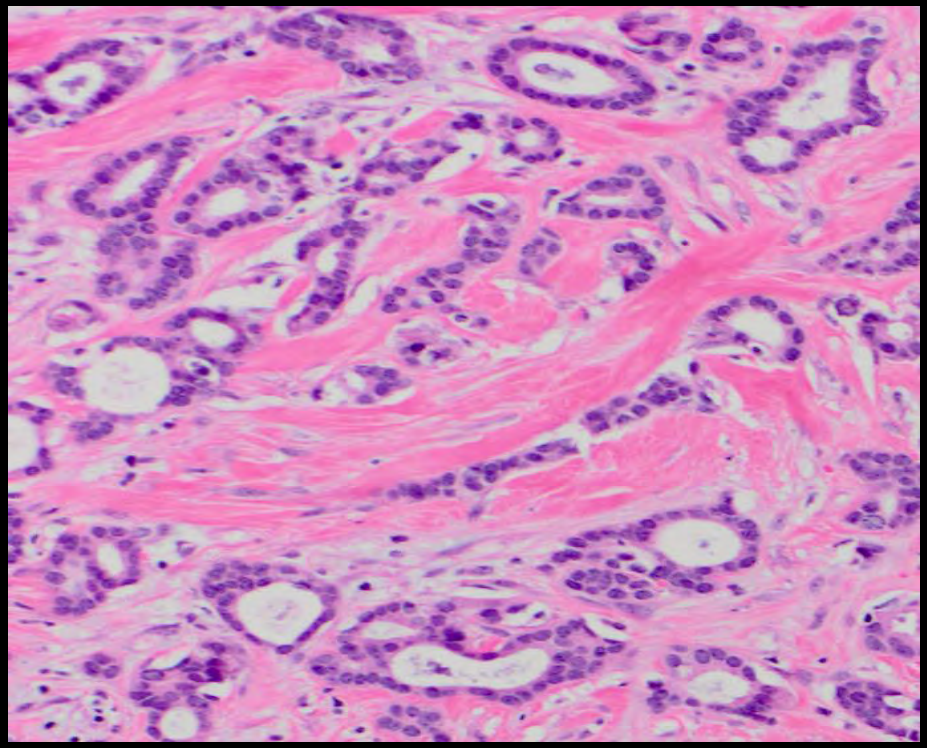

American Cancer Society. Breast Cancer Facts and Figures 2015-2016. Atlanta, GA: American Cancer Society, 2015

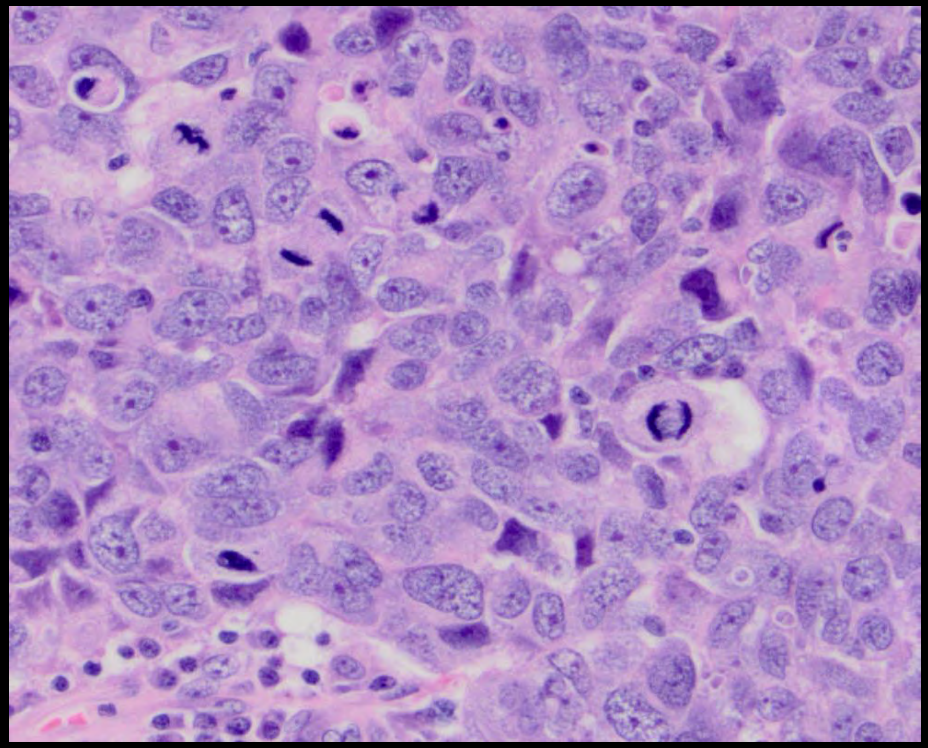



**Danforth D. Disparities in breast cancer outcomes between Caucasian and African American women: a model for describing the relationship of biological and nonbiological factors. *Breast Cancer Res*. 2013;15:1-13**

# Breast Tumor Biology

- **Morphology**





The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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

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

| Variable | Analysis without Recurrence Score | | Analysis with Recurrence Score | |
|---|---|---|---|---|
| | *P* Value | Hazard Ratio | *P* Value | Hazard Ratio |
| Age at surgery | 0.1 | 0.7 | 0.22 | 0.76 |
| Clinical tumor size | 0.13 | 1.35 | 0.38 | 1.19 |
| **Tumor grade** | | | | |
| **Moderately differentiated** | **0.04** | **1.87** | 0.15 | 1.55 |
| **Poorly differentiated** | **<0.001** | **5.14** | **<0.001** | **3.34** |
| HER2 amplification | 0.89 | 1.04 | 0.06 | 0.51 |
| Estrogen-receptor protein | | | | |
| 50-99 fmol/mg | 0.23 | 0.71 | 0.32 | 0.75 |
| 100-199 fmol/mg | 0.38 | 0.78 | 0.72 | 0.9 |
| >200 fmol/mg | 0.9 | 0.97 | 0.94 | 1.02 |
| **Recurrence Score** | | | **<0.001** | **2.81** |

**Paik et al.** A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004;351:2817-2826.

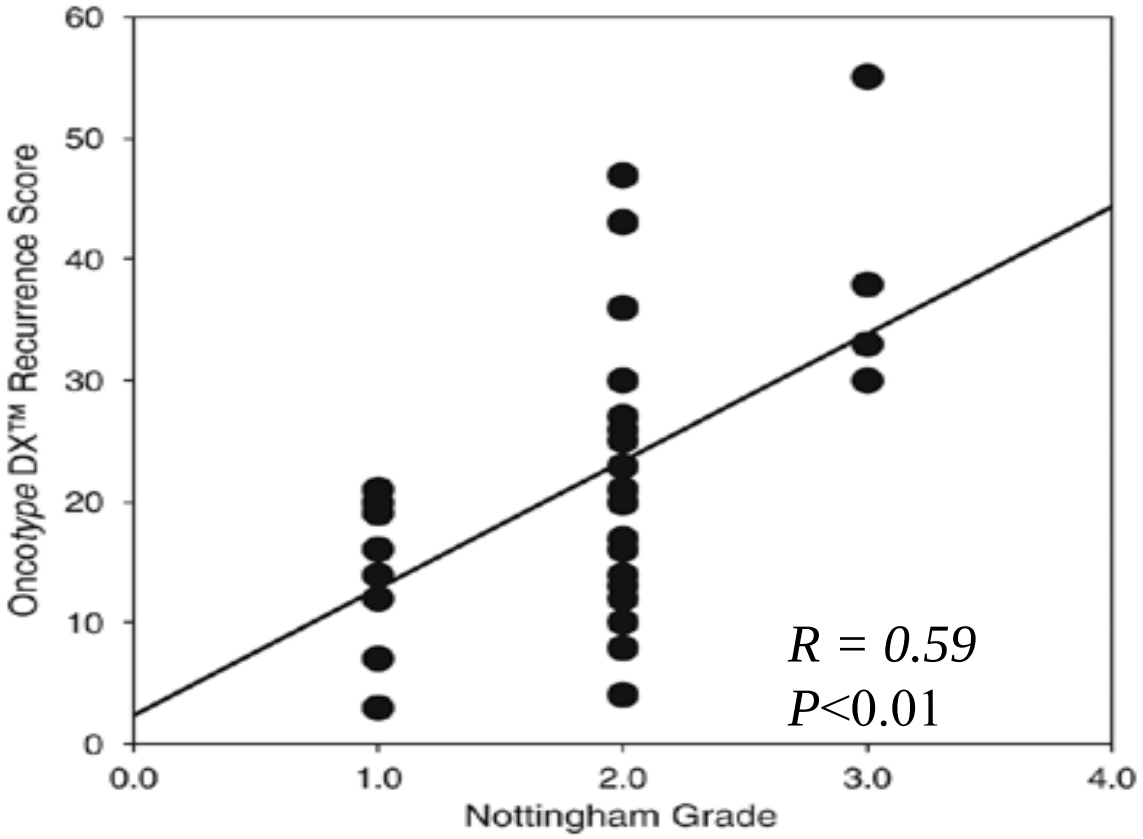
Modern Pathology (2008) 21, 1255–1261

www.modernpathology.org

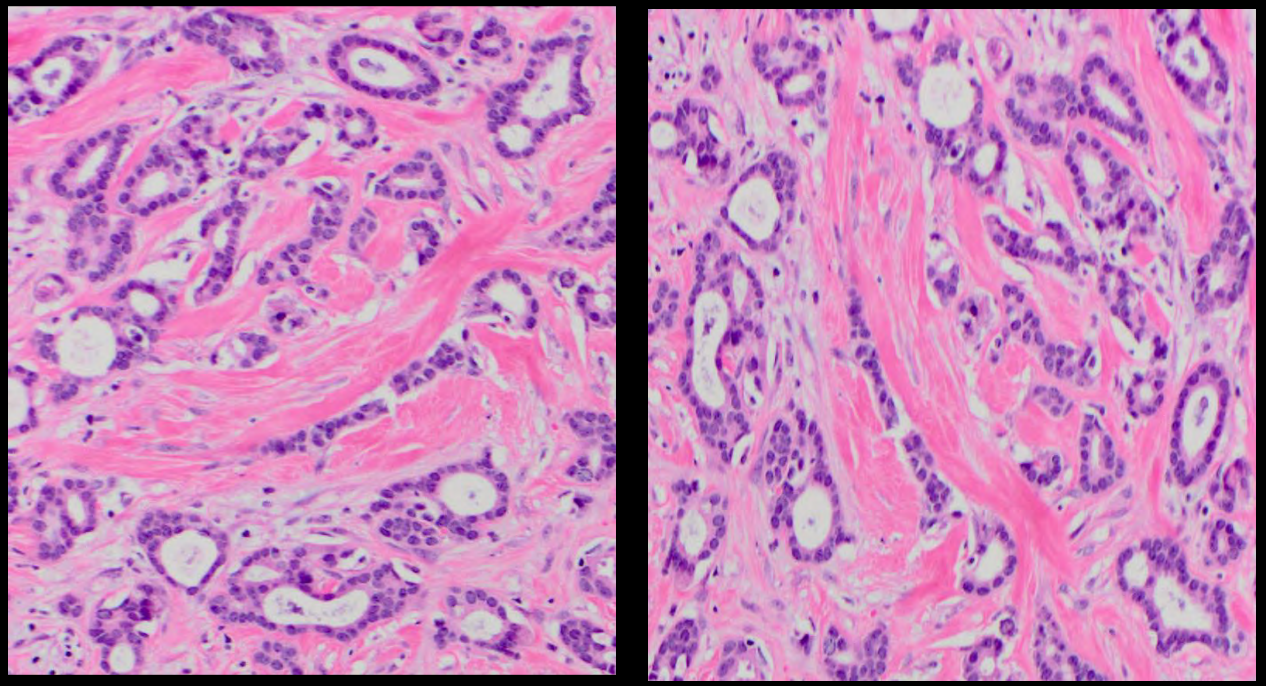
# Histopathologic variables predict Oncotype DX™ Recurrence Score

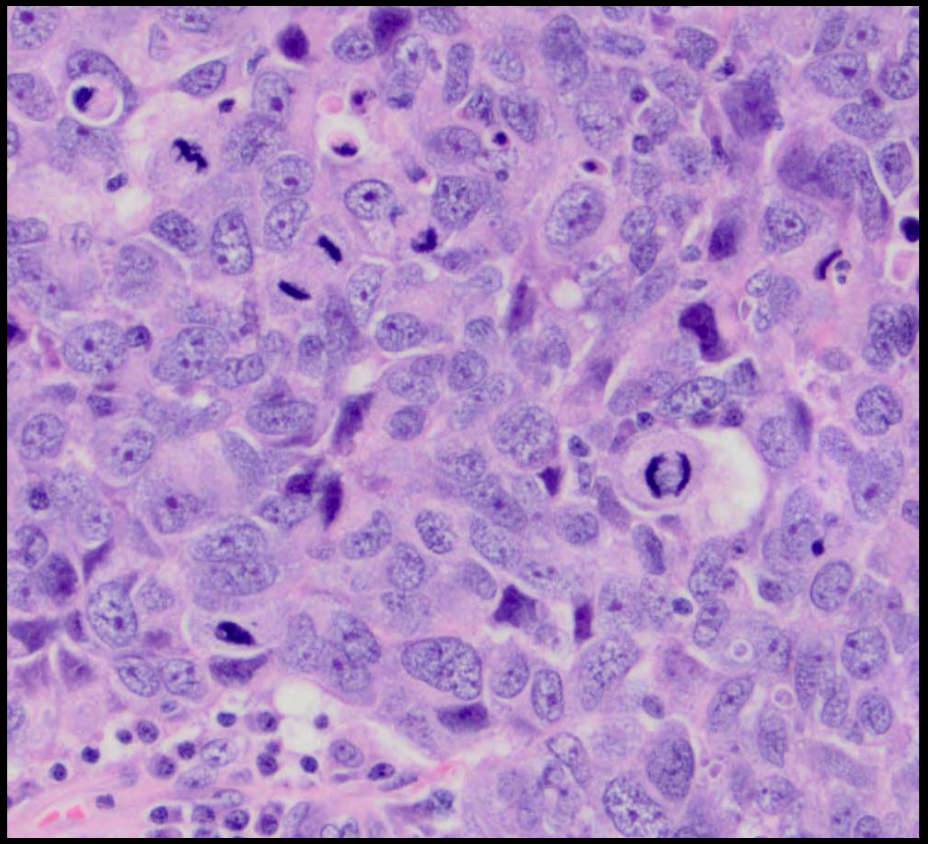
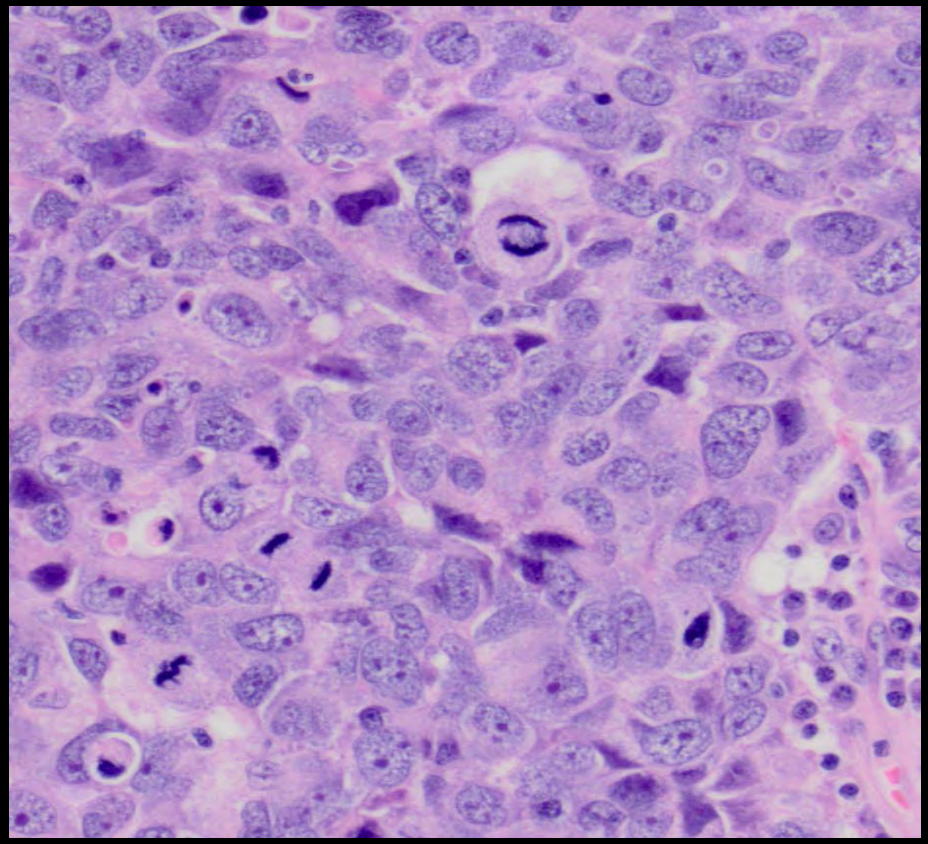
Melina B Flanagan[1], David J Dabbs[1], Adam M Brufsky[2], Sushil Beriwal[3] and Rohit Bhargava[1]

