NEW BREAST CANCER CLASSIFICATION:

Traditional pathology and molecular subtypes
Prognostic and predictive factors

Frédérique Penault-Llorca

Preceptorship in breast cancer 2019
DISCLOSURE OF INTEREST

Frédérique Penault-Llorca

- **Personal financial interests:** Abbvie, Astrazeneca, Bayer, BMS, Genomic Health, Lilly, MERCK lifa, MSD, Myriad, Nanostring, Novartis, Pfizer, Pierre-Fabre, Roche, Tesaro

- **Institutional financial interests:** Astrazeneca, Bayer, BMS, Genomic Health, MSD, Myriad, Nanostring, Roche

- **Congress invitations:** Abbvie, Astrazeneca, BMS, MSD, Novartis, Roche
Objectives
To learn about the biology of breast cancer and its implication in the management of BC patients
Preinvasive
Ductal carcinoma in situ (DCIS)
- Spreads through ducts and distorts ductal architecture; can progress to invasive cancer; unilateral
- Lobular carcinoma in situ (LCIS)
- Does not distort ductal architecture; can be bilateral
- Risk factor rather than precursor

Invasive
Ductal carcinoma no special type (NST)
- Develops from DCIS; fibrous response to produce a mass; metastasizes via lymphatics and blood
- Lobular carcinoma (ILC)
- Isolated tumor cells (CDH1 mutations) minimal fibrous response; metastasizes preferentially via viscera
“What is new”: changes in the practice of breast cancer diagnostics

- **Mass screening**: smaller tumours at diagnosis
- Pre-surgery “strategic biopsy”: less frozen sections for breast cancer diagnosis
- Therapeutic **de-escalation** in surgery: **sentinel lymph node assessment**
- Personalized medicine:
  - Treatment driven by tumour biology ("intrinsic" classification) rather than by stage
  - Reflex testing of predictive factors (hormonal receptors, HER2)
- Therapeutic **de-escalation** in oncology: prognostic and predictive molecular **signatures**
Outlines

• Breast cancer pathology: the basics revisited
• Molecular pathology
• Specific subtypes
• Molecular signatures
• Molecular stratification of metastatic breast cancer
THE CLASSICS
Classical prognosis and predictive factors

- Age
- Grade
- Histological subtypes
- ER/PR and HER2 status
- Ki67 +/- mitotic index
- Vascular invasion
- Tumor margins

Oldies but goldies
CLASSICAL PARAMETERS
TIPS AND TRICKS
Multifocality vs multicentricity

Fisher Cancer 1975

With the use of MRI: 13-70% of multiple lesions
Multiple sites?
Clarification of the AJCC 7th edition

Staging multiple tumors

• If in same breast:
  – T category is based on **single largest tumor focus**
  – Don’t include satellite foci when measuring tumor size
  – If multiple foci of microinvasion, report the # of foci and **the size of the largest focus** (don’t combine)
  – Use (m) modifier

• If bilateral:
  – Stage each side separately
Size post macrobiopsies
Scars, compare with radiologic size
Clarification of the AJCC 7th edition in the 8th edition

• Correlate gross, microscopic and imaging findings to assign correct pT when necessary.
  - For small tumors diagnosed by core biopsy, measuring only the residual tumor in the excision may result in understaging.

• Example:
  – 6 mm mass by imaging; largest focus in biopsy core – 4 mm
  – 2 mm focus of residual carcinoma in excision: categorize as pT1b (not pT1a)
  – No residual cancer in excision: categorize as pT1a (not pTX)

• Same rule applies when tumor is present in multiple fragments: Use clinical and imaging findings to assign pT

• pTX should rarely be used
Initial concepts for the use of SLNB in Breast Cancer

- Obtention of prognostic information
- Therapeutic role (!)
- Avoid full axillary dissection for pN0 patients

Consequences:
- Better management of the nodes (full node assessment)
- Changes in the TNM
Facts about SLNB

• Completion **ALND is not providing benefit** of OS and DFS in **microscopic** metastatic SLN [pN0(i+) and pN1mi].

• Even **macrometastasis** in 1 or 2 SLN(s) in ACOSOG Z0011 did not affect OS.

• SLN biopsy alone can be a **standard practice** demonstrating its efficacy, accuracy **in staging** and equivalent survival outcome when compared to complete ALND and SLNB alone in **T1–T2** breast cancer.
SLNB conclusion

• **No longer systematic intraoperative assessment**
• In case of + SLN, ALND is no longer systematic and as to be discussed in MDTB

• **Ultra-stadification:**
  – balance between what is useful for the patient or not, and should not be deleterious (over treatment)
  – Careful in case of use of molecular signatures (not validated ith SLNB)
  – Balance between what is possible or not in the lab
  – Depends upon guidelines (adjuvant TT and RTT)

• **NACT: 2 options are possible**
  • Pre NACT> post NACT
GRADE
SBR grade modified by Elston and Ellis

- Standardization of tumor grading
- France 2010: Gr I 25%, Gr II 50%, Gr III 25%
- Genomic grade: not confirmed

SBR grade and RFS in operable BC (57% N-) treated by adjuvant therapy
VASCULAR OR LYMPHATIC EMBOLIES
Peripheral lymphovascular invasion and BCSS in N- operable BC treated by adjuvant therapy (from Lee)
HISTOLOGIC SUBTYPES
Histologic subtypes of epithelial breast cancer WHO 2012

