Welcome to the PHC Webinar Series

This lecture on “Molecular Markers in Breast Cancer” is presented by David G. Hicks, MD, FCAP

Your host is Jill Kaufman, PhD. For comments about this webinar or suggestions for upcoming webinars, please contact Jill Kaufman at jkaufma@cap.org

THE WEBINAR WILL BEGIN MOMENTARILY. ENJOY!
David G. Hicks, MD, FCAP

- Professor of Pathology and Laboratory Medicine, University of Rochester School of Medicine
- Director of Surgical Pathology at the University of Rochester Medical Center
- Current research interests focus on the molecular genetic profiling of clinical samples from patients with cancer
- Authored or co-authored over 120 peer reviewed articles
Prognostic/Predictive Factors and Breast Cancer Classification: Toward Clinical Relevance and Therapeutic Implications

David G. Hicks, MD
Professor of Pathology
Director of the Surgical Pathology Unit
University of Rochester Medical Center

www.cap.org
Breast Cancer: Clinical, Morphologic and Molecular Heterogeneity

Factors used to stratify patients to understand diversity/predict behavior

- Age/menopausal status
- Tumor size/tumor burden
- Histologic features: type, grade, LVI, necrosis, margin status
- Lymph node status
- Immunophenotype: ER, PR, HER2
- Proliferation
- Genomic gains and losses
- Gene expression profiling
- QRT-PCR profiling

Clinical Utility

- Classify patient’s (subsets)
- Predict outcome
- Predict treatment response
Breast Cancer Classification: What do Clinicians Want?

• Ability to distinguish different prognostic groups among patients with similar tumor characteristics

• Ability to predict response to various therapeutic agents in individual patients
  o Select out patients with higher likelihood of response
  o Spare patients cost and potential toxicity who are unlikely to respond
Breast Cancer Management: Decisions on Adjuvant Therapy Based on Risk Assessment

- Weigh background level - **risk** of recurrence against benefits & **burdens** of adjuvant therapy, standard clinical factors

- **Patient factors** (clinically validated)
  - Age, menopausal status and co-morbidities

- **Tumor-related factors** (clinically validated)
  - Tumor size, grade, LN, LVI

- Factors are robust prognostic markers, weaker in predicting for Rx response
Breast Cancer Staging & Prognosis

**Tumor stage using AJCC/UICC TNM system**
- Useful in determining risk of recurrence
- Staging affects choice of treatment

<table>
<thead>
<tr>
<th>Tumor – T</th>
<th>Nodes – N number positive</th>
<th>Metastases - M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tis</td>
<td>in situ</td>
<td>M0 None</td>
</tr>
<tr>
<td>T1</td>
<td>( \leq 2 \text{ cm} )</td>
<td>M1 Distant Mets</td>
</tr>
<tr>
<td>T2</td>
<td>2.1 – 5 cm</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>&gt; 5 cm</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>Skin / chest wall</td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>0 pos</td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>1 – 3</td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>4 – 9</td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>( \geq 10 ) and / or supraclavicular or IM nodes</td>
<td></td>
</tr>
</tbody>
</table>
**Tumor Size** (Measure Greatest Diameter X3)

Firm gritty mass with irregular infiltrating borders

<table>
<thead>
<tr>
<th>T1</th>
<th>up to 2.0cm</th>
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<tbody>
<tr>
<td>T2</td>
<td>2.1 - 5.0cm</td>
</tr>
<tr>
<td>T3</td>
<td>&gt;5cm</td>
</tr>
<tr>
<td>T4</td>
<td>chest wall invasion</td>
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For node (-)patients: tumor size next most significant prognostic factor
•used for adjuvant treatment decisions

Lymph Node Status: Correlation with Recurrence & Survival

"Classic" ILC, LN involvement

<table>
<thead>
<tr>
<th>Nodal stage</th>
<th>Nodal involvement</th>
<th>5 year survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td>Negative lymph nodes</td>
<td>82.8%</td>
</tr>
<tr>
<td>N1</td>
<td>1–3 positive nodes</td>
<td>73%</td>
</tr>
<tr>
<td>N2</td>
<td>4–9 positive nodes</td>
<td>45.7%</td>
</tr>
<tr>
<td>N3</td>
<td>10 or more positive nodes</td>
<td>28.4%</td>
</tr>
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DFS vs LN status (pN stage)

Histologic Grade: (Elston & Ellis modification of SBR)
Correlate with Prognosis in Breast Cancer

Modified SBR Grade
3-5: Grade I - Well
6-7: Grade II - Moderate
8-9: Grade III – Poor