*[1]Department of Pathology, Magee-Women's Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA, USA; [2]Department of Medical Oncology, Magee-Women's Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA, USA and [3]Department of Radiation Oncology, Magee-Women's Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA, USA*




**Flanagan MB. et al**. Histopathologic variables predict Oncotype DX® recurrence score. *Mod Pathol.* 2008;21:1255-61



# Breast Tumor Biology

- Morphology
- Immunohistochemistry
  - **Estrogen receptor (ER)**
  - **Progesterone receptor (PR)**
  - **Human epidermal growth factor receptor-2 (HER-2)**
  - **Ki-67**

# ER

- Perhaps the most successful example of the use of a biomarker for guiding cancer therapy
- Important member of a family of intracellular steroid hormone receptors
- Involved in the expression of several genes associated with breast tumor proliferation.
- Clinical benefit appears to be positively correlated with the degree of ER expression

Modern Pathology (2008) 21, 1255–1261

www.modernpathology.org

# Histopathologic variables predict Oncotype DX™ Recurrence Score

Melina B Flanagan[1], David J Dabbs[1], Adam M Brufsky[2], Sushil Beriwal[3] and Rohit Bhargava[1]

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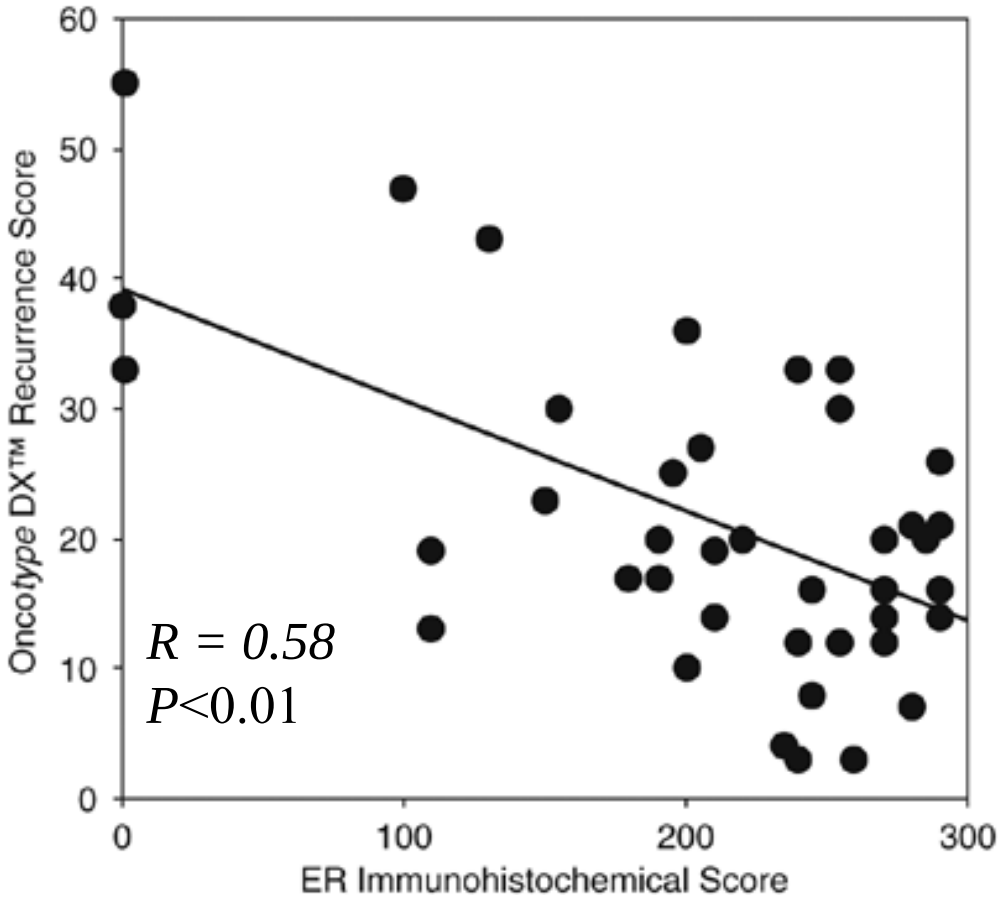


**Flanagan MB. et al**. Histopathologic variables predict Oncotype DX® recurrence score. *Mod Pathol.* 2008;21:1255-61

# PR

- Also a steroid hormone
- Regulated by ER
- Also reported to possibly self-regulate
- An indicator of a functionally intact ER pathway,
- Also suggested to be involved in the expression of several genes associated with breast tumor proliferation
- Clinical benefit also appears to be positively correlated with the degree of PR expression

*Cancer Investigation*, 28:978–982, 2010
ISSN: 0735-7907 print / 1532-4192 online

DOI: 10.3109/07357907.2010.496754

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ORIGINAL ARTICLE
Imaging, Diagnosis, Prognosis

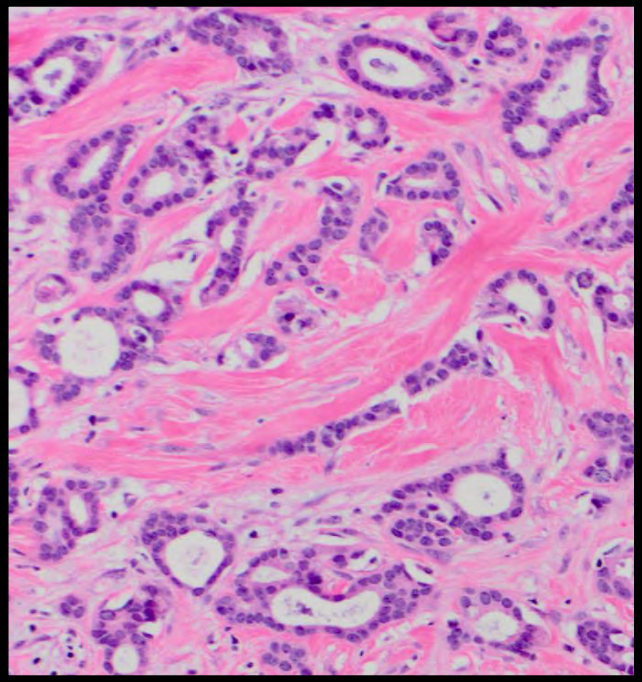
# A Lower Allred Score for Progesterone Receptor Is Strongly Associated With a Higher Recurrence Score of 21-Gene Assay in Breast Cancer

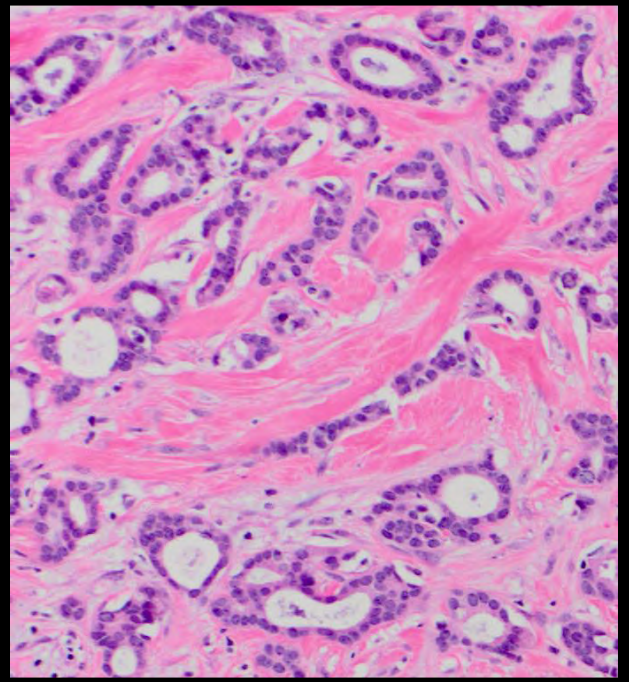
Ping Tang,[1] Jianmin Wang,[2] David G. Hicks,[1] Xi Wang,[1] Linda Schiffhauer,[1] Loralee McMahon,[1] Qi Yang,[1] Michelle Shayne,[1] Alissa Huston,[1] Kristin A. Skinner,[1] Jennifer Griggs[3] and Gary Lyman[4]

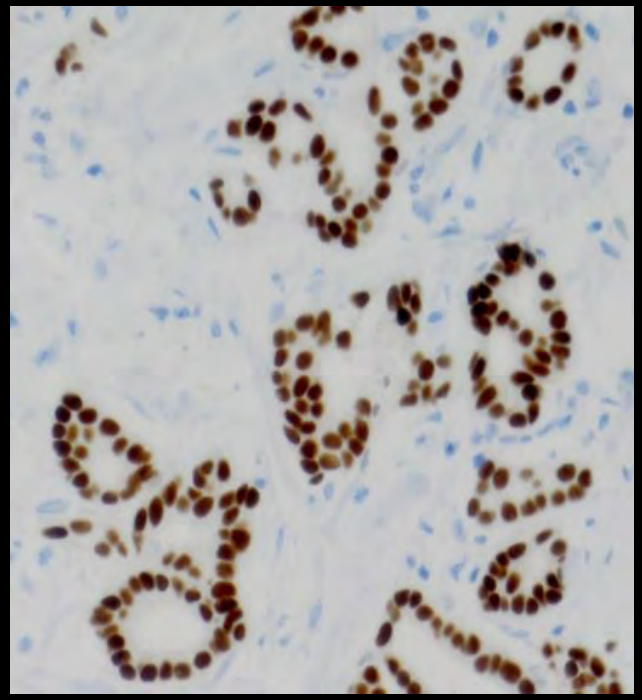
*Department of Pathology, University of Rochester of Rochester Medical Center, Rochester, New York, USA*[1]
*RTI Health Solution, Research Triangle Park, North Carolina, USA*[2]
*Department of Medicine, University of Michigan, Ann Arbor, Michigan, USA*[3]
*Department of Medicine, Duke University School of Medicine, Durham, North Carolina, USA*[4]

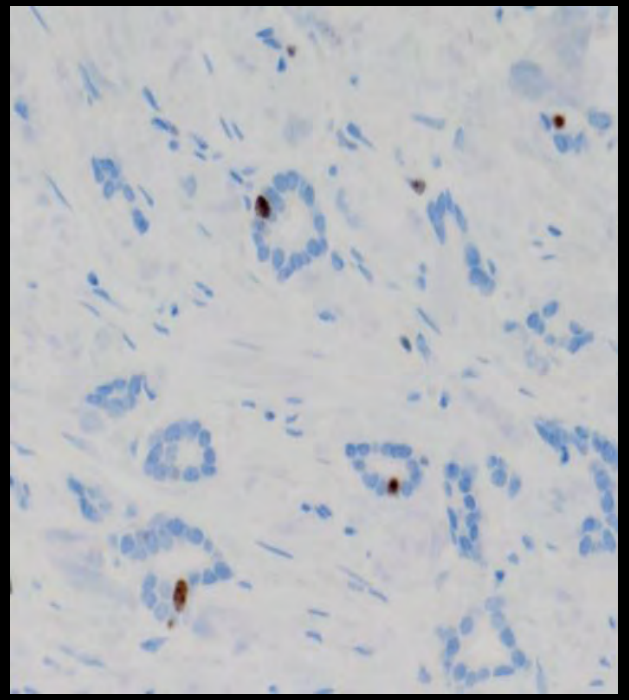
## ABSTRACT

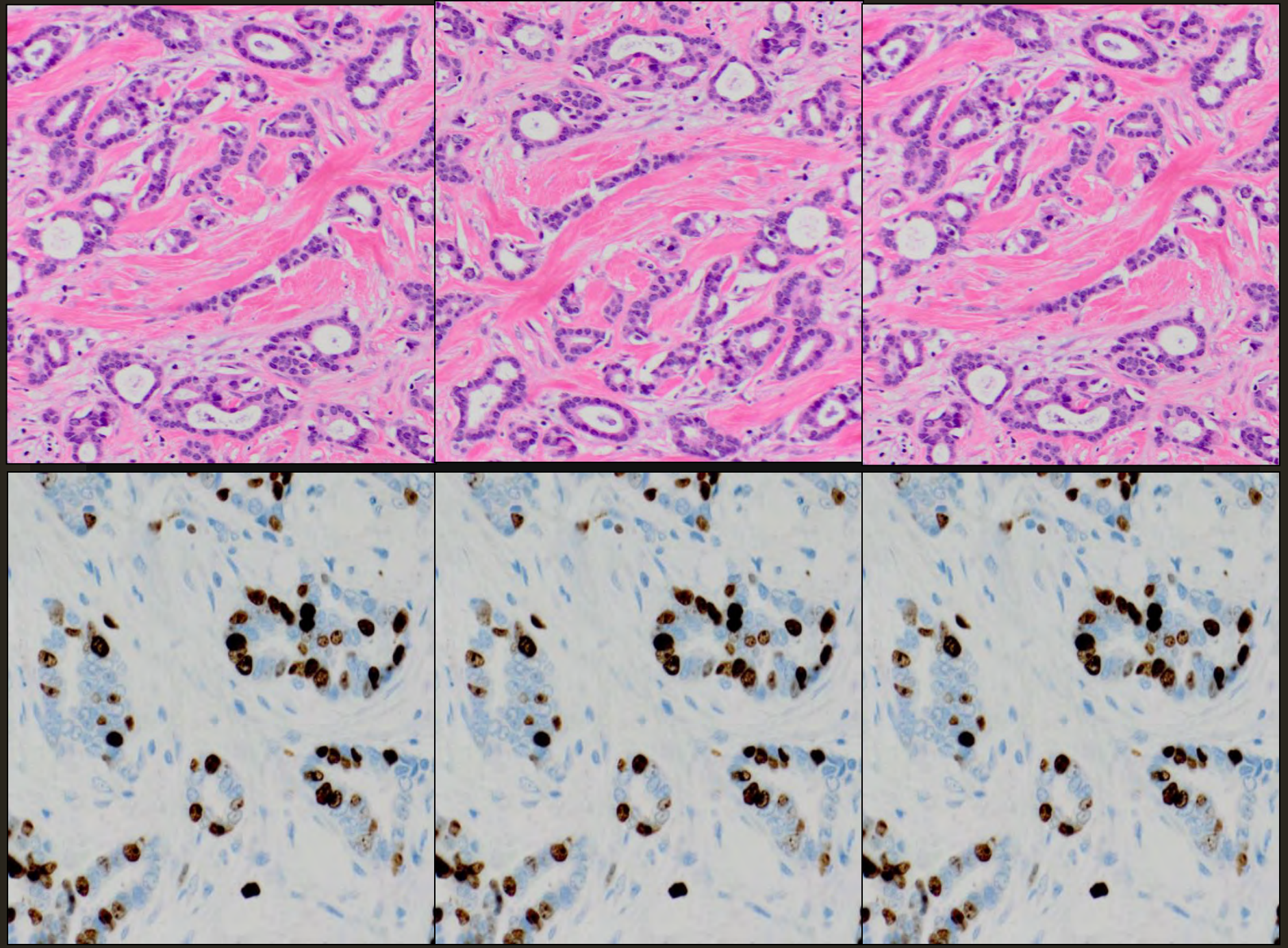
Among the 77 infiltrating breast carcinomas, we found that progesterone receptor (PR) expression was inversely associated with recurrence score (RS, $p < .0001$). RS is also significantly associated with tubule formation, mitosis, and luminal B subtype. The equation of RS = 17.489 + 2.071 (tubal formation) + 2.926 (mitosis) – 2.408 (PR) – 1.061 (HER2) + 7.051 (luminal A) + 29.172 (luminal B) predicts RS with an $R^2$ of 0.65. In conclusion, PR negativity, luminal B subtype, tubal formation, and mitosis are strongly correlated with a higher RS.