Infiltrative carcinoma

Ductal
Lobular
Tubular
Cribriform
**Medullary**
Mucinous
Neuroendocrine
Papillary
Micropapillary
Apocrine

Metaplastic
Secretory
Lipid Rich
Oncocytic
Adenoid Cystic
Acinar
Clear Cell
Sebaceous
Inflammatory

[Image: WHO Classification of Tumours of the Breast]
19 Histological types: **morphology matters!**

- **Group 1 - Excellent prognosis:**
  Tubular, invasive cribriform, mucinous

- **Group 2 - Good prognosis:**
  Tubular mixed, mixed ductal NST and special type like adenoid cystic, secretory

- **Group 3 - Average prognosis:**
  Medullary, classical lobular, lobular mixed

- **Group 4 - Poor prognosis**
  Ductal NST, solid lobular, mixed ductal NST and lobular, micropapillary
Special types

“Tubular and cribriform carcinoma may be suitable for observation without therapy or for endocrine therapy alone”

Tubular carcinoma and DFS (Rakha)
CLASSICAL TNM AND HISTOPATHOLOGICAL PARAMETERS MATTER!
Minimal items in a pathology report
NOT ALL INVASIVE BREAST CANCERS ARE BEHAVE AS INVASIVE.... ENCAPSULATED PAPILLARY BC
Carcinomatous lesions with papillary architecture

• Papilloma with DCIS
• DCIS papillary type
• Encapsulated papillary carcinoma ➔ consider as a DCIS, no theranostic IHC if low grade
• Papillary carcinoma massive type (solid papillary carcinoma) ➔ consider as DCIS
• Infiltrative papillary carcinoma ➔ pT
Encapsulated papillary carcinoma

- Post menopausal patient (>60yrs)
- Palpable or infraclinical lesion
- Capsule +/- thick
- If low grade:
  - \( \Rightarrow pTis \)
  - Treat as a DCIS + SLNB
- If high grade
  - \( \Rightarrow pT \)
  - Treat as an invasive carcinoma (RE, PR, HER2)
NOT ALL BREAST NODULES ORIGINATE FROM BREAST
Metastasis to the breast

• 0.2 to 1.3% = rare
  – lymphomas
  – Melanoma
  – carcinomas (lung, GYN, kidney, digestive tract, prostate ...)
  – non-mammary neuroendocrine tumors
• 1st clinical sign of the disease in 30% of cases
• Delay between primary tumor and metastasis sometimes very long (22 years) especially for melanoma and ovary.
• Often large masses, fast growing, well limited and round, sometimes superficial
• May mimic benign lesions (ACR3)
• Often unique
Most frequent primary tumors

- Carcinoma (58%, 49/85)
- Mélanoma (21%, 18/85)
- Sarcoma (21%, 18/85)

Among carcinoma:
- GYN cancer (39%, 19/49)
- Including ovarian K (29%, 14/49)

Non-mammary metastases to the breast and axilla: a study of 85 cases, DeLair and al, Modern Pathology, 2014
Looks like a lobular
But triple negative
Mammary or not?

- triple negative (?!?) very unusual for a lobular
- So we did GATA3 ➔ neg

➔ CK7 & EMA neg
Metastasis from a melanoma
Always question unusual triple negative tumors

• Unusual clinical presentation
• Clinical history
• The pathologist plays an important role
  – Unusual microscopic aspect
  – Unusual phenotype
  – Absence of DCIS.....
But sometimes the metastasis is HR+!

1) Tumors usually expressing HR:
   Significant expression of ER, PR +/-
   -Breast carcinoma: 80%
   -Carcinoma of **gynecological origin**: endometrioid carcinoma and serous carcinoma: > 80%

2) Tumors rarely expressing HR:
   Often low expression
   -Bronchopulmonary adenocarcinoma (5%)
   -Salivary gland tumor: a minority can express HR (ER and PR): weak expression
   -Neuroendocrine carcinomas and pseudopapillary solid carcinomas of the pancreas: PR only
Take home message: Is it a primary breast cancer?

• Clinical presentation **large nodules**, growing fast, well demarcated sometimes they are superficial.
• **Can mimic benign lesions** (ACR3)
• Frequently unique
• ➔ **beware of triple negative lesions with an unusual presentation** (mucinous for instance)
• ➔ **beware of ER+ with papillary aspects and psammomas**
• ➔ **aspect of lobular carcinoma in a men**
Prediction
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Prognostic</th>
<th>Predictive</th>
<th>Technical validation</th>
<th>Clinical validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>++</td>
<td>+++</td>
<td>YES LOE Ib</td>
<td>YES</td>
</tr>
<tr>
<td>PgR</td>
<td>+++</td>
<td>++</td>
<td>YES LOE Ib</td>
<td>NO</td>
</tr>
<tr>
<td>HER2</td>
<td>++</td>
<td>+++</td>
<td>YES LOE Ib</td>
<td>YES</td>
</tr>
<tr>
<td>Ki67</td>
<td>++</td>
<td>+</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

**Test and scoring recommendations**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>IHC ≥1%</td>
</tr>
<tr>
<td>PgR</td>
<td>IHC ≥1%</td>
</tr>
</tbody>
</table>
| HER2      | IHC ≥10% cells with complete membrane staining  
ISH: number of HER2 gene copies ≥4 or the ratio HER2/chromosome 17 ≥2  
IHC no final consensus on cut-off around 20% (Ki67< 10% = low ; Ki67>30% = high) |
Low ER+ Breast Cancer
Is This a Distinct Group?