Significantly correlates with DFS & OS

Genomic Grade Index

Challenge clinical relevance intermediate grade

Low Risk Recurrence

High Risk Recurrence

Low Grade Breast Neoplasia Pathway

- ADH
- Low-grade DCIS
- Low-grade IDC

High Grade Breast Neoplasia Pathway

- High-grade DCIS
- High-grade IDC

GEP
- Low-grade Tumors
- Intermediate-grade Tumors
- High-grade Tumors

Normal Breast

+1q
-16q

+17q12
+11q13
Adjuvant Therapy Based on Risk Assessment (Recurrence risk versus benefit & burden of adjuvant treatment)

### ER+ (~70%)
- **Age range**: tends to be older patients
- **Histology**: tends to be better differentiated
- **Clinical course**: more indolent
- **Therapeutic targets**: responsive to hormonal therapy
- **Significant variability among group**

### ER– (~30%)
- **Age range**: tends to be younger patients
- **Histology**: tends to be more poorly differentiated
- **Clinical course**: more aggressive
- **Therapeutic targets**: responsive to chem & trastuzumab for HER2+ tumors
- **Significant variability among group**

**ER – Prognostic and predictive of benefit from endocrine therapy**

*Other biomarkers useful for individual risk prediction?*
New Approaches to an Old Problem: Prognosis, Risk & Genomic Profiling

• **Genomics** - potential to identify breast cancer patients at **high risk** for recurrence

  – State-of-the-art molecular technology can be used to analyze **global genomic changes** in breast cancer tissue

  – Genomic activity in early-stage breast cancer

    – Refine breast cancer classification

    – Assess prognosis

    – Assess response to therapy

Two Complementary RNA Analysis Methods for the Study of Gene Expression in Clinical Samples

**DNA Arrays (Chips)**
- Unfixed, frozen tissue
- 1000s of genes
- Limited dynamic range and difficult to control

**RT-PCR Assay (e.g., Genomic Health)**
- Fixed paraffin or unfixed tissue
- 10s-100s of genes
- Wide dynamic range, high sensitivity, specificity, reproducibility

**RNA Analysis Methods:** Study of gene expression in clinical samples

**DNA Arrays (Chips):**
- Unfixed, frozen tissue
- 1000s of genes
- Limited dynamic range and difficult to control

Gene Expression Profiling of Breast Cancer: Types of Analysis

- **Unsupervised “Cluster” Analysis**
  - Sorts tumors into related clusters based upon similarities in gene expression profiles
  - “Dendrograms” illustrate the degree of relatedness
  - Sorts tumors into groups of imputed biologic significance

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Ramaswamy and Golub JCO 20:1932,2001
Molecular Classification – Expression Profiling
Basal, HER2 over-expression, Luminal A, Luminal B, Normal Breast Like

Breast Cancer Classification: Gene Expression Profiling

<table>
<thead>
<tr>
<th>ER+</th>
<th>ER-</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2 subsets of ER+ breast cancer</strong></td>
<td><strong>3 subsets of ER- breast cancer</strong></td>
</tr>
<tr>
<td><strong>Luminal A:</strong> high expression ER-alpha, GATA binding protein-3, x-box binding protein-1, low expression of cell cycle and proliferation genes</td>
<td><strong>HER2-over-expression:</strong> HER2, GRB7, high expression of cell cycle and proliferation genes</td>
</tr>
<tr>
<td><strong>Luminal B:</strong> lower levels of ER expression, may over-express HER2 and other GFR, high expression of cell cycle and proliferation genes</td>
<td><strong>Basal-Like Carcinoma:</strong> HER2-, express basal cytokeratins (5, 6, 14, 17), laminin, cell motility proteins, cytokines, high expression of cell cycle and proliferation genes</td>
</tr>
<tr>
<td><strong>Normal-Like Carcinoma:</strong> Little known about this subset (small % of breast cancers)</td>
<td></td>
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Gene Expression Profiling and Breast Cancer Subtypes: Defined by Genetic Composition of Breast Tumor

All Breast Cancer

Clinical and biologic heterogeneity of disease

- Clinically relevant tumor classification?
- Better assessment of prognosis?
- Better prediction of response to therapy?