# Breast Tumor Biology

- Morphology
- Immunohistochemistry
  - Estrogen receptor (ER)
  - Progesterone receptor (PR)
  - **Human epidermal growth factor receptor-2 (HER-2)**

# Her-2

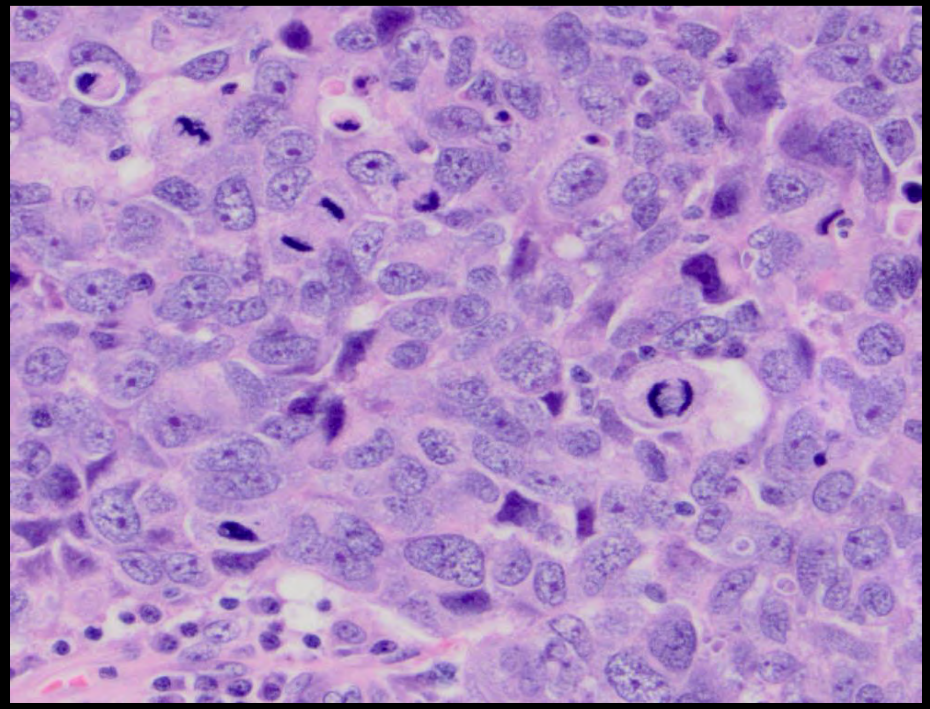
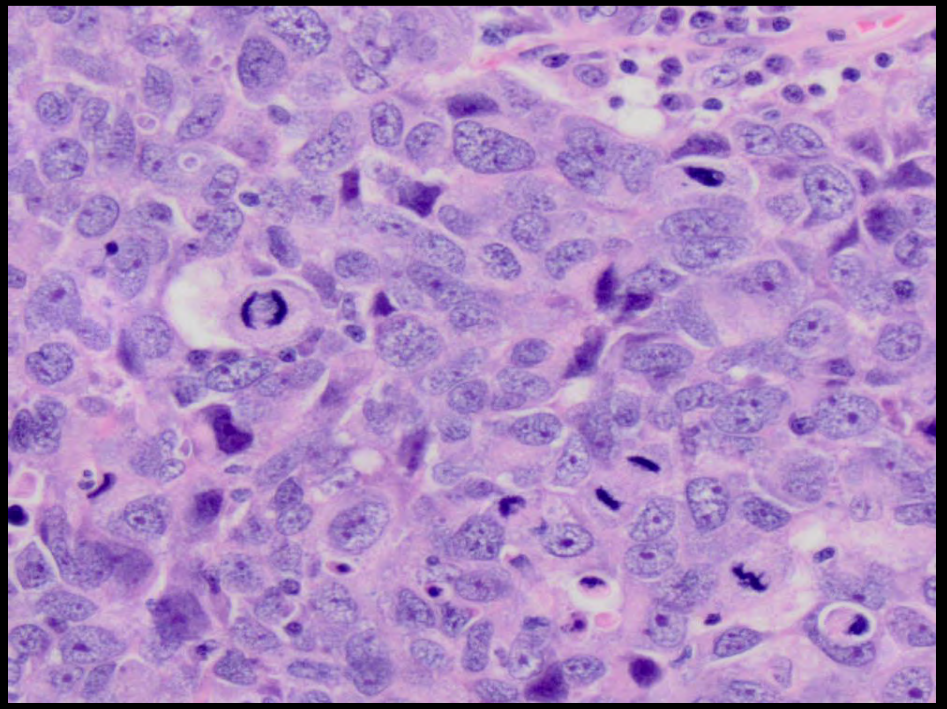
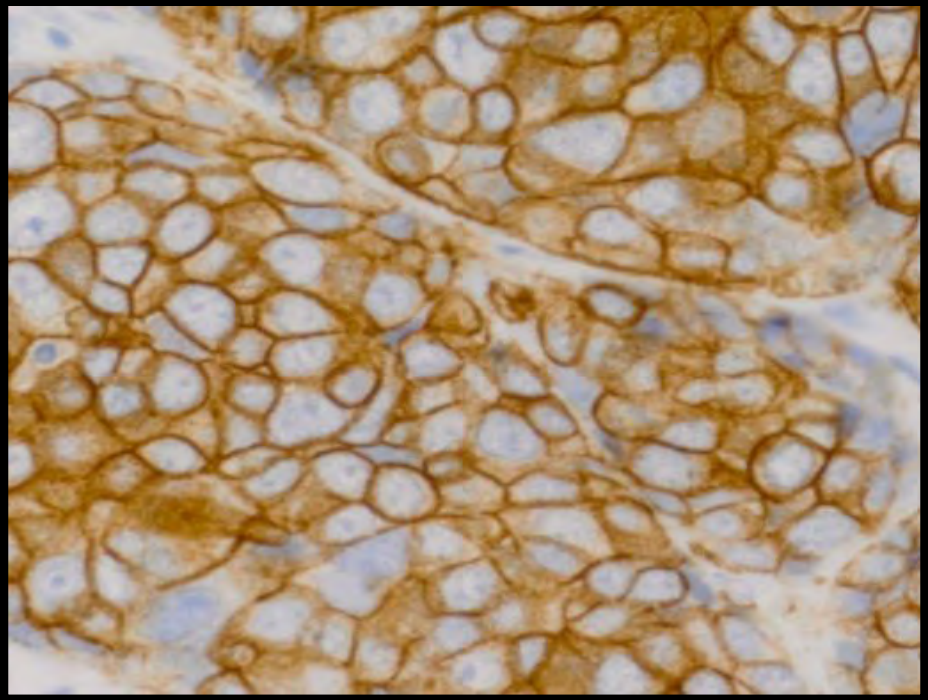
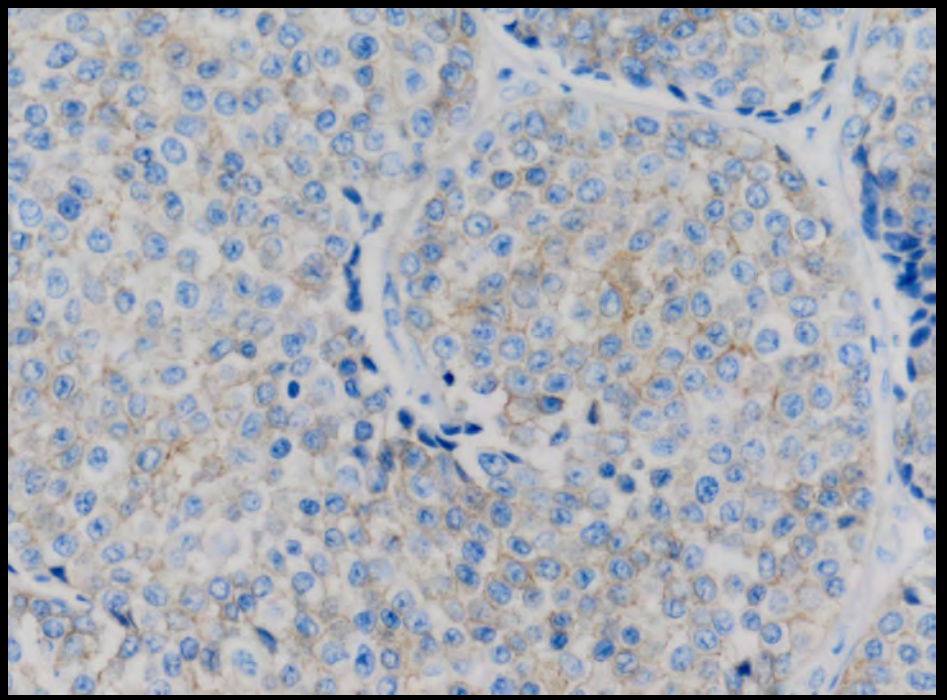
- A tyrosine kinase receptor that is a member of the Human Epidermal family of growth receptors.
- Involved in the complex regulation of cell proliferation and angiogenesis, and in enhancing cell survival pathways.
- Amplified in approximately 12%-18% of breast cancers.

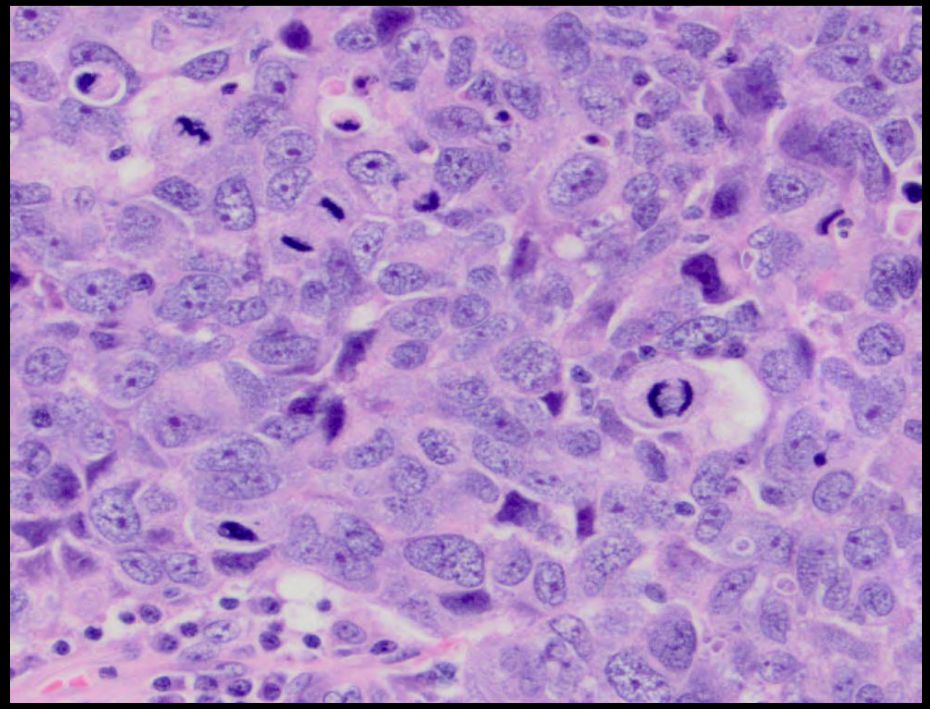
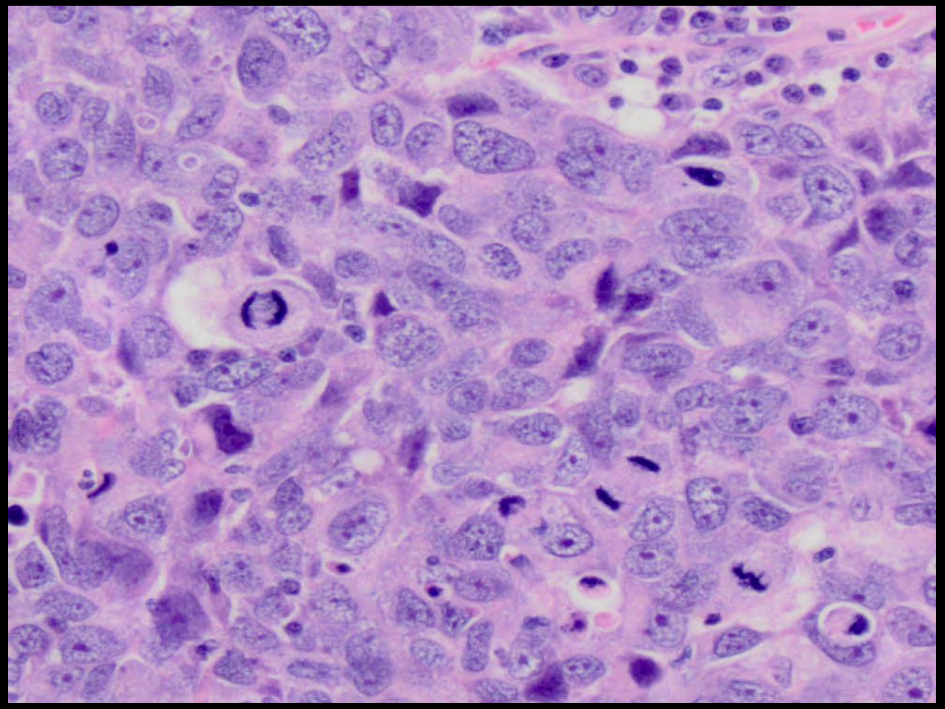
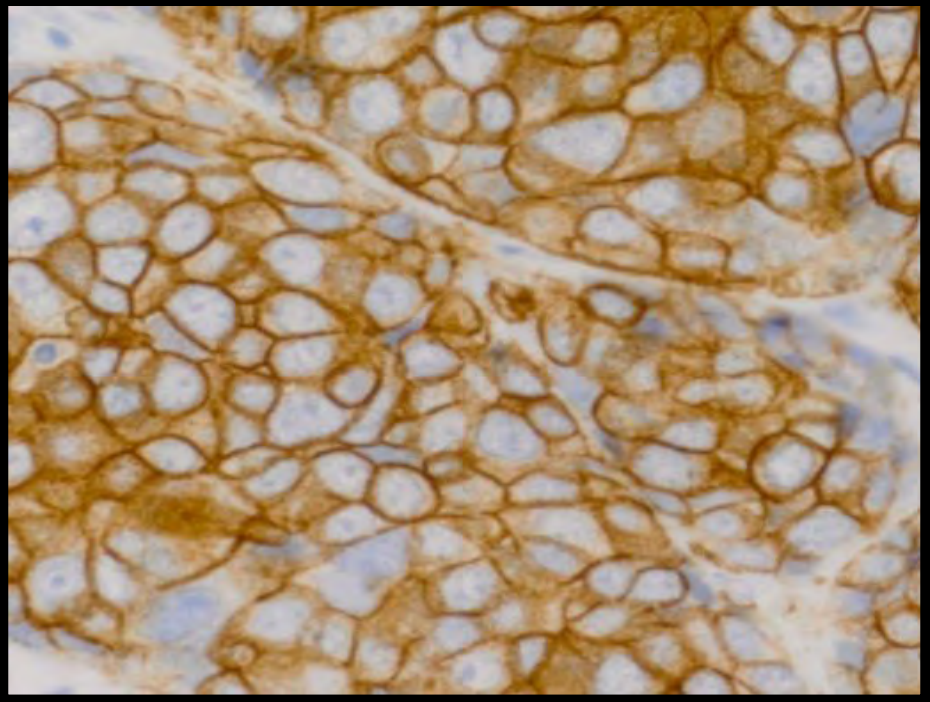
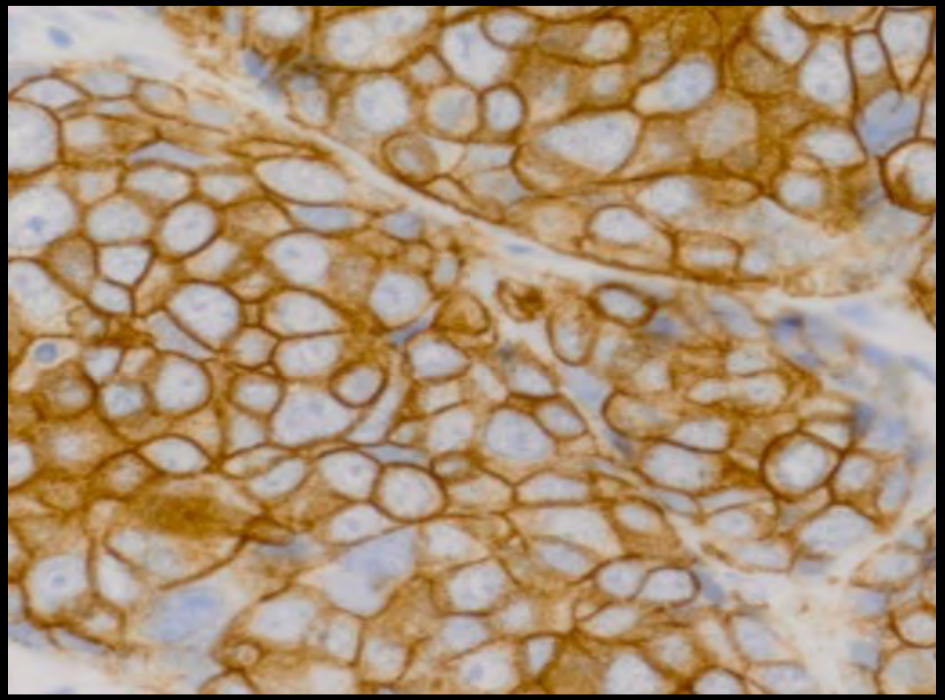
# Her-2