Nika C. Gloyeske, MD, David J. Dabbs, MD, and Rohit Bhargava, MD

From the Magee-Womens Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA.

Key Words: Low ER+/HER2--; Morphology; Response to neoadjuvant chemotherapy

Am J Clin Pathol May 2014;141:607-701
DOI: 10.1093/ajcpqjv060

Conclusions: The low ER+/HER2-- cases have morphologic features and a response to the chemotherapy rate that are more similar to triple-negative tumors than the usual type of ER+ tumors.

- 5% of BC, usually Grade 3
- Solid and necrotic T
- 80% Ki67> 50%
- 94% PR-
- 33% of pCR if NACT
Prognostic and predictive value of low estrogen receptor expression in breast cancer

A. Bouchard-Fortier MD MSc, L. Provencher MD MA, C. Blanchette MSc, and C. Diorio PhD

- Cytosolic
- tamoxifen
- Follow up >20 yrs
  - 17% (383) 0–3 fmol/mg cytosol protein
  - 12% (266) 4–9 fmol/mg cytosol protein.
- 56% 20 yrs OS vs 71% for high ER
A Majority of Low (1-10%) ER Positive Breast Cancers Behave Like Hormone Receptor Negative Tumors


- 240 cases
- 144 high ER (>10%), 75 ER negative and 21 low-ER (1-10%) tumors by IHC
- qRT-PCR test with 6 ER related genes
- ½ low-ER positive tumors ➔ ER negative group based on the probability score
- 95% of ER negative and 92% of the high ER positive tumors classified correctly (p<0.0001).
- Survival of the low-ER group was intermediate between that of the high ER positive and ER negative groups (p<0.05).
In case of weak ER (1-9%) in practise

• On biopsy: redo on surgical specimen
• On surgical specimen: take into account also the other parameters
• Role of Gene Expression Signatures (GES)?
Ki67 why?

• Definition of luminal A and B

• Decision of CT for ER+, Grade II tumors
Ki67 = Not standardized
Reproducibility

An International Ki67 Reproducibility Study

Manuscript received April 2, 2013; revised September 3, 2013; accepted September 18, 2013.

Correspondence to: Torsten Nielsen, MD, PhD, FRCPC, University of British Columbia, Pathology and Laboratory Medicine, Anatomical Pathology, 5641, Vancouver Hospital & Health Science Centre, 888 W 12th Ave, Vancouver, BC, V5Z 1M9, Canada. e-mail: torsten@mai.ubc.ca.

PLOS ONE | DOI:10.1371/journal.pone.0125131 May 1, 2015
An Interobserver Reproducibility Analysis of Ki67 Visual Assessment in Breast Cancer
Ruohong Shui1,2, Baohua Yu1,2, Rui Bi1,2, Fei Yang1,2, Wentao Yang1,2,*
1 Department of Pathology, Fudan University Shanghai Cancer Center, Fudan University, Shanghai 200032, China; 2 Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China.
*yangwe@2000@163.com

Interobserver concordance of Ki67 labeling index in breast cancer: Japan Breast Cancer Research Group Ki67 Ring Study
Yoshiki Miyazaki1,2, Takayuki Ueno1,2, Kenichi Yoshimura1, Hitoshi Tsuda1, Masafumi Kurosawa1, Shinobu Maruda1, Rie Horii1, Masakazu Torii1 and Hironobu Sasan1,2

Departments of 1Diagnostic Pathology, 2Breast Surgery, Kyoto University Hospital, Kyoto; 3Department of Pathology, Osaka General Medical Center, Osaka; 4Department of Pathology, Osaka University Graduate School of Medicine, Osaka; 5Department of Pathology,flat Hospital, Osaka

Modern Pathology (2015) 28, 778–786
An international study to increase concordance in Ki67 scoring
FOCUS ON HER2 GUIDELINES
Huge benefit from anti HER2 therapies for patients with mBC and eBC

13-15% HER2+ in eBC
18% in mBC if previously treated
~20-25 if naive


Reply to E.A. Rakha et al
Heterogeneity: Where to count?
Act III?

1. Simplification of IHC 2+ definition (moderate/weak)
2. Re-testing on surgical specimen if a biopsy is HER2 - :
   “may” in instead of “should”
3. Revision and/or definition of difficult ISH categories
   (monosomies, co-amplification, “equivocal”) ➔ avoid as much as possible “equivocal/eligible” cases

Based on IHC results
Messages for HER2 ASCO/CAP new guidelines

• **Simplification of HER2 2+ 2**
• No longer systematic re-testing
  • **Difficult ISH categories**: between 4-6 copies +/- ratio HER2/CEP17<2
    • Interpretation with IHC++++
    • Independant (second reader for ISH) for 2+
    • Disparition of equivocal ISH category
      • Category 2 (monosomy): rather negative
      • Category 3 3 (co-ampl): rather positive
      • Category 4(ex-equivocal): rather negative
• Avoid single probe ISH
THE PATHOLOGY REPORT
Box 4 | The pathology report for breast cancer

- Histological type according to the current WHO classification
- Histological grade according to the Elston- and Ellis-modified Scarff–Bloom–Richardson system
- Peritumoral vascular or lymphatic emboli
- Hormone receptor status (oestrogen receptor (ER) and progesterone receptor)
- Human epidermal growth factor receptor 2 (HER2) status
- Excision margins (mm)
- Tumour size, single or multiple tumours
- Ductal carcinoma in situ component type, grade and percentage
- Lymph node status
- Pathological stage according to the Union for International Cancer Control TNM system
- Ki67 score according to the international group guidelines

