NEW BREAST CANCER CLASSIFICATION: TRADITIONAL PATHOLOGY AND MOLECULAR SUBTYPES

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Disclosures; last 10 years

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- Astellas, AstraZeneca, BMS, Eisai, Genentech, GSK, Ipsen, Janssen/JnJ, Lilly, Medivation, Merck, Novartis, Pfizer, Roche, XING Technologies
Outline

- Traditional Pathology
  - Haematoxylin & Eosin
  - Descriptors; Scarff Bloom Richardson
  - Proliferation & Apoptosis
  - Protein IHC classification
  - Immune context
- Molecular Subtypes
  - DNA classification
  - RNA classification
  - Protein classification
- Liquid Biopsy
  - ctDNA/CTC

Aim

Classification
Prognosis
Prediction
Monitoring
Screening/
Early detection
Evolution in Breast Cancer Classification

Morphological Diagnosis

Classical Diagnosis
Ductal infiltrating carcinoma of breast with high grade of nuclear atypia

Immunohistochemical assessment

Protein Expression
ErbB2 over expressing breast tumour

DNA microarray analysis

Gene Expression Profiling
Partial two dimensional cluster analysis of 17 breast tumours

CIRCOS Plot

Integrated Analysis
Chromosome, SNV, LSV, indel, amplification miRNA, RNA

Baselga and Norton, 2002 updated
Combining T/N/M with biology: grade, proliferation, ER/PgR/HER2/GEP

- NB GEP only apply to LN negative disease; T1a-bN0M0
- OncotypeDx, Mammaprint, EndoPredict, PAM50, Breast Cancer Index
Treatment strategies based on “T, N, M classification”

AJCC Cancer Staging Manual

Classification:
- T: Tumor Size
- N: Lymph Node Metastasis
- M: Distant Metastasis

Treatment:
- Chemo Tx

Tsuchida Int J Clin Onc 2019

Treatment strategies based on “subtype classification”

AJCC Cancer Staging Manual

Classification:
- Immunohistochemistry
  - Estrogen receptor
  - Progesterone receptor
  - HER2
  - Ki-67 labeling index
- Gene Assay
  - Oncotype-DX
  - MammaPrint
  - PAM50

Treatment:
- Hormone Tx
- Anti-HER2 Tx
- Chemo Tx

SEER; Site Specific Survival

A: HR+/HER2- (n=9538)

B: HR+/HER2+ (n=2497)

C: HR-/HER2+ (n=1309)

D: TN (n=2067)

Treatment strategies based on “genomic classification”

Classification:
- Next-generation sequencer
  - Gene panels
  - Whole genome sequence
  - Whole exome sequence
  - Liquid biopsy (Circulating tumor DNA)

Treatment:
- Targeted Tx
- Precision Medicine

Tsuchida Int J Clin Onc 2019

Wang BMC 2019
**EBC**

- ER/PR positive
  - ER positive, PR negative
  - ER negative, PR positive
    - HER2 negative
    - HER2 positive
- HER2 negative
- HER2 positive

**ABC5**

- LABC
  - Core biopsy to evaluate histology and biomarker expression (ER, PgR, HER2, proliferation/grade)

**MBC**

- Biopsy of metastatic lesion to confirm ABC diagnosis, particularly if first incidence of metastatic disease

**Diagnosis**

**Staging**

- Minimal staging work-up: history and physical examination, haematology, biochemistry and imaging of chest, abdomen and bone with CT, bone scan or PET-CT