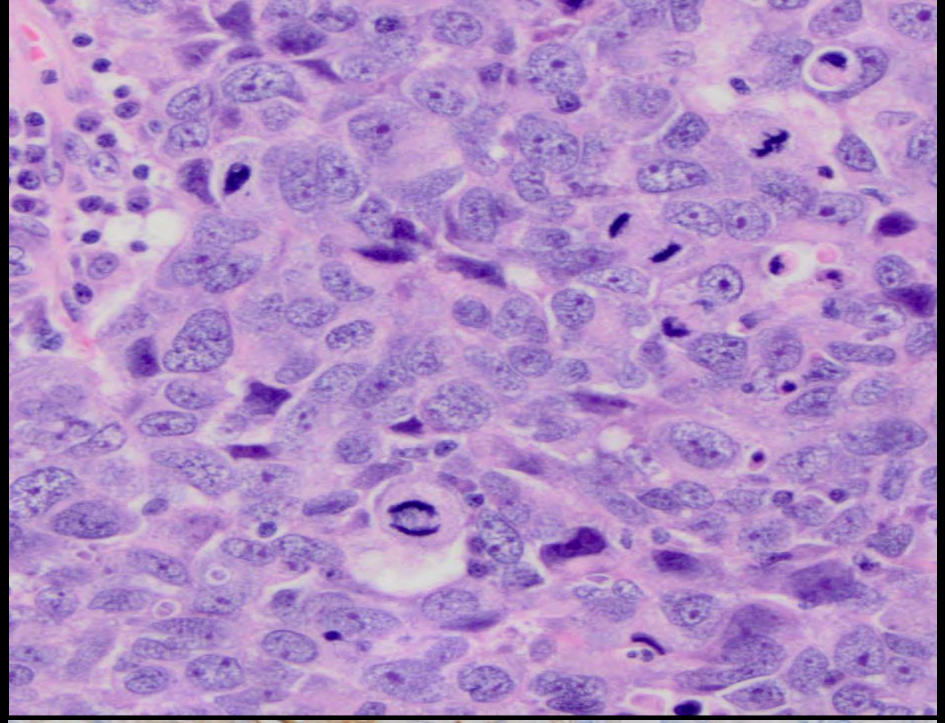
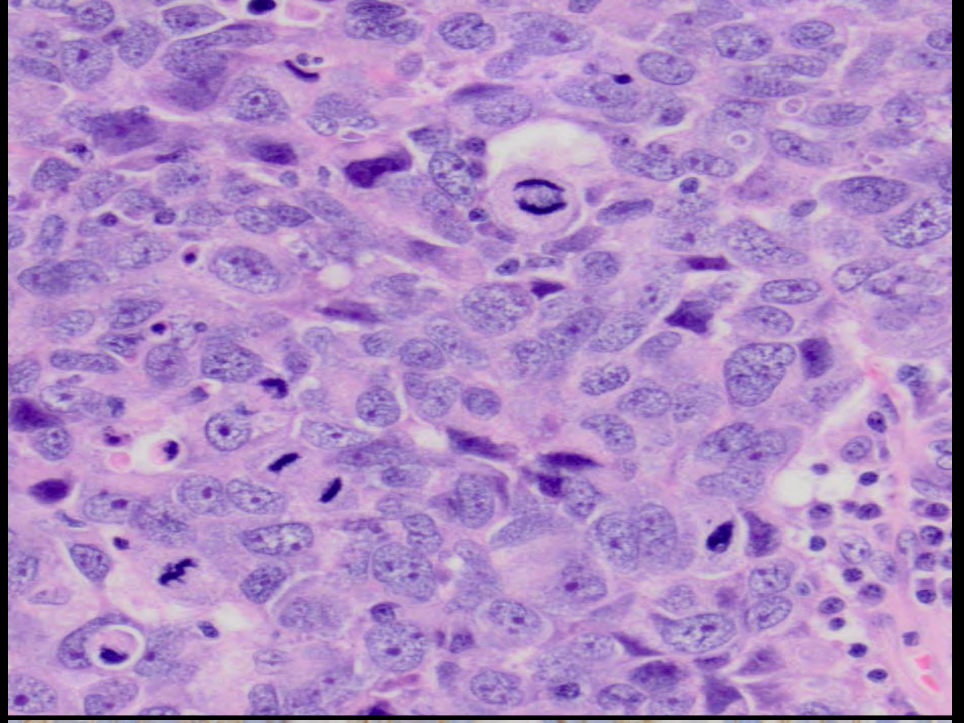
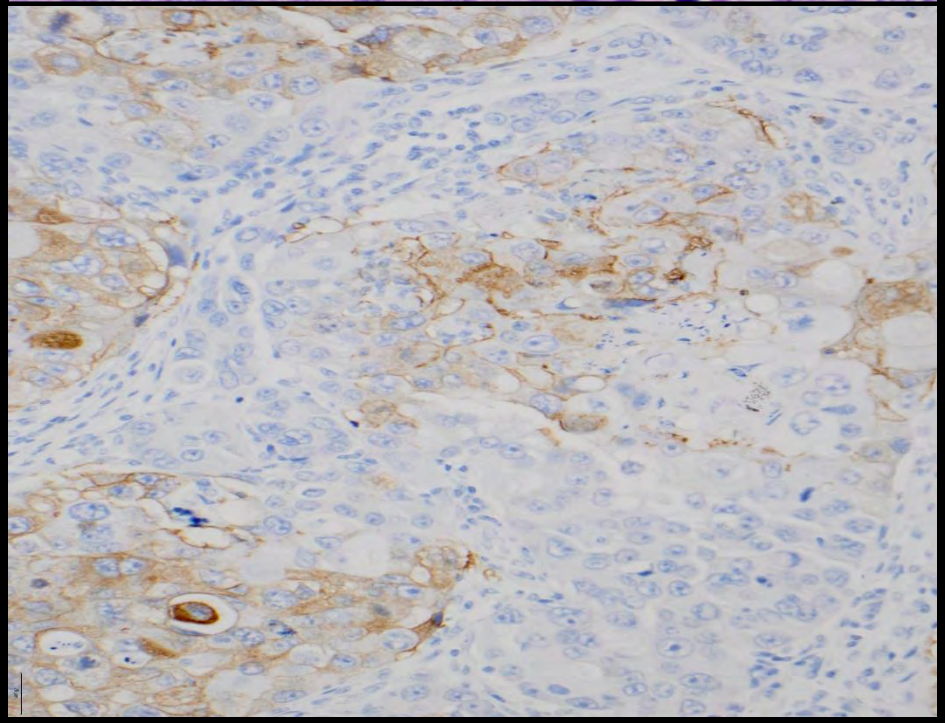
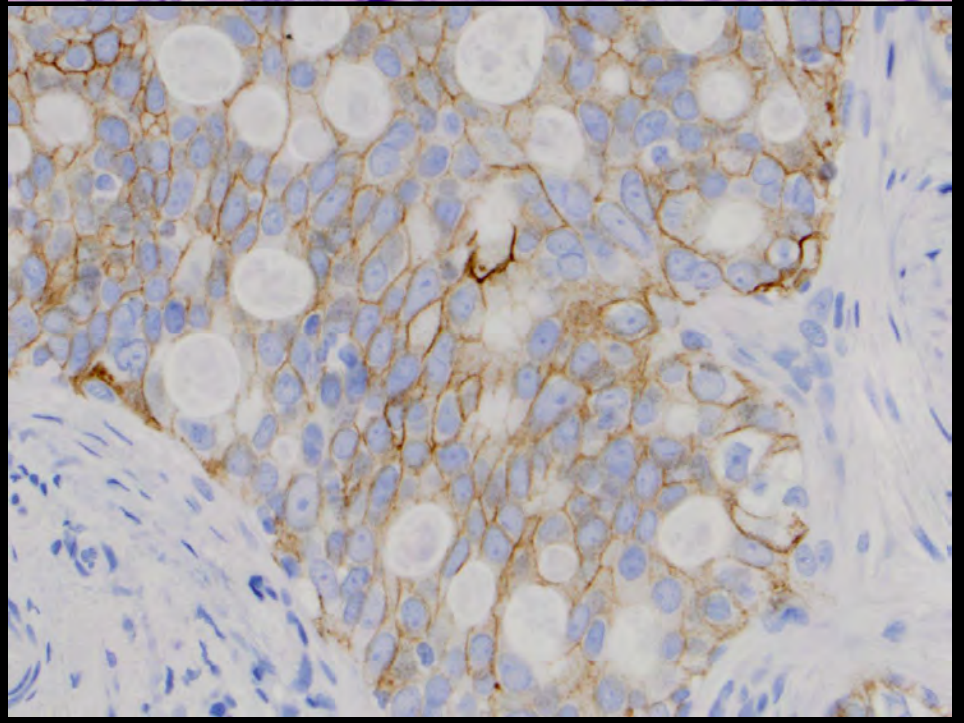
- Gene amplification increases the likelihood of a more aggressive tumor biology and an association of higher recurrence and mortality rates.

- Trastuzumab, Lapatinib, Pertuzumab l which target the HER-2 pathway in Her-2 positive breast cancer and other solid tumors

- Protein overexpression occurs when the Her-2 gene is amplified, thus making HER-2 immunohistochemistry a surrogate for gene amplification.

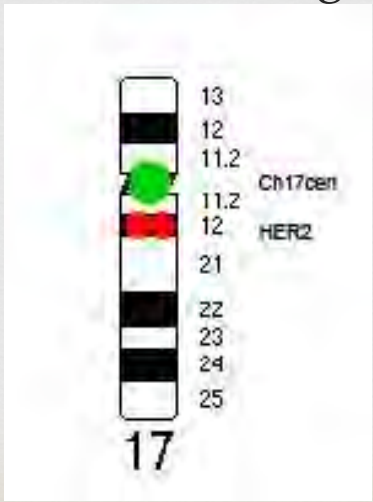





# Breast Tumor Biology

- Morphology
- Immunohistochemistry
  - Estrogen receptor (ER)
  - Progesterone receptor (PR)
  - Human epidermal growth factor receptor-2 (HER-2)
    - **Her-2 in-situ hybridization**
    - **Fluorescence in-situ hybridization (FISH)**

# Her-2 ISH

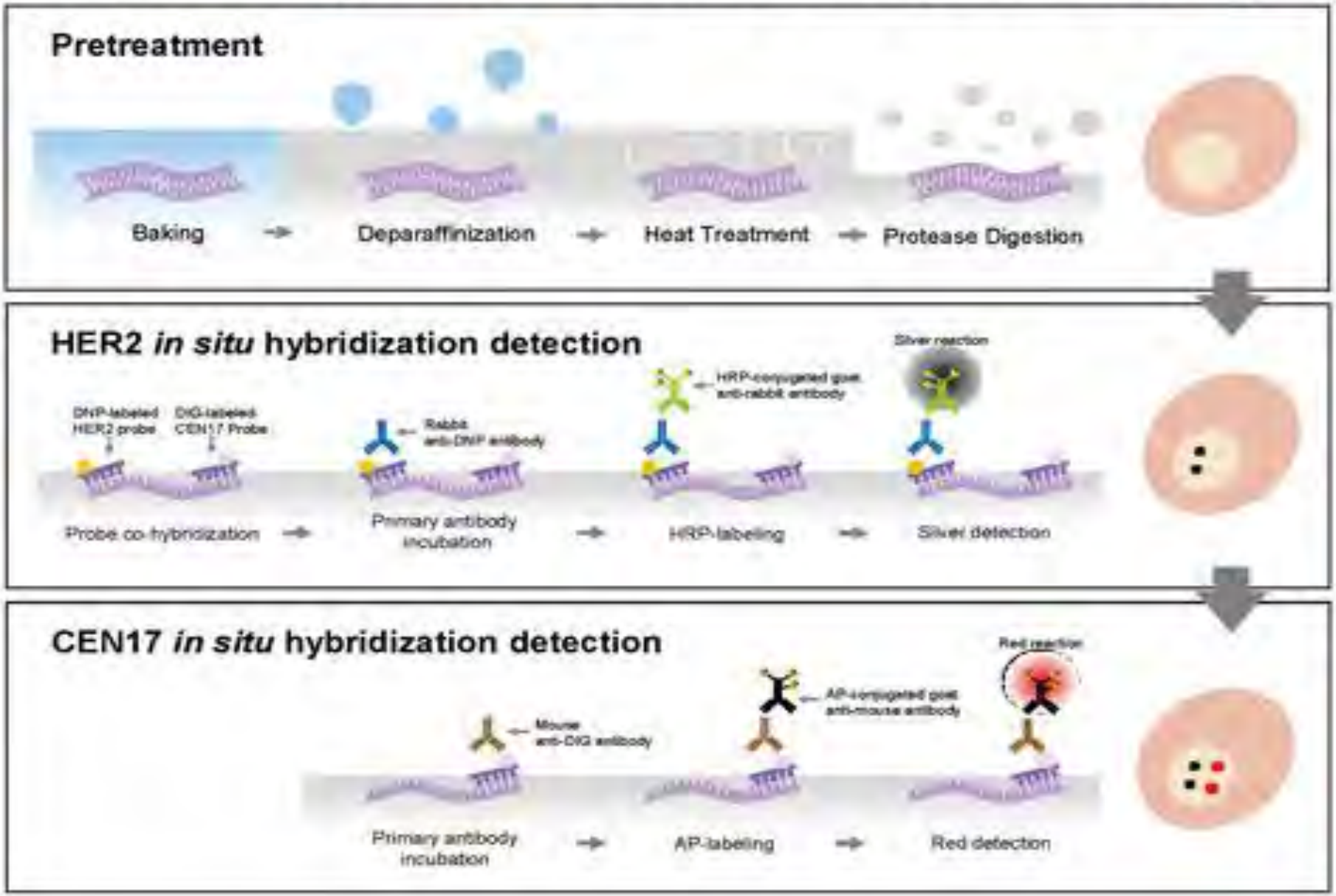
- **Quantitatively** determines Her-2 gene amplification in formalin-fixed paraffin embedded (FFPE) tissue
  - Single color ISH for the HER2 gene only
  - Dual color ISH for the HER2 gene and CEN17 (chromosome 17 centromere).
    - HER2 status is determined with the ratio of HER2 gene copy numbers to CEN17 copy numbers



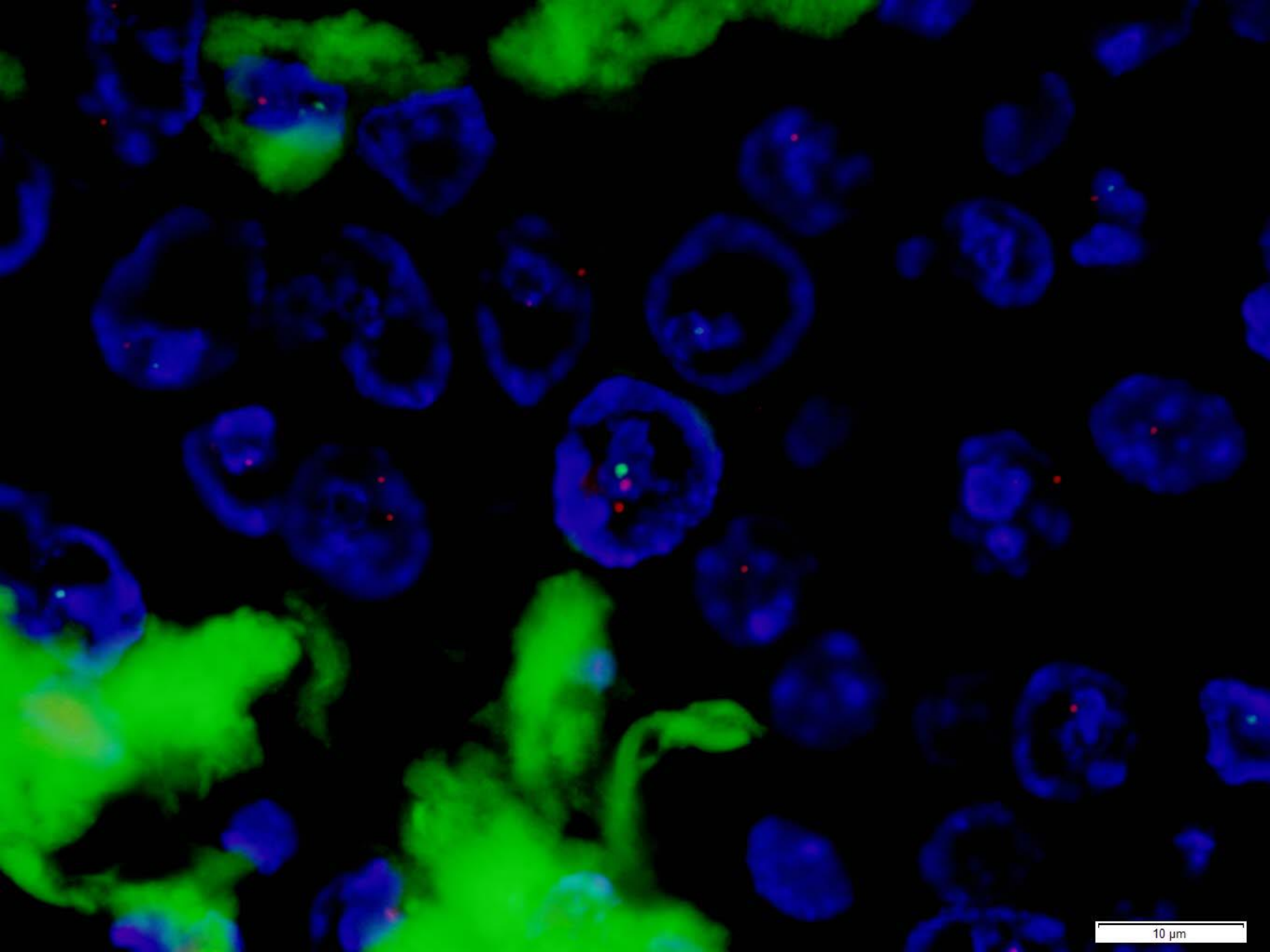

http://www.genemed.com/products/Fluorescent-In-Situ-Hybridization-Probes/HER2-Red-and-Chromosome-17-Centromere-Green-FISH-Probe-Cocktail