- Normal-Breast Like?
- Basaloid 15%
- HER2+ 15-20%
- ER+ 65-75%
- High ER expression
- Low ER expression

→ Phenotypes of interest to clinicians have clear therapeutic implication
Unsupervised Analysis of Breast Cancer Gene Expression Profiles (Intrinsic Molecular Subtypes)

- Identify reproducible, biologically distinct subgroups of breast cancers
  - Validated across multiple patient cohorts
  - Significantly different outcomes between subtypes
  - Differences in likelihood/patterns of recurrence

- Established drug targets (ER, HER2) and proliferation gene help define subgroups
  - Closely correlates with conventional histologic classification and IHC phenotypes of breast cancer
  - No new clinically validated novel drug targets (yet)

Proc Natl Acad Sci USA 100:8418-8423, 2003
Gene Expression Profiling of Breast Cancer: Types of Analysis

- **Supervised Analysis**
  - Initial step is to separate tumors into groups
    - e.g., relapsed vs. not
  - Compare signatures of groups
  - Identify genes identifying tumor as belonging to one group or the other
  - Multiple tests leads to many false positives
  - Validation is necessary

![Supervised Learning Diagram]

Class Prediction
70-Gene Prognostic Signature Assay

- Developed Netherlands Cancer Institute
- Requires fresh/frozen tumor
- Training set, matched BC cases with good & bad outcome
  - Node (-), tumors <5cm, <55 years age
  - Developed 70 gene prognosis signature
  - Distinguish patient’s with good and bad outcome
- Validation study:
- 295 patients with breast cancer
- Independent predict outcome


Probability Patients Would Remain Free of Distant Metastases & Probability of OS According to Whether They Had Good-Prognosis or Poor-Prognosis Signature

All Pts  All Pts  Node -

Overall Good vs. Poor HR=5.1

HR=5.5  Node +  Node +
RNA Analysis Methods: Study of Gene Expression in Clinical Samples

RT-PCR Assay (e.g., Genomic Health)

- Fixed paraffin or unfixed tissue
- 10s-100s of genes
- Wide dynamic range, high sensitivity, specificity, reproducibility

**Oncotype DX™ Technology:** Candidate Gene Selection Approach

*From ~40,000 genes:*

- **250 cancer-related candidate genes**
  
  *Sources include: van’t Veer et al. Nature. 2002;415:530-536.
  Sorlie et al. PNAS. 2001;98:10869-10874.

- Develop RTPCR FFPE
- Test candidates in 3 studies
- 21 final gene set with algorithm
- Calculate Recurrence Score (RS)
The recurrence score defined as:

\[ RS = 0.47 \times \text{HER2 Group Score} - 0.34 \times \text{ER Group Score} + 1.04 \times \text{Proliferation Group Score} + 0.10 \times \text{Invasion Group Score} + 0.05 \times \text{CD68} - 0.08 \times \text{GSTM1} - 0.07 \times \text{BAG1} \]

Scaled 0 to 100

<table>
<thead>
<tr>
<th>Category</th>
<th>RS (0 – 100)</th>
</tr>
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<tbody>
<tr>
<td>Low risk</td>
<td>RS &lt; 18</td>
</tr>
<tr>
<td>Intermed. risk</td>
<td>RS ≥ 18 and &lt; 31</td>
</tr>
<tr>
<td>High risk</td>
<td>RS ≥ 31</td>
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Best RT-PCR performance and most robust predictors
**Oncotype DX® Clinical Validation: B-14 Results – DRFS**

**DRFS Over Time – All 668 Patients**

10-year DRFS = 85%

**DRFS for the three distinct cohorts**

- Low Risk (RS <18) n = 338
- Intermediate Risk (RS 18-30) n = 149
- High Risk (RS ≥31) n = 181

### B-14 Results – DRFS, Multivariate Analysis

Only RS and poor tumor grade are independently associated with recurrence.

B-20 Results: Tam Alone Versus Tam + Chemo (B20 - modest benefit from addition of chemo to Tam, ER+/node-)

- **Low Risk Patients (RS < 18)**
  - TAM + Chemo: 218 events, 8
  - TAM: 135 events, 4
  - DRFS
  - p = 0.61

- **Intermediate Risk Patients (RS 18-31)**
  - TAM + Chemo: 89 events, 9
  - TAM: 45 events, 4
  - DRFS
  - p = 0.39

- **High Risk Patients (RS ≥ 31)**
  - TAM + Chemo: 117 events, 13
  - TAM: 47 events, 18
  - DRFS
  - p < 0.001

28% absolute benefit from tam + chemo

Most of chemotherapy benefit seen in high RS

Histopathologic Variables Predict Oncotype DX™ Recurrence Score

- RS - significantly correlated with tubule formation, nuclear grade, mitotic count, ER IHC score, PR IHC score & HER2 status (Flanagan MB, et al. Mod Pathol. 2008)

\[ R = 0.59 \]
\[ P < 0.01 \]

\[ R = 0.58 \]
\[ P < 0.01 \]
Phenotypes of Interest to Clinicians have Clear Therapeutic Implication

Goals of breast cancer profiling
- Clinically relevant tumor classification
- Better assessment of prognosis
- Better prediction of response to therapy

Limitations of GEP
- Tissue requirement (fresh/frozen)
- Cost
- Availability
- Clinical utility

Can GEP data be translated into IHC markers that are clinically useful and have therapeutic implications???
Different subsets show distinctive morphologic feature:

- Grade
- Architecture
- Growth pattern

Can be recognized or suspected in some cases.