*aInformation obtained at surgical resection. bParticularly relevant for ER-positive, HER2-negative breast cancers.
When to question a pathology report

- PgR+, ER-
- Lobular, tubular carcinoma HER2+
- Grade 1, ER+++ , PgR+++ , HER2+
- Grade 3, ER-, ki67 <5%
- Grade 3 ER+++, PgR+++
- Medullary carcinoma is extremely rare and has been removed from WHO classification

➔ May redo HER2 (and ER) on surgical specimen if grade 3, ER- or ER+
➔ If ER and/or PgR is negative on a biopsy redo on surgical specimen
Does Estrogen Receptor–Negative/Progesterone Receptor–Positive Breast Carcinoma Exist?


Estrogen receptor negative/progesterone receptor positive breast cancer is not a reproducible subtype

Marco M Hefti1, Rong Hu2, Nicholas W Knoeblauch1, Laura C Collins1, Benjamin Haibe-Kains3, Rulla M Tamimi2 and Andrew H Beck17
**ER-/PR+**

- Approximately 70% of breast cancers are ER+,
  - ER+/PR+ 57% EBC
  - 25% ER+/PR− with a more aggressive biological behavior than ER+/PR+ tumors [8]
- ER−/PR+ controversial +++ breast cancers incidence of 1% to 4%
  - Technical artifact arising from inadequate tissue fixation or failure of the immunohistochemical assay?
  - Others argued that even using optimally fixed tissues and any level of nuclear immunoreactivity of tumor cells as a positive result, the ER−/PR+ was still retained as a unique entity
- ER−/PR+ classification was too rare to be of clinical use?
• 5374 consecutive breast cancers
• **2.3% ER−/PR+ tumors**
• High grade and significantly seen in younger patients and African American women (vs ER+/PR+ and ER+/PR−)
• **Similar to ER−/PR− phenotype** (P <0.0001).
A significantly prolonged relapse-free survival (RFS) was associated with the ER+/PR+ subtype when compared with the ER+/PR− (P =.0002) or ER−/PR+ (P = .0004) tumors, whereas all 3 groups showed a superior outcome to that of the ER−/PR− phenotype.

RFS in patients HR+ treated with endocrine therapy.
- ER+/PR+ associated with a significantly prolonged RFS when compared with the ER+/PR− group p=0.001
- No significant difference was found between ER+/PR+ and ER-/PR+
- Same trends for disease specific deaths p=0.005
4,111 cases from 20 published studies with gene expression microarray (GEM) and clinicopathological data (ER + / PR +, ER + / PR -, ER - / PR -, ER - / PR +) and basis of 2011 Nurses' Health Study (NHS) with ER / PR data, clinical data and molecular analysis. The ER - / PR + subtype is rare (1 to 4%) and not reproducible in the molecular classes. Most patients classified as ER - / PR + in the clinical databases (97 and 94% respectively) were reclassified by a second method. The expression of PR in RNAm in the GEM base was associated with prognosis for ER + (P <0.001) but not for ER - (p = 0.21).
ER-/PR+ what to do in practise?

- Re-test the case, check internal controls
- In case of absence of + internal controls Re-test on a other block
- If still ER-/PR+  
  - If available require a GES  
  - The prognosis of those lesions appears less favorable than ER+:PR+ but the positivity of PR receptor remains a strong prognostic factor in case of hormonal treatment
TILS
Assessing Tumor-infiltrating Lymphocytes in Solid Tumors: A Practical Review for Pathologists and Proposal for a Standardized Method From the International Immunoncology Biomarkers Working Group Part 1 Assessing the Host Immune Response, TILs in Invasive Breast Carcinoma and Ductal Carcinoma In Situ, Metastatic Tumor Deposits and Areas for Further Research

Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers

9 essais randomisés TILs et survie globale dans les CSTN
Different TILs infiltrates in different categories of breast cancer

http://dx.doi.org/10.1016/j.semcancer.2017.11.003
Immunogenicity of breast cancers

Tubular BC
Lobular BC
Mucinous BC
Carcinoma with medullary features

P<0,0001

http://dx.doi.org/10.1016/j.semcancer.2017.11.003
TILs assessment requires standardized approaches

Lymphocyte predominant breast cancer can be used as a descriptive term for tumors that contain “more lymphocytes than tumor cells.” However, the thresholds vary between 50% and 60% stromal lymphocytes.
MOLECULAR AND HISTOLOGIC CLASSIFICATION
Towards a simplified taxonomy of breast cancer? « definition of intrinsic subtypes has proven efficient in defining prognosis for breast cancer patients »
<table>
<thead>
<tr>
<th>Intrinsic subtypes (PAM50)</th>
<th>Basal-like</th>
<th>Claudin-low</th>
<th>HER2-enriched</th>
<th>Luminal B</th>
<th>Luminal A</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53 mutations; genetic instability; BRCA mutations; medullary-like histology; poorly differentiated</td>
<td>largely triple-negativity; metastatic</td>
<td>HER2 amplification; GRB7 amplification; PIK3CA mutations; TOP2a and/or MYC amplification; NST, pleomorphic lobular and micropapillary histology</td>
<td>PI3KCA mutations (40%); ESR1 mutations (30–40%); ERBB2 and ERBB3 mutations; NST, micropapillary and atypical lobular histology</td>
<td>Activation of ERS1, GATA3, FOXA1, XBP1; NST, tubular cribriform and classic lobular histology</td>
<td></td>
</tr>
</tbody>
</table>