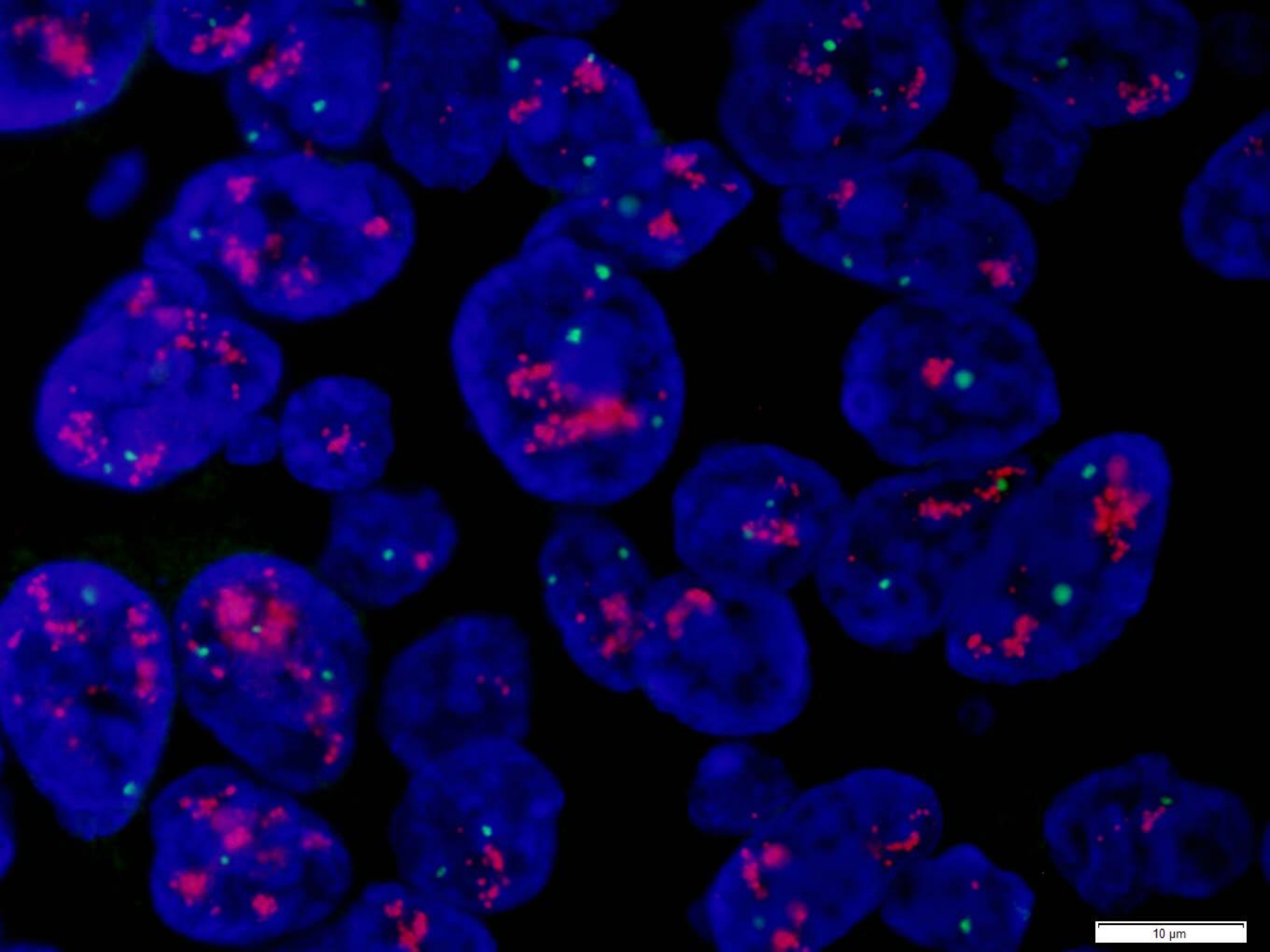
# Her-2 ISH

- Locate Her-2 equivocal areas on the Her-2 immunohistochemistry slide
- Evaluate the same area on the ISH stained slide




**Nitta H.** Automated HER2 testing: Personalized healthcare for breast cancer patients enabled by novel molecular morphology. Medical Laboratory Observer. June 20 2013.


10 µm


10 µm

# Breast Tumor Biology

- Morphology
- Immunohistochemistry
  - Estrogen receptor (ER)
  - Progesterone receptor (PR)
  - Human epidermal growth factor receptor-2 (HER-2)
    - **Her-2 Fluorescence in-situ hybridization (FISH)**
  - Ki-67

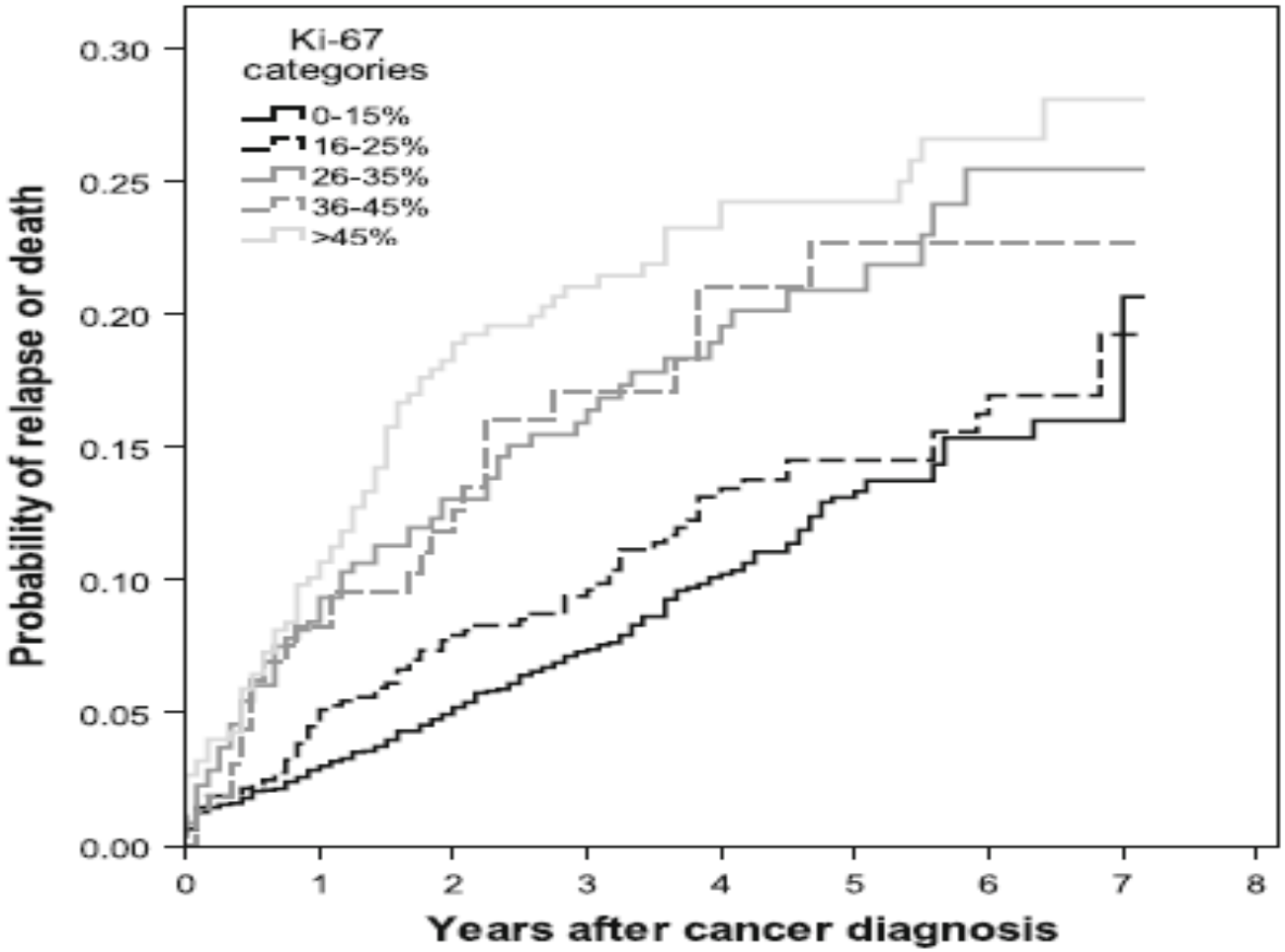
# Ki-67

- The proliferation marker Ki-67 is one of the most controversially discussed parameters for treatment decisions in breast cancer patients.

- Ki-67 positivity correlates with a higher risk of recurrence and a worse survival rate in patients with breast cancer.

# Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry

E. C. Inwald · M. Klinkhammer-Schalke · F. Hofstädter · F. Zeman · M. Koller · M. Gerstenhauer · O. Ortmann




**Inwald EC. et al.** Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry Breast Cancer Res Treat. 2013 Jun;139(2):539-52.

**Short Communication**

# Ki 67 is a major, but not the sole determinant of Oncotype Dx recurrence score

**S Sahebjam[1], R Aloyz[1,3], D Pilavdzic[2], M-L Brisson[2], C Ferrario[1], N Bouganim[1], V Cohen[1], WH Miller Jr[1,3] and LC Panasci[*,1,3]**

*[1]Department of Medical Oncology, Jewish General Hospital, McGill University, 3755 Côte Ste-Catherine Road, Montreal, QC H3T 1E2, Canada; [2]Department of Pathology, Jewish General Hospital, McGill University, Montreal, QC H3T 1E2, Canada; [3]Segal Cancer Center, Lady Davis Institute, Jewish General Hospital, Montreal, QC H3T 1E2, Canada*

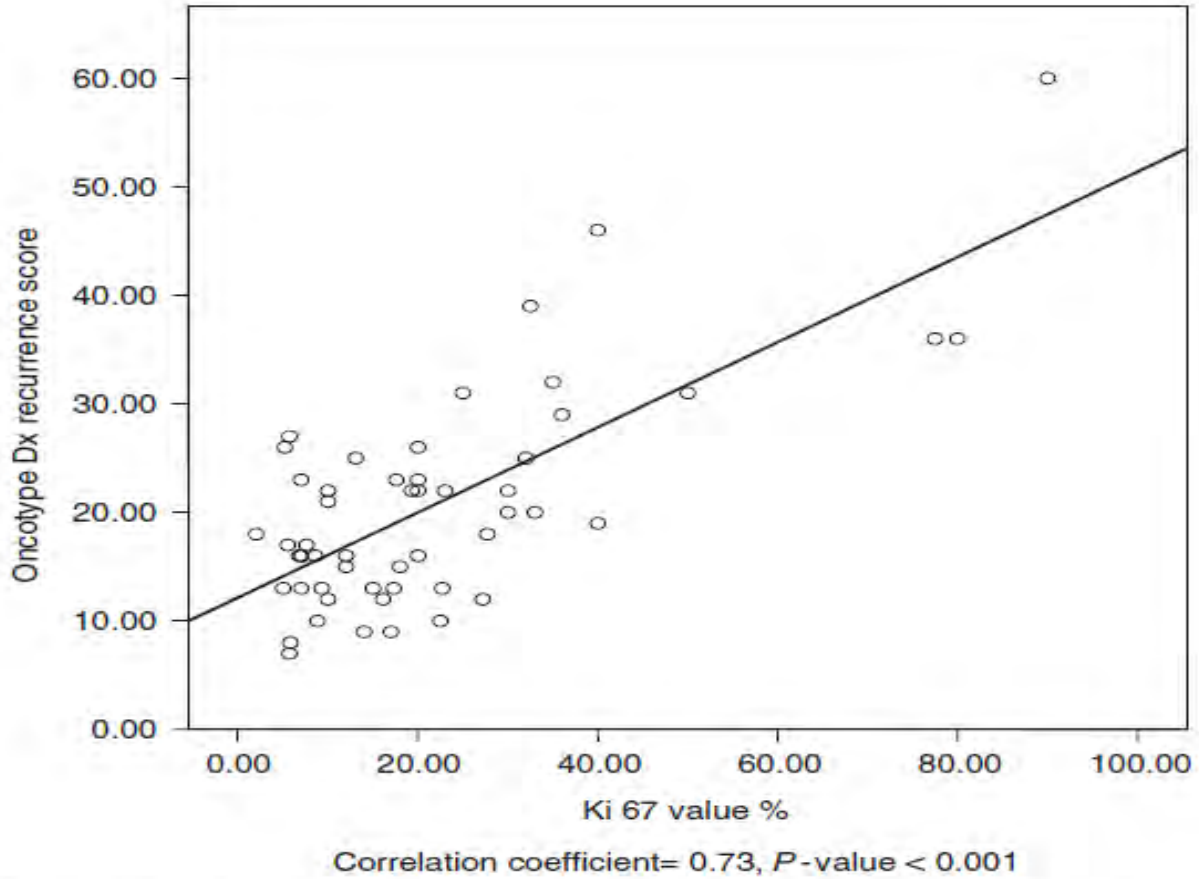
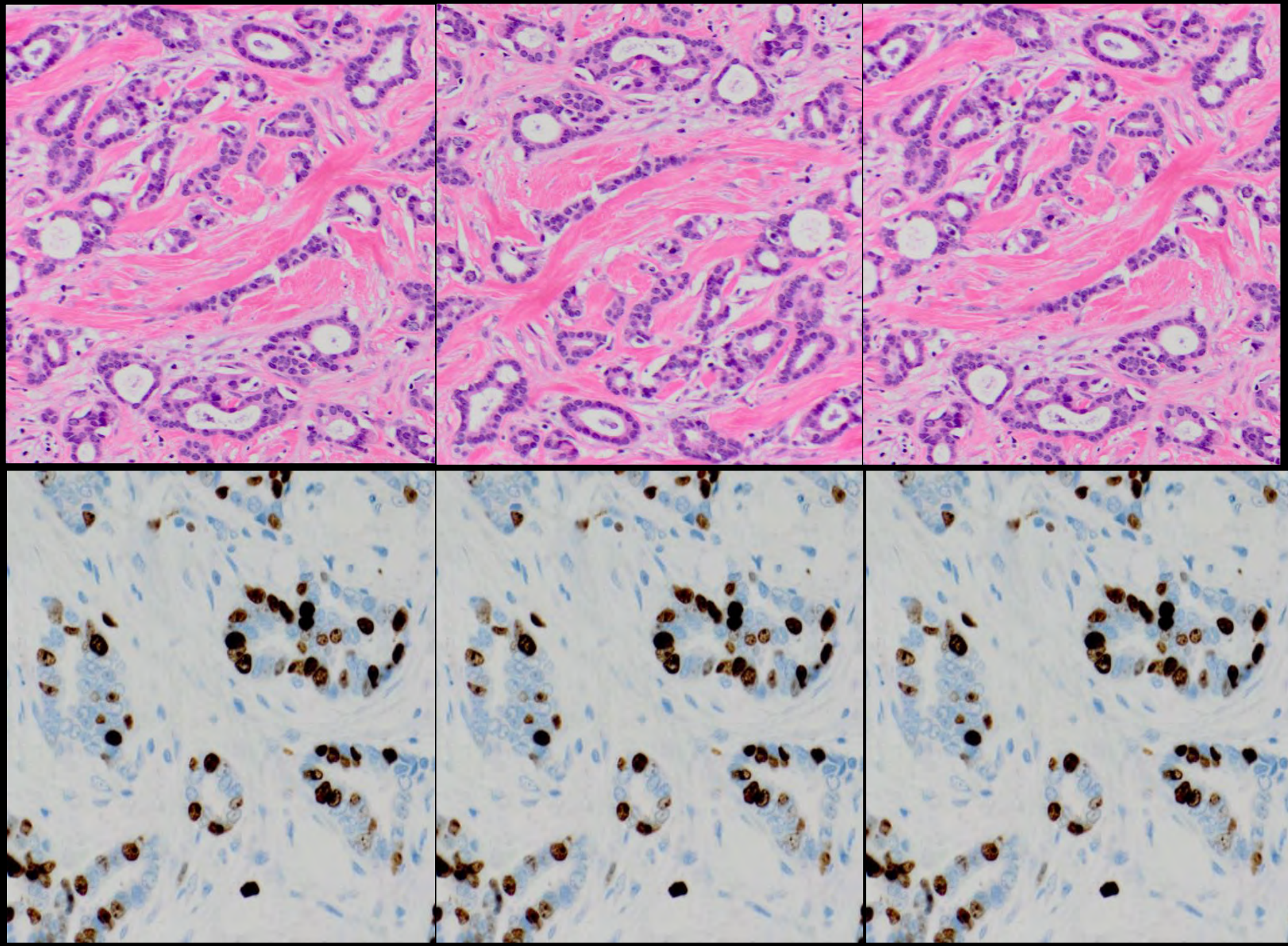



**Figure 1** Correlation of Oncotype DX RS with Ki 67 value. Correlation coefficient = 0.73, *P*-value < 0.001.



# Ki-67

- To date no standard operating procedure or generally accepted cut-off definition for Ki-67 exists.