Key factors include:

- Luminal A
- Luminal B
- HER2
- Basal-like
- ER Expression
- Proliferation (KI67)
- HER2 Over-Expression
- “Triple Negative”
Proposed IHC Surrogates for Molecular Classification of Breast Cancer

Morphologic features

Infiltrating breast cancer

IHC phenotype: ER, HER2, proliferation

- **ER (-)**
  - HER2 (+)
    - **HER2 classic**
      - ER-/HER2+
  - HER2 (-)

- **HER2 (+)**
  - CK5/6
    - ER (+)
      - ER+/HER2+
    - HER2 (-)
      - **Luminal HER2**
        - Luminal B
        - Luminal A
  - CK5/6+ &/or EGFR+

- **CK5/6- & EGFR-**
  - Basal Phenotype
  - Non-Basal, Normal-Breast-Like Phenotype
Luminal A Breast Tumors: Clinical Features

• Demonstrate higher level of expression of ER & ER related genes
  o Lower histologic grade
  o Lower levels of proliferation related genes

• More indolent clinical course
  o May experience later recurrences

• Better prognosis compared with Lum-B and other subtypes
ER+ Breast Cancer (Luminal A)
Luminal A Breast tumors

- Implications for therapy
  - Good prognosis
  - Respond well to endocrine therapy
  - May be adequately treated with hormonal therapy alone
  - Tend to respond poorly to adjuvant and neo-adjuvant chemotherapy
    - Typically low recurrence score by Oncotype Dx
Luminal-B Breast Tumors: Clinical Features

- Lower levels of ER expression and ER-related genes
  - May be PR negative
  - May over-express GFR (HER2 & EGFR)
  - 30% of Luminal-B tumors over-express HER2 (IHC & FISH+)

- Higher histologic grade

- More aggressive clinical course, worse prognosis
  - More likely lymph node positive

- Higher expression of proliferation related genes
  - KI-67 proliferation index may be useful in separating Lum B from Lum A tumors
ER+ Breast Cancer (Luminal-B)
ER+ Breast Cancer (Luminal-B/Luminal-HER2)
Luminal-B/Luminal HER2 Breast Tumors

• **Implication for therapy**
  - Aggressive clinical course
  
  - May be less responsive to tamoxifen
  
  - More likely to respond to chemotherapy added to endocrine treatment
    - Typically - high Recurrence Score by Oncotype Dx
  
  - For HER2+ Luminal-B tumors
    - Similar benefit from HER2-targeted therapy compared with HER2+/ER- tumors in adjuvant clinical trials
**HER2+ Breast Tumors**

- HER2+ breast tumors by GEP are ER-
  - Over-expression of other genes in HER2-amplicon (GRB7, TOP2A)
  - High proliferative index
  - More likely to harbor p53 mutations
  - Higher histologic grade
  - Younger age at presentation

- Aggressive clinical course, poor prognosis
HER2 Classic Breast Cancer Subtype

- 39 year old female
- Size: 4 cm
- Lymph nodes: (2/4)
- ER: negative
- PR: negative
- KI-67: 60%
- P53: positive
- Modified-SBR: 3/3
HER2+ Breast Tumors

- **Implications for therapy**
  - More likely to respond to anthracyclines
    - May be explain by co-amplification of TOP2A
  - Good response to trastuzumab therapy in combination with chemotherapy (adjuvant and metastatic)
  - More likely to show pCR to neo-adjuvant chemotherapy + trastuzumab
  - ER+/HER2+ tumor may show Tam resistance
Basal Subtype Breast Tumors: Clinical Features

• **GEP reminiscent of normal myoepithelial cells**
  - Lack of expression of ER and related genes, low HER2
  - High expression of basal cytokeratins (CK5,6,14,17)
  - High expression of proliferation-related genes
  - Amplification/over-expression of EGFR (other GFR), VEGF

• **Aggressive clinical course, poor prognosis**
  - Increase likelihood of early systemic recurrence
  - Visceral recurrence & brain mets more likely than other subtypes

• **Hereditary breast cancer**
  - BRCA1 mutation generally develop basal-like breast tumors