**Grade, LVI, proliferation, DCIS (RCB)**
Histology - T
H&E

Neoadjuvant Biopsy

Surgical Specimen

Figure 2. Probability Maps Generated by the Top 3 Algorithms From the CAMELYON16 Competition
Nucleoli reflect chromatin condensation.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Score</th>
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</thead>
<tbody>
<tr>
<td>Tubule formation (%)</td>
<td></td>
</tr>
<tr>
<td>Majority of tumor (&gt;75)</td>
<td>1</td>
</tr>
<tr>
<td>Moderate degree (10-75)</td>
<td>2</td>
</tr>
<tr>
<td>Little or none (&lt;10)</td>
<td>3</td>
</tr>
<tr>
<td>Nuclear pleomorphism</td>
<td></td>
</tr>
<tr>
<td>Small, uniform cells</td>
<td>1</td>
</tr>
<tr>
<td>Moderate increase in size/variation</td>
<td>2</td>
</tr>
<tr>
<td>Marked variation</td>
<td>3</td>
</tr>
<tr>
<td>Mitotic counts (per 10-40 fields)</td>
<td></td>
</tr>
<tr>
<td>0-5 (histo) or 0-1 (cyto)</td>
<td>1</td>
</tr>
<tr>
<td>6-10 (histo) or 2-4 (cyto)</td>
<td>2</td>
</tr>
<tr>
<td>&gt;11 (histo) or &gt;5 (cyto)</td>
<td>3</td>
</tr>
<tr>
<td>Grade 1 (well-differentiated) (sum)</td>
<td>3-5</td>
</tr>
<tr>
<td>Grade 2 (moderately differentiated) (sum)</td>
<td>6-7</td>
</tr>
<tr>
<td>Grade 3 (poorly differentiated) (sum)</td>
<td>8-9</td>
</tr>
</tbody>
</table>

Scarff-Bloom-Richardson (SBR) Grade

Tubule Formation

Nuclear Pleomorphism

Mitotic Count
Differentiation

Well-differentiated

Poorly-differentiated
Lymphovascular invasion

Anti-vascular antibody CD34
Proliferation & Apoptosis

- MIB-1
- Antibody to Ki67
- Chr 10q26.2
- Protein expressed variably through cell cycle not G0; ? Role heterochromosome stability

- Apoptosis
- One form of cell death
- Multiple assays; TUNEL, COMET, etc.

International Ki67 in Breast Cancer Working Group
Visual vs Automated Ki-67 reading

C

Specimen number

Method
- non-standardized automated (maximum) - 4 labs
- standardized visual (hot-spot) - 10 labs

Ki67 (%)

Specimen number

Method
- non-standardized automated (maximum) - 6 labs
- standardized visual (hot-spot) - 10 labs

Ki67 (%)

Specimen number

Rimm Mod Path 2018
Immune Context

Angiogenesis; CD34

Tumour-infiltrating lymphocytes

Step 1: Select tumor area

Step 2: Define stromal area

Step 3: Scan at low magnification

Step 4: Determine type of inflammatory infiltrate

Step 5: Assess the percentage of stromal TILs (examples of percentages shown in figure 4)

Salgado Ann Oncol 2014
Special subtypes

2a Tubular

3c Metaplastic
Residual Cancer Burden Calculator

*Values must be entered into all fields for the calculation results to be accurate.

(1) Primary Tumor Bed

Primary Tumor Bed Area: (mm) X (mm)

Overall Cancer Cellularity (as percentage of area): (%)

Percentage of Cancer That Is in situ Disease: (%)

(2) Lymph Nodes

Number of Positive Lymph Nodes:

Diameter of Largest Metastasis: (mm)

Residual Cancer Burden:

Residual Cancer Burden Class:
Artificial Intelligence

Patient with suspected malignancy has biopsy and/or surgical resection

Pathologist fixes and sections the tissue specimen, and makes multiple whole slides using several stains

Pathologist digitizes physical slide using whole-slide scanner; oncologist collates adjoining database of relevant clinical and/or outcome information

Pathologist provides reference comparison for the region of interest based on the problem

Convolution

Pooling layer

Flattening

Input

Deep learning (deep neural network) approach

Convolutional layer

Output

AI-based approach from both modalities gives a prediction based on input data

Prediction is compared against the reference to evaluate performance of the model