# Ki-67

- Therefore, Ki-67 is not implemented in standard routine pathology so far

# Breast Tumor Biology

- Morphology
- Immunohistochemistry
  - Estrogen receptor (ER)
  - Progesterone receptor (PR)
  - Human epidermal growth factor receptor-2 (HER-2)
    - **Her-2 Fluorescence in-situ hybridization (FISH)**
  - Ki-67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# Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100 000 women in 123 randomised trials

Early Breast Cancer Trialists' Collaborative Group (EBCTCG)

- It has been shown repeatedly that the ***traditional method*** of using clinical and histopathologic factors in making treatment decisions regarding systemic chemotherapy has lead to overtreatment, with little if any impact on outcome

# Qualitative versus Quantitative approach

- This has primarily been because the evaluation of clinical and histopathologic factors has been a ***qualitative approach,*** as opposed to a more ***quantitative approach***.

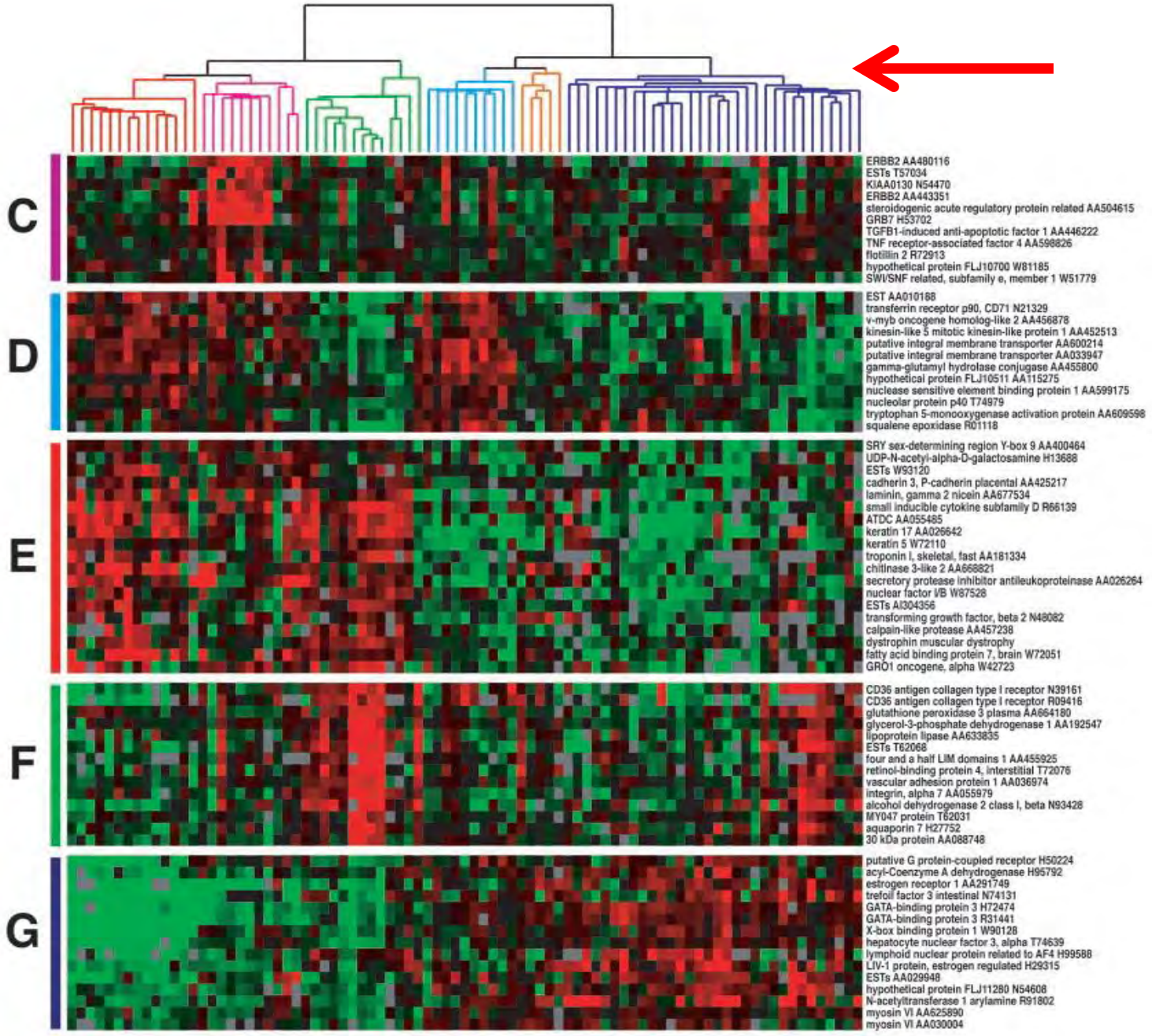

The Next Generation is
Coming...

# Multigene expression assays

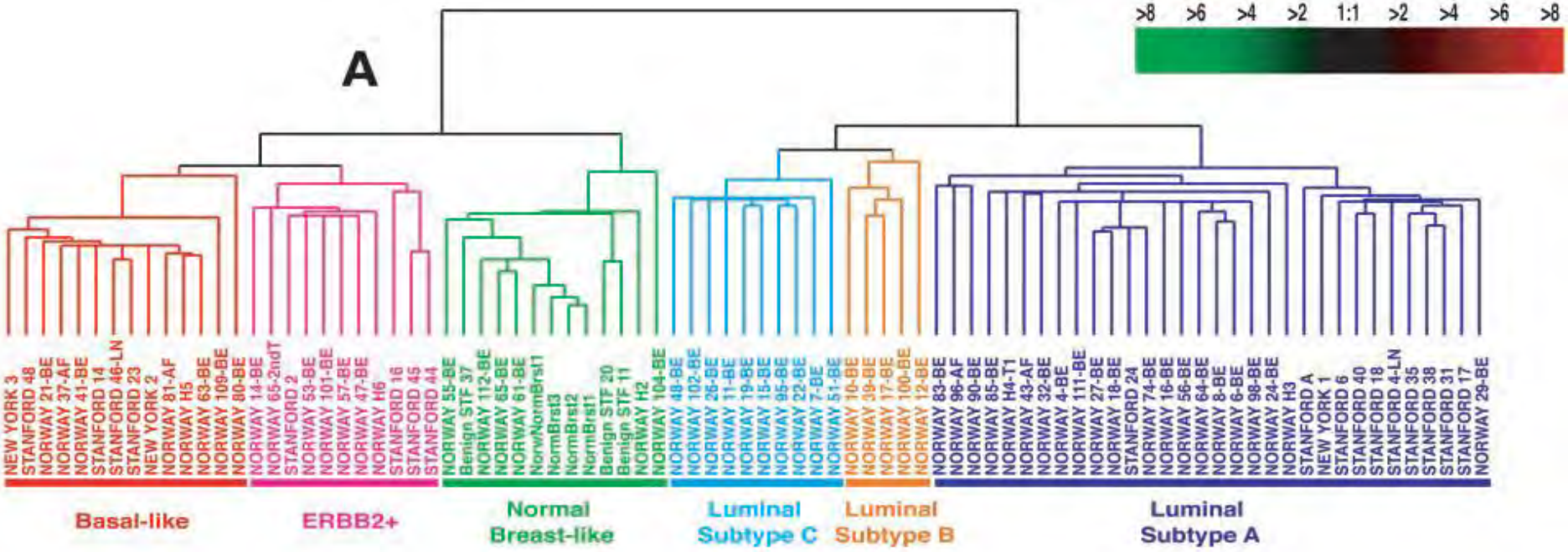
- Multigene assays have entered the picture over the last ten years, based on gene expression studies, and subsequently translated into quantitative results through various methods.

- Using cDNA microarrays and unsupervised clustering analysis, breast cancers can be subdivided into distinct molecular subtypes based on similarities in the patterns of their global gene expression profiles

- The molecularly defined subtypes repeatedly have shown significant differences in prognosis, likelihood and patterns of recurrence, and response to adjuvant therapies




**Sorlie T. et al.** Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. PNAS. 2001:98(19); 10869-10874




**Sorlie T. et al.** Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. PNAS. 2001:98(19); 10869-10874

- **Luminal subtype A**
  - *ER+, PR+, Her-2 negative, lower Ki-67*
- **Luminal subtype B**
  - *ER+, PR+/-, Her-2 positive, higher Ki-67*
- **Her-2 enriched**
  - *ER-, PR-, Her-2 positive, higher Ki-67*
- **Basal-like**
  - *ER-, PR-, Her-2 negative, higher Ki-67*

- **PHENOTYPES MAY OVERLAP**

# Multigene expression assays

1. **MammaPrint**
2. Genomic Grade Index (**GGI**)
3. Prosigna Breast Cancer Prognostic Gene Signature Assay (**PAM/50**)
4. Endopredict
5. Breast Cancer Index (**BCI**)
6. **Oncotype Dx (ODX)**

# MammaPrint

- First gene expression assay
- Uses microarray technology
- 70/25,000 genes were correlated independently with poor prognosis
- These 70 genes were tested in a validation study of 295 patients
  - **Good prognostic gene signature** - < 15% risk of recurrence at 10 years
  - **Poor prognostic gene signature** - 50% risk for distant metastasis.
- Calculates a ***RECURRENCE SCORE***
- Meta-analysis studies have shown that MammaPrint can also be predictive for chemotherapy
- Validated for use on FFPE clinical samples

# GGI

- A 97 gene expression classifier using microarray data
- Driven by proliferation and cell cycle related genes
- Discriminates consistently and reproducibly between two ER positive subgroups of different grades and different outcomes
- Tumors can be divided into low or high genomic grade categories
- Predictive for recurrence in endocrine and chemotherapy treated patients
- Prognostic for neoadjuvant chemotherapy

# PAM/50

- Uses quantitative reverse transcriptase polymerase chain reaction (qRTPCR) to measure the expression levels of 50 genes and 5 control genes

- PAM50 Risk of ***RECURRENCE SCORE***
  - **node-negative cancers** are classified as low (0-40), intermediate (41-60), or high (61-100) risk
  - **node-positive cancers** are classified as low (0-40) or high (41-100) risk

- Validated for use on FFPE clinical samples

- Predictive for complete or near-complete response to neoadjuvant chemotherapy

# Endopredict

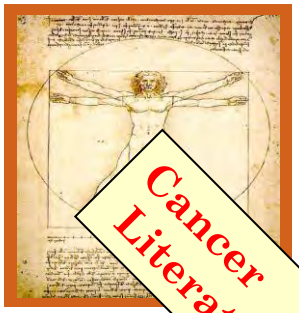
- Uses qRTPCR to analyzes the expression levels of eight genes of interest, and three reference genes
- An EP Risk of ***RECURRENCE SCORE*** is calculated from 0-15
- An EPclin combined Risk of ***RECURRENCE SCORE*** consisting of the EP risk score and clinical parameters, is calculated from 0-15
- Low-risk 0-5
- High risk: > 5
- Validated for use on FFPE clinical samples

# BCI

- Uses quantitative reverse transcriptase polymerase chain reaction (qRTPCR) to measure the expression of:
  - HOXB13, IL17BR (H/I)
  - BUB1B, CENPA, NEK2, RACGAP1, RRM2 (MGI)
  - ACTB, HMBS, SDHA, and UBC (reference genes)
- An algorithmic combination of the H/I and MGI results in the BCI
- Three risk groups: low, intermediate, and high
- Validated for use on FFPE clinical samples
- Predictive and prognostic in ER+ lymph node negative patients

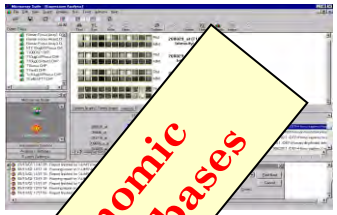
# Oncotype DX™ Technology*: Candidate Gene Selection Approach

***From ~40,000 genes:***

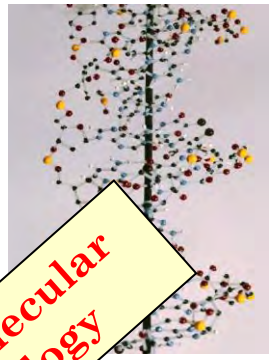


Cancer Literature

Microarray Data*



Genomic Databases



Molecular Biology

**250 cancer-related candidate genes**

Develop RTPCR FFPE

Test candidates in 3 studies

21 final gene set with algorithm

Calculate Recurrence Score (RS)

***Genomic Health Inc.**


***Sources include: van't Veer et al. *Nature.* 2002;415:530-536.**
**Sorlie et al. *PNAS.* 2001;98:10869-10874.**
**Ramaswamy et al. *Nat Genetics.* 2003;33:49-54.**
**Gruvberger et al. *Cancer Res.* 2001;61:5979-5984.**

# Oncotype DX: 21 Gene Recurrence Score (RS) Assay

## 16 Cancer and 5 Reference Genes From 3 Studies

**PROLIFERATION**
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

**ESTROGEN**
ER
PR
Bcl2
SCUBE2

**GSTM1**

**BAG1**

**CD68**

**INVASION**
Stromolysin 3
Cathepsin L2

**HER2**
GRB7
HER2

**REFERENCE**
**Beta-actin**
**GAPDH**
**RPLPO**
**GUS**
**TFRC**

**Coefficient x Expression Level**

**The recurrence score defined as:**

**RS=**
**+ 0.47 x HER2 Group Score**
**– 0.34 x ER Group Score**
**+ 1.04 x Proliferation Group Score**
**+ 0.10 x Invasion Group Score**
**+ 0.05 x CD68**
**– 0.08 x GSTM1**
**– 0.07 x BAG1**

Scaled – 0 to 100

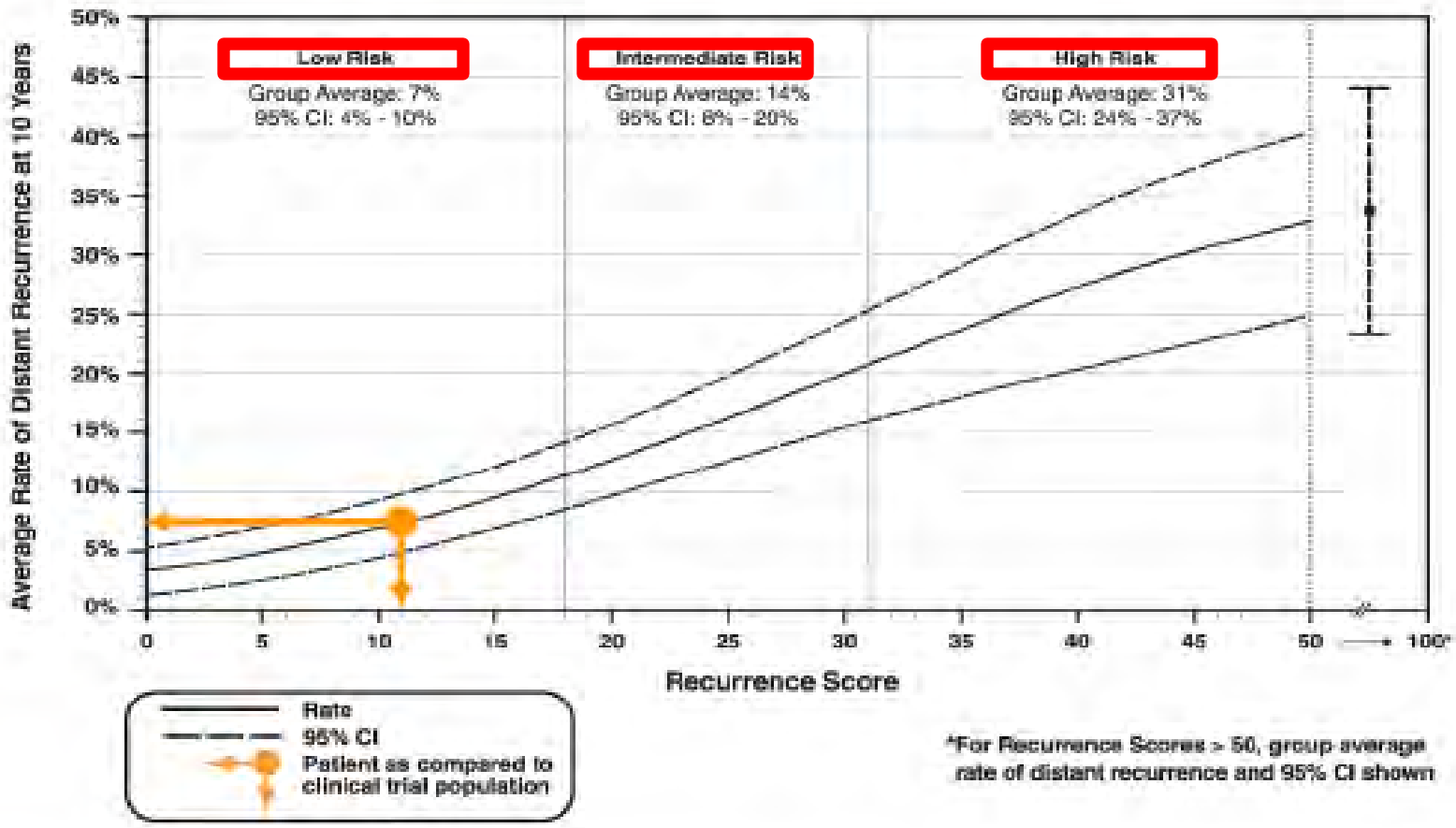
| Category | RS (0 – 100) |
|---|---|
| Low risk | RS < 18 |
| Int risk | RS ≥ 18 and < 31 |
| High risk | RS ≥ 31 |