**Surrogate intrinsic subtypes**

<table>
<thead>
<tr>
<th>Triple-negative</th>
<th>HER2-enriched (non-luminal)</th>
<th>Luminal B-like HER2+</th>
<th>Luminal B-like HER2-</th>
<th>Luminal A-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-, PR-, HER2-; high grade; high Ki67 index; NST histology; special type histology (metaplastic, adenoid cystic, medullary-like and secretory); poor prognosis except for some special types</td>
<td>ER-, PR-, HER2+; high grade; high Ki67 index; NST histology; aggressive disease but responds to targeted therapies; intermediate prognosis</td>
<td>ER+ but lower ER and PR expression than luminal A-like; HER2+; higher grade; high Ki67 index; NST and pleomorphic; responds to targeted therapies; intermediate prognosis</td>
<td>ER+ but ER and PR expression lower than in luminal A-like; HER2-; low proliferation rates; typically low grade; high Ki67 index; high-risk GES; NST, micropapillary and lobular pleiomorphic histology; intermediate prognosis</td>
<td>Strongly ER+ and PR+; HER2-; low proliferation rates; typically low grade; low Ki67 index; low-risk GES; NST, tubular cribriform and classic lobular histology; good prognosis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proliferation</th>
<th>High grade</th>
<th>Basal-like genes</th>
<th>HER2 expression</th>
<th>ER expression</th>
<th>Low grade</th>
</tr>
</thead>
</table>

2013 St Gallen International Expert Consensus

Quote: “Panel endorsed gene expression signatures that permit avoidance of chemotherapy in many patients with ER-positive breast cancer”.

- Endocrine Therapy (chemo in selected cases)
- Endocrine + Chemo (most)
- Endocrine + Chemo + anti-HER2
- Chemo + anti-HER2
- Chemo
Biology of breast cancer varies with aging

de Kruijf Mol Oncol 2014, Jenskins Oncologist 2014
Molecular biology of BC is influenced by ethnies and country of residence

- African (mean age = 45 y)
- African American (premenopausal)
- African American (postmenopausal)
- White in US (premenopausal)
- White in US (postmenopausal)
- White in Poland (mean age = 56 y)
- Japanese (median age = 54 y)

- Basal
- Luminal A
- HER2
- Luminal B
- Non classées
The picture of basal-like breast cancer

- Low ER (and related genes) expression
- Low HER2 cluster expression
  → usually “triple negative”
- High basal cluster
  - basal cytokeratins
  - EGFR
  - c-kit
  - others...
- Very proliferative
- Often p53 mutant (>90%)
- Evidence of genomic instability
Triple negative BC by IHC and molecular subtypes: a 80% concordance

- Basal-like
- Triple-negative

TNBC subtypes of excellent prognosis

- Medullary
- Low grade squamous
- Adenoid cystic
- Secretory carcinoma

• ER and PgR absent
• HER2 negative
• ~80% overlap between TNBC & intrinsic « basal-like »
Triple-Negative Breast Carcinomas: Prototypical Features

• Clinical features
  – Younger patients (47-55 years)
  – African American women
  – Interval cancers
  – BRCA-1 mutations
  – Prevalence of brain and lung metastases
  – Early metastasis (2-3 years)

90% of Triple negative breast tumors: invasive ductal NST

- Pushing borders
- Large areas of necrosis
- Myofibroblastic stroma
- Lymphocytic stroma
- Poor differentiation
- Atypia, proliferation
- ER 0%
- PgR 0%
- HER2 negative
TN Tumors Are Heterogeneous

- IDC NOS, high grade
- ILC high grade, pleomorphic
- Metaplastic, high grade
- Myoepithelial carcinoma
- High-grade (oat-cell) neuroendocrine
- Apocrine
- Adenoid-cystic
- Juvenile Secretory
- Carcinoma with rich lymphoid stroma
- Metaplastic, low grade
  - Low-grade adenosquamous
  - Fibromatosis-like

- Poor prognosis
- Good prognosis
Identify special types with better prognosis

Amplicons chr 10, 12
10p+, 9p+, 16q+, 4p-

t(12;15) (ETV6; NTRK3)

Basal like carcinoma

EGFR amplification
WNT pathway alterations

t(6;9) (q22-23; p23-24) (MYB ;NFIB)
Uncertain prognosis: Apocrine carcinoma

in ½ cases: HER2+
HER2 POSITIVE
Surrogate definition of intrinsic subtypes of breast cancer

«HER2 enriched»

- HER2 positive ➔ 3+ by IHC or amplified by FISH
- And ER and PgR negative
HER2+ diseases

- Rare +++ lobular, tubular carcinoma
- ~ 50% are ER+  ➔ completely different disease

HER2+, prognostic value of pCR in HR-
2 different HER2+ groups /HR status

Less pCR in HER2+, ER +
LUMINAL BREAST CANCER
Luminal breast cancer

Luminal A
- ER+
- And all
  - PR +
  - Ki67 low
- Low molec risk

Luminal B
- ER+
- And at least
  - PR low
  - Ki67 high
- High molec risk

Luminal B HER2 +
- ER+, HER2 3+
- Whatever PR
- Whatever Ki67
Intermediate category