Performance evaluation is done by reporting area under the curve as well as survival analysis using hazard models

Construct a hand-crafted model to build the AI-based prediction: classification approach for the clinical problem

Pathologist, oncologist, and AI expert use intrinsic domain knowledge to engineer features to be analysed with AI

Hand-crafted AI approach

Patient with suspected malignancy has biopsy and/or surgical resection
Biology – Standard Immunohistochemistry & RPPA
ER; cutoff <1% vs <10%

ER-positive

ER-positive

ER-negative

ER-negative; positive GATA3

ERα, ERβ & G protein-coupled estrogen receptor GPER

ER gene

ER co-activators

Lobular
PR

- Cytoplasmic

**ER & PgR Pathway signature retrospective analysis neo/adjuvant trials discriminate de novo/acquired resistance**
In women with invasive breast cancer, the ECIBC Guidelines Development Group suggests administration of adjuvant endocrine therapy if 1% or greater of tumour cells show oestrogen receptor positivity rather than applying a threshold of 10% tumour cell oestrogen receptor positivity (conditional recommendation, very low certainty in the evidence).

**Recommendation strength**

- **Same wording for progesterone receptor**

- 

  - **Strong recommendation against the intervention**
  
  - **Conditional recommendation against the intervention**

  Ongoing work 1-9 % cut-off; esp clinical trials
• Associated with ER-pos
• Nurses health study
  - 806 deaths from breast ca
  - If AR-positive (continuous)
  - 27% reduction in breast cancer mortality overall
  - 47% reduction for ER-pos cancers
  - 62% increase for ER-neg cancers

Positive cells – continuous score
H-score = combination intensity + no cells

Kensler JNCI 2018
HER2 and FISH images showing HER2 levels from 0 to 3.

0 = Normal
1 = Amplified
2 = Amplified
3 = Amplified

Must see Jenkins SABCS 2017 presentation.
Basal

Cytokeratin 5/6 (CK 5/6)

EGFR
Proliferation

## Analytical Validity

- **ER/HER2**
- **Ki67 14%**
- **PAM50

## Clinical Validity

- **ER/HER2**
- **Ki67 14%**
- **PAM50

## Clinical Utility

- Convincing
- Adequate
- Inadequate

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**Focke BCRT 2015**

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**IMPACT 2012**
St Gallen Criteria

Table 2. Treatment-oriented classification of subgroups of breast cancer

<table>
<thead>
<tr>
<th>Clinical grouping</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triple-negative</td>
<td>Negative ER, PgR, and HER2</td>
</tr>
<tr>
<td>Hormone receptor-negative and HER2-positive</td>
<td>ASCO/CAP guidelines</td>
</tr>
<tr>
<td>Hormone receptor-positive and HER2-positive</td>
<td>ASCO/CAP guidelines</td>
</tr>
<tr>
<td>Hormone receptor-positive and HER2-negative</td>
<td>ER and/or PgR positive ≥ 1%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>luminal disease as a spectrum:</td>
<td>Multiparameter molecular marker ‘favorable prognosis’ if available. High ER/PgR and</td>
</tr>
<tr>
<td>High receptor, low proliferation, low tumor</td>
<td>clearly low Ki-67&lt;sup&gt;b&lt;/sup&gt;. Low or absent nodal involvement (N 0–3), smaller T size (T1 T2).</td>
</tr>
<tr>
<td>burden (luminal A-like)</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>Multiparameter molecular marker ‘intermediate’ if available.</td>
</tr>
<tr>
<td></td>
<td>Uncertainty persists about degree of risk and responsiveness to endocrine and</td>
</tr>
<tr>
<td></td>
<td>cytotoxic therapies.</td>
</tr>
<tr>
<td>Low receptor, high proliferation, high tumor</td>
<td>Multiparameter molecular marker ‘unfavorable prognosis’ if available. Lower ER/PgR</td>
</tr>
<tr>
<td>burden (luminal B-like)</td>
<td>with clearly high Ki-67&lt;sup&gt;b&lt;/sup&gt;. More extensive nodal involvement, histological</td>
</tr>
<tr>
<td></td>
<td>grade 3, extensive lymphovascular invasion, larger T size (T3).</td>
</tr>
</tbody>
</table>

<sup>a</sup>ER values between 1% and 9% were considered equivocal. Thus, endocrine therapy alone cannot be relied upon for patients with these values.