# RESULTS

**Recurrence Score = 11**

# CLINICAL EXPERIENCE

Patients with a Recurrence Score of **11** in clinical validation study had an Average Rate of Distant Recurrence at 10 years of 7.4% (95% CI: 4.9%, 9.8%)



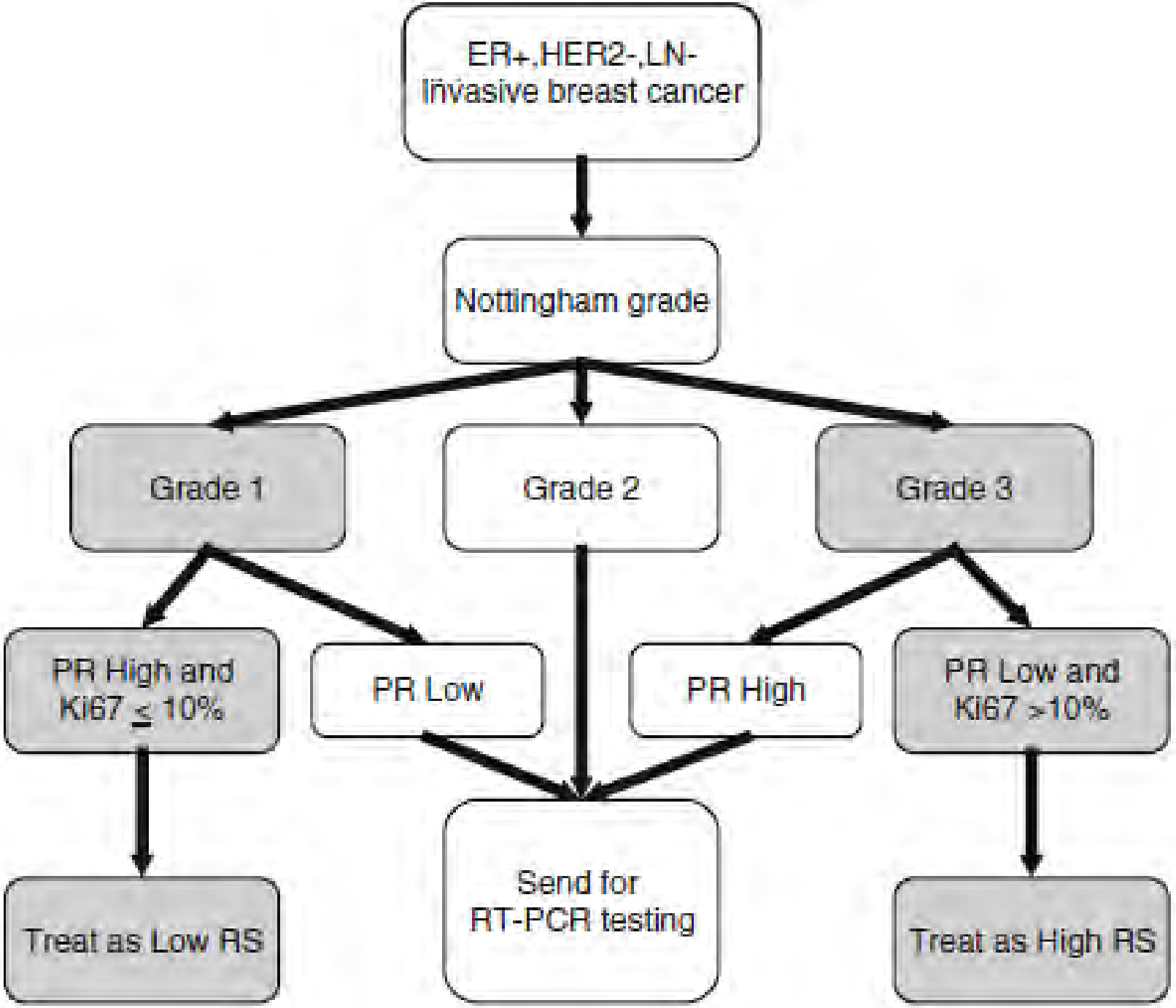
# Concerns

- Gene expression variables overlap with the pathologic and clinical evaluation of breast cancer cases
- Population data
- Cost (Oncotype DX - $4.350.00; MammaPrint - $4200.00)
- Can similar information be provided by information already generated in the laboratory, without sending out the specimen for additional costly testing?


Everything Old
is new again !
SOUTH OF THE BORDER
25
Mi.

# IHC and protein profiling of breast cancer

- Reports increasingly have suggested that the application of selective antibody panels and routine IHC also can be used to predict clinical behavior and outcomes in subsets of breast cancer patients.


ER+,HER2-,LN-
Invasive breast cancer
Nottingham grade
Grade 1
Grade 2
Grade 3
PR High and
Ki67 ≤ 10%
PR Low
PR High
PR Low and
Ki67 >10%
Send for
RT-PCR testing
Treat as Low RS
Treat as High RS

# IHC and protein profiling of breast cancer

- Mammostrat
- IHC4 score
- Linear regression equations

# Mammostrat

- Five antibody IHC panel
- Independent of ER, PR,Her-2 and proliferation (Ki-67)
- Markers related to nutrient transport, cell cycle progression, hypoxia and embryonic differentiation
- Calculates a ***Risk Index (RI):*** low, moderate, high
- Validated for use on FFPE clinical samples

# IHC4 score

- Incorporates ER, PR, Ki-67, and HER-2 results into a risk score using weighting factors and an algorithm.

VOLUME 29 · NUMBER 32 · NOVEMBER 10 2011

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

# Prognostic Value of a Combined Estrogen Receptor, Progesterone Receptor, Ki-67, and Human Epidermal Growth Factor Receptor 2 Immunohistochemical Score and Comparison With the Genomic Health Recurrence Score in Early Breast Cancer

*Jack Cuzick, Mitch Dowsett, Silvia Pineda, Christopher Wale, Janine Salter, Emma Quinn, Lila Zabaglo, Elizabeth Mallon, Andrew R. Green, Ian O. Ellis, Anthony Howell, Aman U. Buzdar, and John F. Forbes*

- **IHC4 =** 94.7 x {0.100 $ER_{10}$ - 0.079 $PgR_{10}$ + 0.586 HER2 + 0.240 ln (1  10  Ki67)}
- **Clinical score =** 100 x {0.417$N_{1-3}$ - 1.566$N_4$ + 0.930(0.497$T_{1-2}$ + 0.882$T_{2-3}$ + 1.838$T_{>3}$ + 0.559$Gr_2$ + 0.970$Gr_3$ + 0.130Age $_{\geq 65}$ -0.149Ana0}
- **Shrinkage factors to allow for overfitting:**
  - $0.947(35.1/39.1)^{1/2}$ for theIHC4score
  - $0.930(45.1/52.1)^{1/2}$ for the non-nodal part of clinical score

# Linear regression equations

- Recently been reported in the literature to be of possible value using histology and selective immunohistochemistry panels to predict clinical behavior in subsets of breast cancer patients

Modern Pathology (2008) 21, 1255–1261

www.modernpathology.org

# Histopathologic variables predict Oncotype DX™ Recurrence Score

Melina B Flanagan[1], David J Dabbs[1], Adam M Brufsky[2], Sushil Beriwal[3] and Rohit Bhargava[1]

[1]*Department of Pathology, Magee-Women's Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA, USA;* [2]*Department of Medical Oncology, Magee-Women's Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA, USA and* [3]*Department of Radiation Oncology, Magee-Women's Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA, USA*

**Oncotype DX™ is a commercially available reverse transcriptase-polymerase chain reaction based assay that provides a Recurrence Score (RS) and has been shown to provide prognostic and predictive information in estrogen receptor-positive lymph node-negative breast cancers. Independent studies of its utility in routine practice are lacking. Slides and surgical pathology reports from 42 cases of breast carcinomas evaluated by Oncotype DX™ were retrospectively reviewed to determine patient age, tumor size, histologic grade, estrogen and progesterone receptor (ER and PR) and *ERBB2* (HER-2/neu) data, with ER and PR reported as a semi-quantitative score reflecting both intensity of staining and proportion of positive cells. We show here that Recurrence Score is significantly correlated with tubule formation, nuclear grade, mitotic count, ER immunohistochemical score, PR immunohistochemical score, and HER-2/neu status, and that the equation RS = 13.424 + 5.420 (nuclear grade) + 5.538 (mitotic count) − 0.045 (ER immunohistochemical score) − 0.030 (PR immunohistochemical score) + 9.486 (HER-2/neu) predicts the Recurrence Score with an $R^2$ of 0.66, indicating that the full model accounts for 66% of the data variability. Although the Oncotype DX™ Recurrence Score holds potential, further validation of its independent value beyond that of histopathologic analysis is necessary before it can be implemented in clinical decision making.**

*Modern Pathology* (2008) **21**, 1255–1261; doi:10.1038/modpathol.2008.54; published online 21 March 2008

*Cancer Investigation*, 28:978–982, 2010
ISSN: 0735-7907 print / 1532-4192 online

DOI: 10.3109/07357907.2010.496754

informa
healthcare

ORIGINAL ARTICLE
Imaging, Diagnosis, Prognosis

# A Lower Allred Score for Progesterone Receptor Is Strongly Associated With a Higher Recurrence Score of 21-Gene Assay in Breast Cancer

Ping Tang,[1] Jianmin Wang,[2] David G. Hicks,[1] Xi Wang,[1] Linda Schiffhauer,[1] Loralee McMahon,[1] Qi Yang,[1] Michelle Shayne,[1] Alissa Huston,[1] Kristin A. Skinner,[1] Jennifer Griggs[3] and Gary Lyman[4]

*Department of Pathology, University of Rochester of Rochester Medical Center, Rochester, New York, USA*[1]
*RTI Health Solution, Research Triangle Park, North Carolina, USA*[2]
*Department of Medicine, University of Michigan, Ann Arbor, Michigan, USA*[3]
*Department of Medicine, Duke University School of Medicine, Durham, North Carolina, USA*[4]

## ABSTRACT

Among the 77 infiltrating breast carcinomas, we found that progesterone receptor (PR) expression was inversely associated with recurrence score (RS, $p < .0001$). RS is also significantly associated with tubule formation, mitosis, and luminal B subtype. The equation of RS = 17.489 + 2.071 (tubal formation) + 2.926 (mitosis) – 2.408 (PR) – 1.061 (HER2) + 7.051 (luminal A) + 29.172 (luminal B predicts RS with an $R^2$ of 0.65. In conclusion, PR negativity, luminal B subtype, tubal formation, and mitosis are strongly correlated with a higher RS.

MODERN PATHOLOGY (2013) 26, 658–664



OPEN

# Prediction of the Oncotype DX recurrence score: use of pathology-generated equations derived by linear regression analysis

Molly E Klein[1], David J Dabbs[2], Yongli Shuai[3], Adam M Brufsky[4], Rachel Jankowitz[4], Shannon L Puhalla[4] and Rohit Bhargava[2]

*[1]Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, USA; [2]Department of Pathology, Magee-Womens Hospital of UPMC, Pittsburgh, PA, USA; [3]University of Pittsburgh Cancer Institute Biostatistics Facility, Pittsburgh, PA, USA and [4]Department of Medical Oncology, Magee-Womens Hospital of UPMC, Pittsburgh, PA, USA*

**Oncotype DX is a commercial assay frequently used for making chemotherapy decisions in estrogen receptor (ER)-positive breast cancers. The result is reported as a recurrence score ranging from 0 to 100, divided into low-risk (<18), intermediate-risk (18–30), and high-risk (≥31) categories. Our pilot study showed that recurrence score can be predicted by an equation incorporating standard morphoimmunohistologic variables (referred to as original Magee equation). Using a data set of 817 cases, we formulated three additional equations (referred to as new Magee equations 1, 2, and 3) to predict the recurrence score category for an independent set of 255 cases. The concordance between the risk category of Oncotype DX and our equations was 54.3%, 55.8%, 59.4%, and 54.4% for original Magee equation, new Magee equations 1, 2, and 3, respectively. When the intermediate category was eliminated, the concordance increased to 96.9%, 100%, 98.6%, and 98.7% for original Magee equation, new Magee equations 1, 2, and 3, respectively. Even when the estimated recurrence score fell in the intermediate category with any of the equations, the actual recurrence score was either intermediate or low in more than 80% of the cases. Any of the four equations can be used to estimate the recurrence score depending on available data. If the estimated recurrence score is clearly high or low, the oncologists should not expect a dramatically different result from Oncotype DX, and the Oncotype DX test may not be needed. Conversely, an Oncotype DX result that is dramatically different from what is expected based on standard morphoimmunohistologic variables should be thoroughly investigated.**

*Modern Pathology* (2013) **26,** 658–664; doi:10.1038/modpathol.2013.36; published online 15 March 2013

## *New Magee equation 1 calculating predicted RS*:

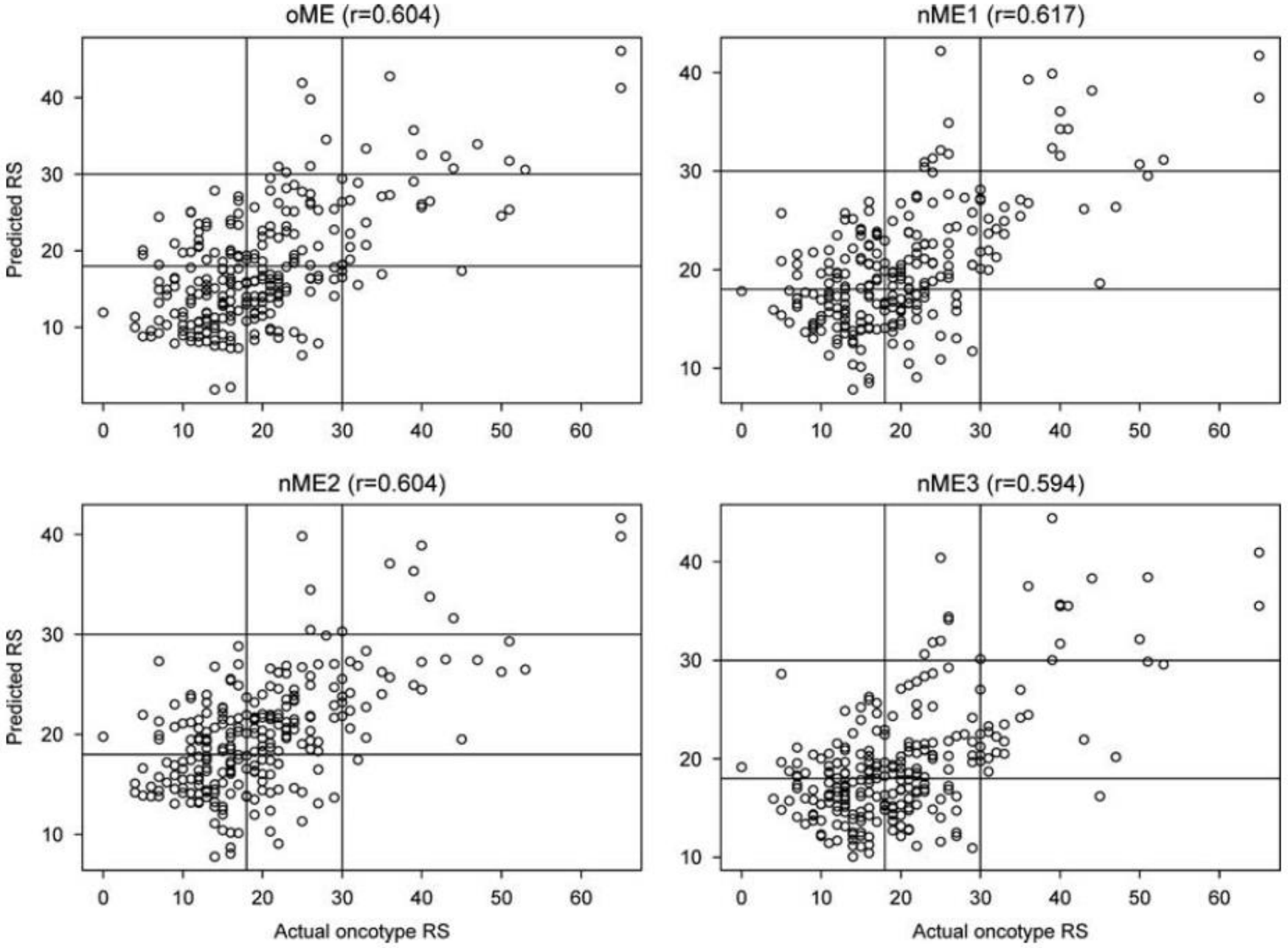
- Recurrence score = 15.31385+ Nottingham score* 1.4055 **+ERIHC***(−0.01924)**+PRIHC***(−0.02925)+(0 for HER2 negative, 0.77681 for equivocal, 11.58134 for HER2 positive) +tumor size* 0.78677 +Ki-67 index* 0.13269.