**LUMINAL A**
- Grade 1
- ER⁺
- PR⁺ (> 20%)
- Ki67 low (< 20%)
- NOS, tubular, cribriform, mucinous mol low risk, simplex genomic profile
- Low activation PI3K/AKT
- Hormonosensitivity

**LUMINAL B**
- Grade 3
- ER⁺
- PR⁺/⁻ (≤ 20%)
- Ki67 high (≥ 20%)
- HER2⁺/⁻
- NOS, micropapillary
- Mol high risk, complex genomic profile
- Activation growth factor R
- Hormonosensitivity, chemosensitivity

→ Heterogeneous tumours defined by the expression of ER
→ **Current detection method is IHC** (issues on threshold, standardization)
→ ER⁺ tumours and HER2⁺ classified as luminal B
→ Major role of proliferation
→ **Potential over/undertreatment / late recurrences**
LOBULAR CARCINOMA
12-14% of BC, poor limitation
Frequent metastasis to serous tissues (pleura, peritoneum, pericardia
Lobular carcinoma

- E-cadherin Inactivation in 95% of cases
- ER+ > 90% of cases
- Low proliferation
- HER2 score 3+ < 5% of cases
  - HER2 Mutations:
    - 6% classical ILC
    - 15% ILC high grade
- **PIK3CA Mutations in 48% of the cases**
- Mutations TP53, GATA3, FOXA1, RUNX1 ~ 5 -10% of the cases
  PTEN/AKT pathway activation mutually exclusive with mutuellement PIK3CA mutations.
- 3 or 2 transcriptomic groups have been identified
  - « reactive-like » (good prognostic), « Immune-related » &« proliferative »
  or « immune-related » & « hormone – related »

Michaut et al Scientific report 2016
Ciriello et al Cell 2015
Denizialt et al Oncotarget 2016
TCGA. Nature 2012.

Targeted anti-HER2 therapies
- Low chemosensitivity
- mTOR inhibitor
- PIK3CA i
Micropapillary carcinoma a very aggressive luminal tumor

- Embolies -70-80%) and frequent node invasion (pure 60%, mixed 40%)
- SBR II or III Recurrent abnormalities in 8p11-22, involving FGFR1, NGR1 / neuregulin
- HR + 70-90%
- HER2 + 35-50%
- C-MYC amplification
Specific/frequent molecular alterations

• **Mucinous carcinomas** (2%)  
  - Characterized by **increased frequency** of **GATA3** (23%) mutations, and **decreased frequency** of **PIK3CA** (8%) and TP53 (8%) alterations compared to IDC.

• **One third of all BC primary tumors do not present any reported driver mutation.**
LUMINAL KEY MESSAGES
CLASSICAL PARAMETERS ARE IMPORTANT, BUT.......
Classical prognosis and **predictive** factors

- Age
- Grade
- Histological subtypes
  - ER/PR and HER2 status
  - Ki67 +/- mitotic index
- Vascular invasion
- Tumor margins

Oldies but goldies
TREATMENT DESCALATION IN HR+ HER2- ➔ MOLECULAR SIGNATURES
Yes, we have molecular biology!

- Age
- Grade
- Histological subtypes
- ER/PR and HER2 status
- Vascular invasion
- Tumor margins
4 signatures, 4 different worlds

<table>
<thead>
<tr>
<th></th>
<th>Oncotype DX</th>
<th>MammaPrint</th>
<th>Prosigna</th>
<th>EndoPredict</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene number</strong></td>
<td>16 + 5 reference</td>
<td>70</td>
<td>50 + 8 reference</td>
<td>8 + 3 reference</td>
</tr>
<tr>
<td><strong>Patient Type</strong></td>
<td>Pre or postmenopausal HR+, HER2 Node -/+ (1-3) early stage</td>
<td>Pre or postmenopausal ER+/ Node -/+ early stage tumor &lt;5cm</td>
<td>Postmenopausal HR+, HER2- Node -/+ (1-3) Stage I to IIIA BC</td>
<td>Postmenopausal HR+, HER2- Node -/+</td>
</tr>
<tr>
<td><strong>Individual Risk</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Classification</strong></td>
<td>Continuous score 0-100; reports individualised</td>
<td>Low, High</td>
<td>Continuous score reported as Low, Inter, High</td>
<td>Low, High</td>
</tr>
<tr>
<td><strong>Prognostic</strong></td>
<td>Yes level 1A</td>
<td>Yes level 1A</td>
<td>Yes level 1B</td>
<td>Yes level 1B</td>
</tr>
<tr>
<td><strong>Predictive of chemotherapy benefit</strong></td>
<td>Yes level 1A</td>
<td>No clinical evidence</td>
<td>No clinical evidence</td>
<td>No clinical evidence</td>
</tr>
<tr>
<td><strong>Technology</strong></td>
<td>Quantitative RT-PCR</td>
<td>Microarray</td>
<td>direct mRNA hybridization</td>
<td>Quantitative RT-PCR</td>
</tr>
</tbody>
</table>

Centralized tests
MammaPrint
(Agendia, NL)

HR+ ET HR- / HER2- , T < 5cm, N ≤ 3

Fresh frozen=> FFPE
DNA array
70 GENES
CELL CYCLE/ PROLIFERATION
SIGNAL TRANSDUCTION
INVASION, METASTASIS, ANGIogenesis

« CENTRALIZED » TEST
RECENTLY ADAPTED TO FFPE

Group of genes (« signatures »)
EARLY RECURRENCE (Dg < 5 ans)
PROGNOSTIC
GOOD SIGNATURE :
LOW RISK
POOR SIGNATURE :
HIGH RISK
OncotypeDX
(Genomic Health, USA)