<sup>b</sup>Ki-67 scores should be interpreted in the light of local laboratory values: as an example, if a laboratory has a median Ki-67 score in receptor-positive disease of 20%, values of 30% or above could be considered clearly high; those of 10% or less clearly low.

<sup>c</sup>Not all multiparameter molecular marker tests report an intermediate score.
Histology - N
Nodal Metastasis

Micrometastasis

Extracapsular spread

Isolated Tumour Cells
Molecular Classification
Now 5 years on
From the initial publications
Germ-line Genetics

- TNBC
- ER-neg
- ER-Pos

50/50

BRCA1
BRCA2
etc
DNA Classification

- Point mutation SNV/SNA vs SNP; indel (30bp); amplification; LSV

CNV > SMG

- Age 60%
- APOBEC 14%
- BRCA1/2 10%
- APOBEC 2%

You need to know the language

Nik-Zainal Nature 2016
Epigenetics

Histone code is mediated by writers, erasers, and readers of histone marks

The language of covalent histone modifications

Nature, 2000
Epigenetic alterations in cancer cells

A. Normal epigenome

B. Cancer epigenome

- Hypomethylated domain (28 kb–10 Mb)

- Hypomethylated region
- Hypermethylated region
- Hypomethylated region

Genomic instability
Oncogene activation?

Tumor-suppressor gene silencing

Genomic instability
Oncogene activation?

H3K9me3
Histone acetylation
H3K4me3
Unmethylated CpG
DNA methylation

Baylin and Jones, Cold Spring Harb Perspect Biol, 2016
Original Microarray Expression analyses

Perou Nature 2001
Sørlie et al PNAS 2003
RNA Classification

Prognostic and Now Predictive

Esserman
NCI-EORTC-AACR
Nov 2018
Increasing Complexity

Figure A: Comparison of relapse-free survival across different cancer subtypes using various classification methods: SCMGENE, PAM50, and IntClust. Each graph illustrates the survival rates over follow-up periods, with distinct lines representing different subtypes and their respective numbers of subjects and events. The graphs highlight the complexity and variability in survival outcomes across different classifications.

Legend:
- SCGEME: Subtype (subjects/events)
  - Luminal A (1326/286)
  - Luminal B (765/299)
  - HER2 (352/145)
  - Basal (534/179)
  - Normal-like (272/58)
- PAM50: Subtype (subjects/events)
  - IntClust 1 (256/92)
  - IntClust 2 (96/40)
  - IntClust 3 (643/157)
  - IntClust 4 (577/160)
  - IntClust 5 (270/104)
  - IntClust 6 (106/42)
  - IntClust 7 (294/65)
  - IntClust 8 (434/111)
  - IntClust 9 (243/96)
  - IntClust 10 (332/103)

METABRIC

Ali Genome Biol 2014
Protein

- Ultimate effector
  - capture the functional state and dynamic properties of a cell
- Proteo-genomics
- Kinome
  - Phospho, other PTMs
- Membrane
- Cytoplasm
- Golgi etc
- Nuclear
TCGA human breast tumors (n=598)

- Luminal A
- Luminal B
- HER2-e
- Basal-like
- Normal-like

IHC (red = pos)

RPPA (red = high)

RNA-seq, PAM50 genes (yellow = high)