• **Premenopausal African American women 2x the risk of developing basal-like tumors as other group of women**
Basal-Like Carcinoma: Morphologic Features & Immunophenotype

EGFR

CK 5/6
Basal Subtype Breast Tumors

• **Implications for therapy**
  o No role for endocrine therapy or trastuzumab
  o Aggressive clinical course = combination cytotoxic chemotherapy
  o More likely to achieve pCR to neoadjuvant chemo
  o More sensitive to conventional chemo than luminal tumors
    - Anthracyclines + taxane

• **Absence or impaired BRCA1 function**
  o Loss or impairment of DNA repair
  o Hypersensitivity to DNA damaging agents
  o PARP inhibitors (target DNA repair pathways) in clinical trials
Which Markers/Molecular Profiles Should Be Tested in Breast Cancer?

- Recent study compared predictions derived from 5 different gene-expression-based models
  - Models had high rates of concordance in outcome predictions for 295 well characterized breast cancer cases with follow-up
  - Different gene sets that were used for prognostication

- Concluded that although different gene sets were being used as predictors
  - Each appears to track a common set of biologic characteristics which impact clinical behavior and outcome
  - Proliferation genes common driving force behind all prognostic breast cancer signatures

Carcinogenesis, Tumor Biology and Clinical Behavior: Clinically Useful Breast Cancer Classification (Implications for Therapy)

DNA errors

- Altered gene expression
- Altered protein expression
- Altered morphology

Tumor Phenotype

Cancer Diagnosis and Risk of Recurrence:

Key drivers:
- Tumor biology
- Clinical behavior
- Therapeutic response

Testing
- FISH/CISH
- Microarray
- RTPCR
- IHC
- H&E Histology

Cancer biomarker levels
- Tumor biology
- Prognosis and outcome
- Predict Tx response

Interrelated
Intrinsic Properties of Breast Cancer & Clinical Course of Disease

- High Tumor Proliferation
- Genomic Grade Index
- Wound signature
- 70 gene signature
- 76 gene signature
- 21 gene recurrence score
- Intrinsic Molecular Classification

Intrinsic Biologic Properties of Tumor

Intrinsic Molecular Classification

Patient Tumor Burden

- Tumor Size & Nodal Status
- Aggressive Clinical Course
- Poor Prognosis

• Proliferation genes common driving force in prognostic signatures
• Tumor burden independently associated with prognosis

Sotiriou and Paccart, 2007
Is Molecular Profiling the Future of Breast Cancer Diagnosis?

- Role of molecular in routine clinical practice is evolving

- Important questions - application of molecular methods to the clinical diagnosis of breast cancer:
  
  - What is the most practical, clinically relevant, cost-effective, & broadly applicable ancillary testing that's useful to help determine prognosis for breast cancer patients in routine practice?
    
    - Can this approach be reliably used to guide the selection of beneficial treatment regimens?
    
    - Can some patients be spared the cost and morbidity of treatments that will be ineffective?
    
    - What is the level of evidence that the testing strategy can reliably answer these question?
Breast Cancer Treatment: Multidisciplinary Team Approach

Breast Surgeon
- Performs biopsy
- Sends biopsy to pathologist
- Diagnosis/communication of diagnosis/prognosis to patient
- Refers patients to medical oncologist

Pathologist
- Confirms diagnosis of cancer
- Provides information including size of tumor, staging, presence/absence of lymphatic involvement, status of margins, histology grading
- Performs ER/PR and HER2 tests and assesses tumor markers and other cancer characteristics
- Reports test results to surgeon, oncologist, and PCP

Imaging Radiologist
- Performs biopsy
- Sends biopsy to pathologist

Medical Oncologist
- Diagnosis/communication of diagnosis/prognosis to patient

Radiation Oncologist
- Seeks information about margins from pathologist

Opportunities for Pathology Community
- Serve as consultant and treatment advisor - team
- Directly impact initiation of appropriate treatments
- Directly impact patient outcomes

Patient contact
- Patient advisor
- Treatment decision-maker
- Treatment advisor
- Directly impacts initiation of treatment
- Directly impacts treatment stop

PCP=primary care physician.

Thank You!
Next in the Series of Free PHC Webinars

- **Critical Role of Pathology in Personalized Health Care—Wednesday, February 9th, 11 am-12 pm CT**
  - Eric Walk, MD, FCAP

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  - Molecular Diagnosis for Lung Cancer
  - Molecular Diagnosis for Colorectal Cancer
  - Endoscopic Microscopy: Bridging the Radiology/Pathology Divide
  - Considerations in Setting up a Biorepository
  - Personalized Pathology: PHC in the General Pathology Practice
  - Introduction to the Medical Home