HR+ / HER2- , T1-3, N-/N+
FFPE specimens
qRT-PCR

21 GENES
PROLIFERATION, OESTROGENE,
HER2, INVASION (16 GENES) + REFS (5 GENES)
« CENTRALIZED » TEST
(recurrence score) RS
Late recurrence (10 years)
Benefit from adjuvant TT
PROGNOSTIC AND PREDICTIVE

LOW RISK 1-25:
HORMONOTHERAPY
HIGH RISK >26:
+ HORMONOTHERAPY / + CHEMOTHERAPY
<table>
<thead>
<tr>
<th>First generation signatures</th>
<th>Prognostic</th>
<th>Predictive</th>
<th>Technical validation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MammaPrint®</strong>&lt;br&gt; All BC, N0-N1-3&lt;br&gt; 70 genes signature&lt;br&gt; 2 categories (low &amp; high risk)</td>
<td>+++</td>
<td>++</td>
<td>YES&lt;br&gt; Gene expression profile&lt;br&gt; Central Lab</td>
</tr>
<tr>
<td><strong>Oncotype Dx®</strong>&lt;br&gt; ER+, HER2- BC, N0-N1-3&lt;br&gt; 21 genes signature&lt;br&gt; Recurrence score RS&lt;br&gt; 3 categories</td>
<td>+++</td>
<td>+++</td>
<td>YES&lt;br&gt; RT-PCR&lt;br&gt; Central Lab</td>
</tr>
</tbody>
</table>

**Clinical validation**

**MammaPrint®**: LOEIA Prospective validation for *prognostic* value of low genetic in clinically high risk: 5yrs DMFS ≥94% (46%N+)<br> 14% reduction in CT prescription up to 46% in high clinical risk

**Oncotype Dx®**: LOEIA prospective validation for RS <26 *prognosis* LO1B validated retrospectively in prospective clinical trials (prediction chemotherapy benefit), prospective clinical validation ongoing for prediction
Decentralized tests
EndoPredict
(Sividon, GE)

HR+ / HER2- , T1-2, N0

FFPE
qRT-PCR
8 GENES SIGNATURE
PROLIFERATION, OESTROGENES

« LOCAL » TEST
(SPECIAL EQUIPMENT IS REQUIRED)

SCORE OF RECURRENCE EP SCORE
LATE AND EARLY RECURRENCES
(5 & 10 YEARS)

PROGNOSIS
LOW RISK
HIGH RISK

UBE2C
BIRC5
DHCR7

STC2
AZGP1
IL65T
RBBP8
MGP

EP-Score
Prosiga (PAM50)  
(NanoString Technology, USA)

IDENTIFICATION OF « MOLECULAR SUBTYPES »  
(LumA, LumB, HER2-enriched, Basal)

FFPE DNA ARRAY WITH BARCODES  
(1 gene = 1 barcode)

50 GENES « LOCAL » TEST  
(SPECIAL EQUIPMENT IS REQUIRED)

LATE AND EARLY RECURRENCES  
(5 & 10 YEARS)

PROGNOSIS

LOW RISK (ROR)

Intermediate risk

HIGH RISK (ROR)
<table>
<thead>
<tr>
<th>Second generation signatures</th>
<th>Prognostic</th>
<th>Predictive</th>
<th>Technical validation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prosigna®</strong></td>
<td>++</td>
<td>++</td>
<td>YES N-Counter® technology Dedicated instrument</td>
</tr>
<tr>
<td>ER+, HER2- BC, N0-N1-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 genes signature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Includes size and N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Endopredict®</strong></td>
<td>++</td>
<td>++</td>
<td>YES RT-PCR Dedicated instrument</td>
</tr>
<tr>
<td>ER+, HER2- BC, N0-N1-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 genes signature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Includes size and N</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Clinical validation**

**Prosigna®**: LOE1B Validated retrospectively in prospective clinical trials of HT
Prognosis
Late recurrences (after 5 years)

**Endopredict®**: LOE1B Validated retrospectively in prospective clinical trials of HT
Prognosis
Late recurrences (after 5 years)
NEW AJCC TNM AND SIGNATURES
8th Edition – “Genomic panels...have become as or more important than the anatomic extent of disease to define prognosis”*
Low risk molecular signature result in lower stage than would be recorded using biologic and anatomic factors alone

8th ed. Prognostic Stage using T,N,M, grade, ER, PR, HER2

If low risk GES, all of these patients are classified as Stage IA
EMERGING BIOMARKERS (FOR METASTATIC DISEASE)
Metastatic breast cancer

Tumor biopsy

ER IHC

PR IHC

HER2 IHC

HER2 FISH

20% of discordancess pos. vers neg. 24% neg. vers pos. 14%

33% of discordancess pos. vers neg. 46% neg. vers pos. 15%

8% of discordancess pos. vers neg. 13% neg. vers pos. 5%

Tumor BRCA testing PARPi

Luminal

HER2+

Triple negative

PIK3CA mutations

40% in luminal BC (stable between primary and mets) PIK3CA inhibitors

ESR1 mutations

20-40% acquired in luminal BC with prior AI therapy Resistance to IA

Liquid biopsy

Blood samples

Germinal BRCA testing PARPi

Tumor BRCA testing PARPi

PDL-1

Immune cells >> tumor cells TNBC > HER2+ > luminal IC ≥1% for IO treatment
Metastatic breast cancer