? 60 – 80%
Metabolome

- Metabolic reprogramming in ER-positive breast cancer
- Super-SILAC mix to quantify over 10,000 proteins with high accuracy
Integration of information

- Sum is greater than the parts
Immune-Context
Immune gene signature

Nagalla Genome Biol 2013
Prognostic Value of TILs in Early-Stage TNBC

9 Randomized Adjuvant Treatment Trials

- N= 2148 TNBC patients samples
- 77% had ≥ 1% sTIL
- Average value of sTIL=23% (SD, 20%)
- Lower sTILs in older patients, larger tumor, more nodal involvement, lower grade
- Each 10%↑ in sTIL was associated with:
  - 13% relative ↑ in iDFS events
  - 16% relative ↓ in deaths

1. Loi S et al. JCO 2019
PD-L1 IHC assays: prevalence and analytical concordance

NPA, negative percentage agreement; OPA, overall percentage agreement; PPA, positive percentage agreement.

a > 97% of SP142+ samples included in 22C3+ or SP263+ samples. b Compared with 41% in ITT (Schmid, New Engl J Med 2018).

SP142 (IC ≥ 1%) and 22C3 (CPS ≥ 1%)

<table>
<thead>
<tr>
<th>Cases</th>
<th>SP142</th>
<th>22C3</th>
<th>SP263</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>SP142</td>
<td>22C3</td>
<td>SP263</td>
</tr>
<tr>
<td>20%</td>
<td>SP142+</td>
<td>22C3+</td>
<td>22C3-</td>
</tr>
<tr>
<td>40%</td>
<td>SP142+</td>
<td>22C3+</td>
<td>22C3-</td>
</tr>
<tr>
<td>60%</td>
<td>SP142+</td>
<td>22C3+</td>
<td>22C3-</td>
</tr>
<tr>
<td>80%</td>
<td>SP142+</td>
<td>22C3+</td>
<td>22C3-</td>
</tr>
</tbody>
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SP142 (IC ≥ 1%) and SP263 (IC ≥ 1%)

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<th>SP263</th>
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<tbody>
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<td>SP142+</td>
<td>SP263+</td>
</tr>
<tr>
<td>20%</td>
<td>SP142+</td>
<td>SP263+</td>
</tr>
<tr>
<td>40%</td>
<td>SP142+</td>
<td>SP263+</td>
</tr>
<tr>
<td>60%</td>
<td>SP142+</td>
<td>SP263+</td>
</tr>
<tr>
<td>80%</td>
<td>SP142+</td>
<td>SP263+</td>
</tr>
<tr>
<td>100%</td>
<td>SP142+</td>
<td>SP263+</td>
</tr>
</tbody>
</table>

PD-L1+ prevalence

<table>
<thead>
<tr>
<th>Cases</th>
<th>SP142 (IC ≥ 1%)</th>
<th>22C3 (CPS ≥ 1%)</th>
<th>SP263 (IC ≥ 1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>SP142+</td>
<td>22C3+</td>
<td>22C3-</td>
</tr>
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<td>22C3+</td>
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<td>22C3-</td>
</tr>
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Liquid Biopsy

- Tumor protein
- ctDNA
- CTC
- EV
- TEP
- RNA signatures
- Alternative splicing
- Fusions
- ctRNA
- miRNA profiles
- mRNA splicing and fusion variants
- IncRNA
- Other ncRNAs
- Genomics
- Transcriptomics
- Cytogenetics
- Surface and intravesicle proteins
- Drug screening
- Proteomics
- Genetic analyses
- RNA profiling
- Clinical protein assays
- Amplifications
- Mutations
- Deletions
- LOH
- Methylation
- Translocations
- Fragmentation
- Coding
- Non-coding
- De Rubis TIPS 2018
**Summary**

- TNM
- DNA
- meDNA
- RNA
- Protein

Significantly improved understanding of individual patient’s biology

Precision medicine + Immuno-Oncology

Need to ensure affordable, accessible access for all