## *New Magee equation 2 calculating predicted RS* :

- Recurrence score = 18.8042+ Nottingham score* 2.34123 **+ERIHC***( −0.03749)**+PRIHC***(−0.03065)+(0 for HER2 negative, 1.82921 for equivocal, 11.51378 for HER2 positive)+ tumor size* 0.04267.

## *New Magee equation 3 calculating predicted RS* :

- Recurrence score = 24.30812 **+ERIHC***(−0.02177) **+PRIHC*** (−0.02884)**+**(0 for HER2 negative, 1.46495 for equivocal, 12.75525 for HER2 positive)+ Ki-67*0.18649.




Klein ME., Dabbs DJ., et al., Modern Pathology 2013

MODERN PATHOLOGY (2015) 28, 921–931



# Use of modified Magee equations and histologic criteria to predict the Oncotype DX recurrence score

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**Oncotype DX (Genomic Health, Redwood City, CA, USA, current list price $4,350.00) is a multigene quantitative reverse transcription-polymerase chain reaction-based assay that estimates the risk of distant recurrence and predicts chemotherapy benefit for patients with estrogen receptor (ER)-positive breast cancers. Studies have suggested that standard histologic variables can provide similar information. Klein and Dabbs *et al* have shown that Oncotype DX recurrence scores can be estimated by incorporating standard histologic variables into equations (Magee equations). Using a simple modification of the Magee equation, we predict the Oncotype DX recurrence score in an independent set of 283 cases. The Pearson correlation coefficient (r) for the Oncotype DX and average modified Magee recurrence scores was 0.6644 ($n=283$; $P<0.0001$). 100% of cases with an average modified Magee recurrence score $>30$ ($n=8$) or an average modified Magee recurrence score $<9$ (with an available Ki-67, $n=5$) would have been correctly predicted to have a high or low Oncotype DX recurrence score, respectively. 86% (38/44) of cases with an average modified Magee recurrence score $\leq 12$, and 89% (34/38) of low grade tumors (NS $<6$) with an ER and PR $\geq 150$, and a Ki-67 $<10\%$, would have been correctly predicted to have a low Oncotype DX recurrence score. Using an algorithmic approach to eliminate high and low risk cases, between 5% and 23% of cases would potentially not have been sent by our institution for Oncotype DX testing, creating a potential cost savings between $56,550.00 and $282,750.00. The modified Magee recurrence score along with histologic criteria may be a cost-effective alternative to the Oncotype DX in risk stratifying certain breast cancer patients. The information needed is already generated by many pathology laboratories during the initial assessment of primary breast cancer, and the equations are free.**
*Modern Pathology* (2015) **28**, 921–931; doi:10.1038/modpathol.2015.50; published online 1 May 2015

**H-score = [proportion (1) x 1] + [proportion (2) x 2] + [proportion (3) x 3]**

- Either an average percentage and intensity are given and/or an Allred score is given
- **Can we substitute an average percentage and intensity and still predict the actual ODX recurrence score for ER+ breast cancer cases with a similar degree of confidence?**

# Materials and Methods

- The Magee equations were tested on a validation set of 283 cases at the University of Rochester Medical Center (URMC) sent for ODX (2009-present)
- Modified Magee Recurrence scores (MMRS's) were calculated by calculating results from the three published Magee equations, with a modification of the H-score for ER and PR

$$\text{Average intensity} = \frac{\begin{array}{c}1\times[\%\text{ staining grade 1}]+\\ 2\times[\%\text{ staining grade 2}]+3\times[\%\text{ staining grade 3}]\end{array}}{100}$$

TURNER ET AL. USE OF MODIFIED MAGEE EQUATIONS AND HISTOLOGIC CRITERIA TO PREDICT THE ONCOTYPE DX RECURRENCE SCORE. MODERN PATHOLOGY (2015) 28, 921–931

- We estimated the H-score by using the ***predominant*** intensity grade (1, 2 or 3) as a surrogate for the average intensity of staining and multiplying this grade by the percentage of cells staining positive

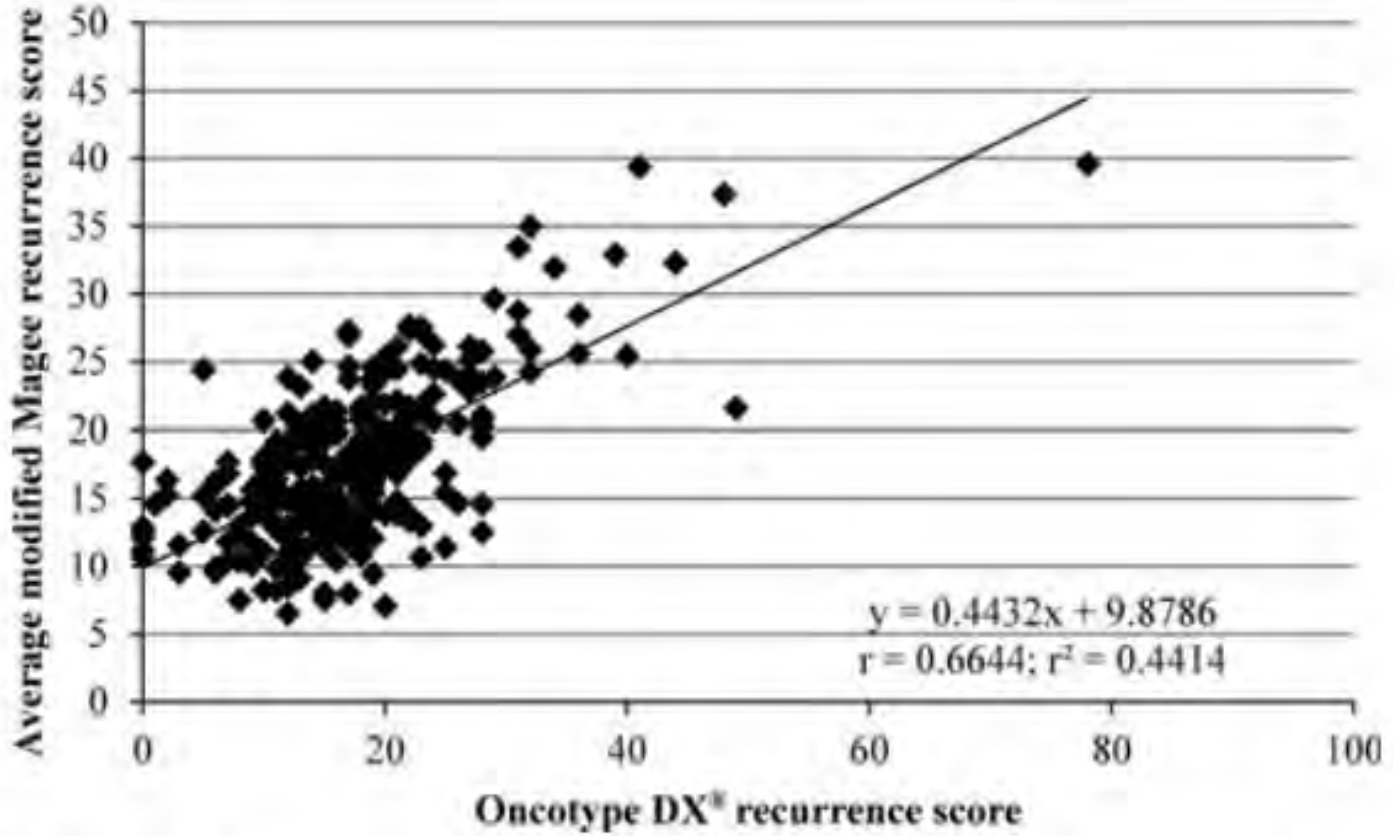
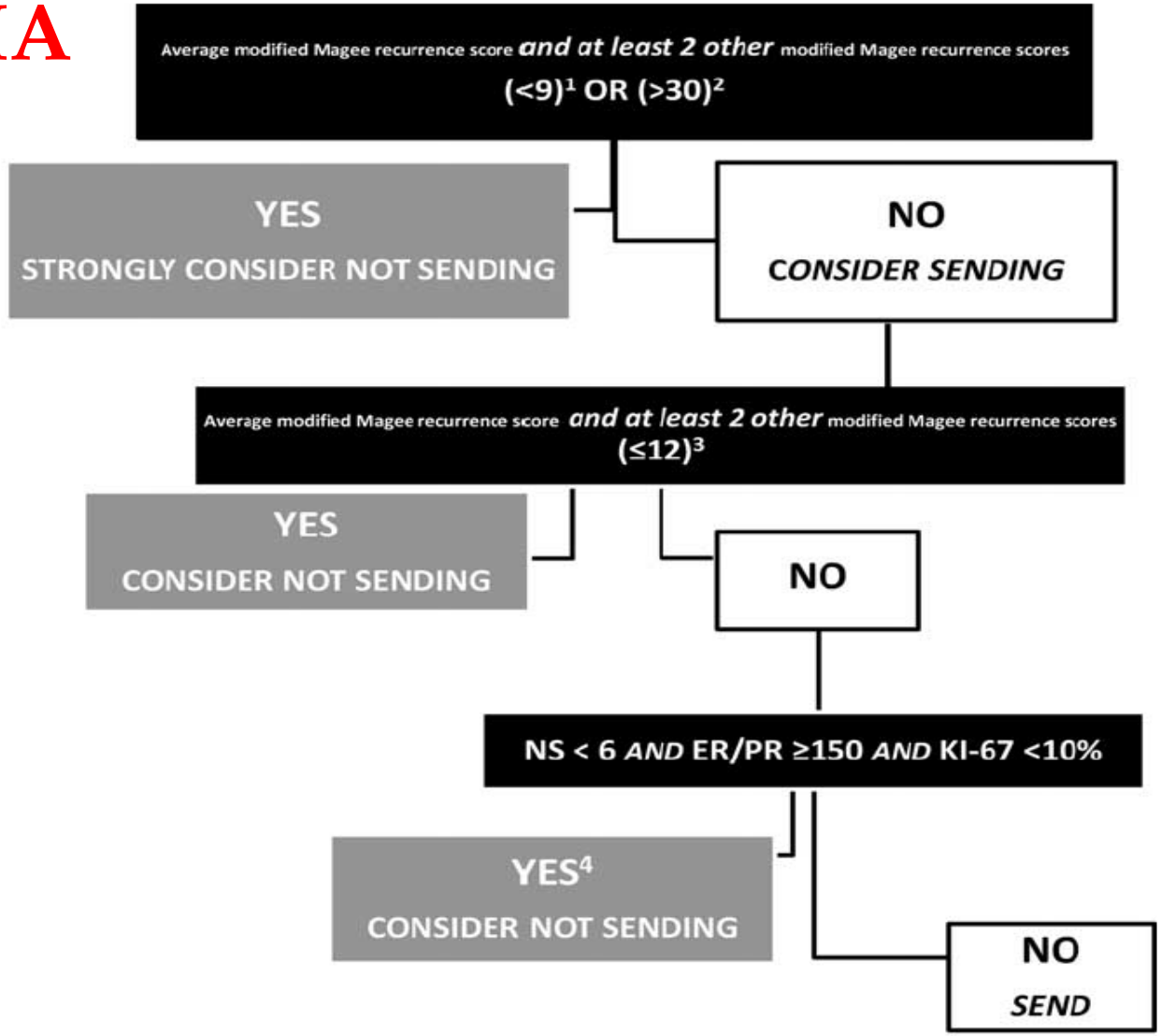



**Figure 1** Correlation of average modified Magee recurrence score and Oncotype DX recurrence score.

**Turner et al. Use of modified Magee equations and histologic criteria to predict the Oncotype DX recurrence score. Modern Pathology (2015) 28, 921–931**

# RUMMA




[1]Ki-67 must be < 10%. If modified Magee recurrence score #2 > 7, *consider sending*

[2]If modified Magee recurrence score #2 < 30, *consider sending*

[3]Ki-67 must be ≤ 15%; If modified Magee recurrence score #2 > 12, *consider sending;* Consider ER/PR in clinical decision making

[4]Consider modified Magee recurrence scores in clinical decision making

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Everything Old
is new again !
SOUTH OF THE BORDER
25
Mi.


CONTINENTAL-AMERICAN
Peter Allen

# Out with the OLD, in with the NEW?

- Does multigene assay testing completely devalue the more traditional approach to diagnoses and treatment of breast cancer?

- How much added value does multigene assay testing add when we consider cost and cancer outcomes?

# Addressing cost……

- Approximately 63,342  tests delivered by ODX in 2012
- Approximately 81,269 tests delivered by ODX in 2014
- The ODX risk stratification of these patients is unknown.

# Theoretical Scenario

- Paik et al. *N Engl J Med*. 2004 (B-14 validation)
  - **16**-34% of cases considered low-grade tumors (depending on the pathologist)
- Our study
  - 50% had low grade tumors
    - **5%** with an average MMRS < 9 which satisfied our algorithmic criteria
- Most conservative scenarios
  - **16%** of the 2012 (10,135) and 2014 (13,003) estimated to be low grade
  - **5%** of these 2012 and 2014 low grade tumors (460 and 650, respectively) estimated to have an average MMRS < 9
  - Estimated cost savings to health care system:
    - **$2,001,000.00 (2012)**
    - **$2,827.500.00 (2014)**


"We'll keep your application on file and if we ever lower our standards, we'll give you a call."

# Future Perspectives
# *Next Generation* Sequencing

- **Permits the simultaneous interrogation of genomic alterations present in a panel of cancer genes at high speed and relatively low cost**

1. Mutational landscape of breast cancer
2. Most recurrently mutated genes
   - ✓ **PIK3CA**
   - ✓ **TP53**
   - ✓ CCND1
   - ✓ FGFR1
   - ✓ Her2
3. Some mutations enriched according to molecular and histopathologic subtype
4. Genomic diversity among breast tumors with multiple combinations of mutations
5. Genomic characterization of circulating tumor DNA(ctDNA)
   - ✓ Monitoring treatment response
   - ✓ Identification of resistance mutations
   - ✓ Surveillance and identification of residual disease

# Future Perspectives
# *Next Generation* Sequencing

- Only a few recurrently mutated genes have been identified as potential targets for new treatments

# Out with the OLD, in with the NEW?

- With each answer, we will have more questions.

# New insights on PI3K/AKT pathway alterations and clinical outcomes in breast cancer

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ABSTRACT

PI3K/AKT signaling pathway plays an important role in tumorigenesis and regulates critical cellular functions including survival, proliferation and metabolism. *PIK3CA* mutations and AKT activation by phosphorylation (pAKT) are often detected in many cancers and especially at high frequencies in breast cancer. Mounting data suggest that *PIK3CA* mutations or pAKT are mostly associated with better or insignificant outcomes in estrogen receptor-positive (ER+) early stage breast cancer and tend to be with worse prognosis in ER− disease. pAKT expression has been identified to predict paclitaxel chemotherapy benefit in node-positive breast cancer. Preclinical and neoadjuvant trial data suggest that *PIK3CA* alterations confer resistance to HER2-targeted therapy and are associated with lower pathological complete response (pCR) rate in HER2-positive breast cancer. However, recent results from randomized clinical trials of adjuvant and metastatic settings show that patients with mutant and wildtype *PIK3CA* tumors derived similar benefit from anti-HER2 therapy. This article, with our new insights, aims to decipher the mixed data and discusses the influence of the potential confounding factors in the assessments. We also share our views for validation of PI3K/AKT alterations in relation to clinical outcome in the context of specific breast cancer subtypes and treatment modalities towards further advance of the precision medicine for breast cancer treatment.

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# Out with the OLD, in with the NEW?

- In our quest for the truth, there are some things we may never know……

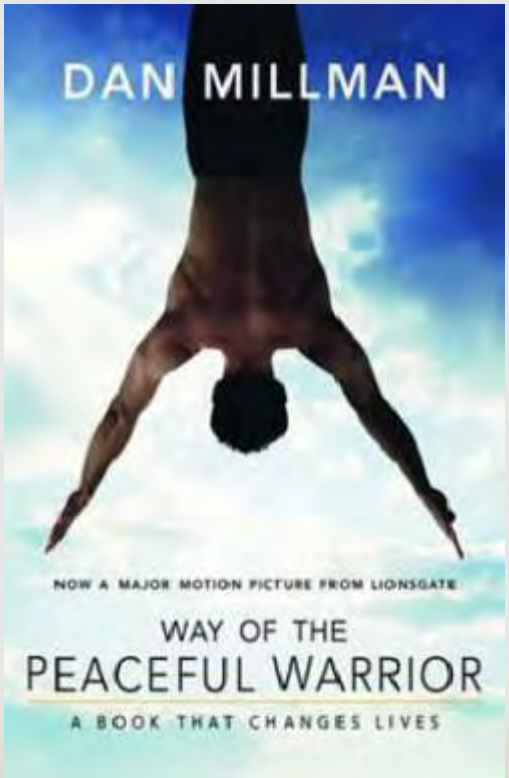
- *True knowledge exists in knowing… … …*
- *that you know nothing*

*- Socrates*

# Change

- " The secret of change is to focus all of your energy not on fighting the old, but building the new"

-



# In with the OLD!!! In with the NEW!!!

- We must continue to ask questions, and use *ALL* of that which we do know, the ***old and the new*** to arrive at the best possible conclusion……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