Tumor biopsy
- ER IHC
- PR IHC
- HER2 IHC
- HER2 FISH

20% of discordances
pos. vers neg. 24%
neg. vers pos. 14%

33% of discordance
pos. vers neg. 46%
neg. vers pos. 15%

8% of discordances
pos. vers neg. 13%
neg. vers pos. 5%

Tumor BRCA testing PARPi

Luminal

PIK3CA mutations
- 40% in luminal BC (stable between primary and mets)
- PIK3CA inhibitors

ESR1 mutations
- 20-40% acquired in luminal BC with prior AI therapy
- Resistance to IA

HER2+

Triple negative

PDL-1
- Immune cells >> tumor cells
  - TNBC > HER2+ > luminal
  - IC ≥ 1% for IO treatment

Liquid biopsy

Blood samples

Germinial BRCA testing PARPi
In IMpassion130, PD-L1 in TNBC is expressed mainly on tumor-infiltrating immune cells.

Prevalence of PD-L1 IC subgroups:
- PD-L1 IC+ (IC1/2/3) 41%
- IC2/3 14%
- IC1 27%
- IC0 59%

Prevalence of PD-L1 TC subgroups:
- PD-L1 TC+ 9%
- PD-L1 TC− 91%

The majority of patients with expression of PD-L1 on TC are included within the PD-L1 IC+ population.

BEP, biomarker-evaluable population.
BEP (IC): n = 960, PD-L1 scoring: IC0: ≤1%; IC1: ≤1% and <5%; IC2: ≥5% and <10%; IC3: ≥10%; TC−: <1% PD-L1 on tumor cells. TC+: ≥1% PD-L1 on tumor cells.

Emens LA, et al. IMpassion130 biomarkers. SABCS 2018 (program GS1-04)
Response to Immunotherapy Alone

**Anti-PD1/PDL1 single agent in TNBC PDL1 +/-, TILs +/-**

- **Atezolizumab (n=115)**
  - 1st line: 26%
  - 2nd line: 11%
  - 1L Keynote 086 Cohort B: 23%
  - 2L Keynote 086 Cohort A: 5%

- **Pembrolizumab (n=222)**

**TNBC**, Triple negative breast cancer

Metastatic breast cancer

Tumor biopsy

- ER IHC
- PR IHC
- HER2 IHC
- HER2 FISH

20% of discordances
pos. vers neg. 24%
neg. vers pos. 14%

33% of discordance
pos. vers neg. 46%
neg. vers pos. 15%

8% of discordances
pos. vers neg. 13%
neg. vers pos. 5%

Tumor BRCA testing PARPi

Luminal

PIK3CA mutations
- 40% in luminal BC (stable between primary and mets)
- PIK3CA inhibitors

ESR1 mutations
- 20-40% acquired in luminal BC with prior AI therapy
- Resistance to IA

Liquid biopsy

HER2+

Triple negative

PDL-1

Immune cells >> tumor cells
TNBC > HER2+ > luminal
IC ≥ 1% for IO treatment

Blood samples

Germinal BRCA testing PARPi
PIK3CA

• Recurrent mutations
  - exon 9: E542K, E545K, Helicase domain
  - exon 20: H1047R, Kinase domain
  - Frequent: 30 to 40% of BC

• Prognostic role?

• Predictive role for specific PIK3CA inhibitors
Metastatic breast cancer

Tumor biopsy

- Tumor BRCA testing PARPi
  - ER IHC: 20% of discordanices
  - PR IHC: 33% of discordanices
  - HER2 FISH: 8% of discordanices

Luminal

- PIK3CA mutations: 40% in luminal BC (stable between primary and mets)
- ESR1 mutations: 20-40% acquired in luminal BC with prior AI therapy

HER2+

- PIK3CA inhibitors
- Resistance to IA

Triple negative

- PDL-1
  - Immune cells > tumor cells
  - TNBC>HER2+>luminal
  - IC≥1% for IO treatment

Liquid biopsy

Blood samples

Germinal BRCA testing PARPi
CONCLUSION
Classical prognosis and predictive factors

- Age
- Grade
- Histological subtypes
- ER/PR and HER2 status
- Ki67 +/- mitotic index
- Vascular invasion
- Tumor margins

Molecular signatures
Yes, we have molecular biology!

- Age
- Grade
- Histological subtypes
- ER/PR and HER2 status
- Vascular invasion
- Tumor margins
Present and Future biomarkers in mBC

**Metastatic breast cancer BIOPSY**

- **Somatic BRCA status ➔ PARPi**
  - Tumor BRCA testing PARPi

- **PIK3CA mutations**
  - 40% in luminal BC (stable between primary and mets)
  - PIK3CA inhibitors

- **ESR1 mutations**
  - 20-40% acquired in luminal BC with prior AI therapy
  - Resistance to AI

- **NTK fusions** enriched in secretory breast cancers ➔ NTRK inhibitors

- **Luminal**
  - PIK3CA mutations

- **HER2+**
  - ESR1 mutations
  - PDL-1 IHC

- **Triple negative**
  - NTRK inhibitors

**CLINICAL TRIALS**

- **AR IHC tumor cells anti-androgen TT**

- **PD-L1 IHC**
  - Immune cells >> tumor cells
  - TNBC>HER2+>luminal ICz1% for IO treatment

**Germinal**

- BRCA testing PARPi
THANK YOU!