New Breast Cancer classification: Traditional pathology and molecular subtypes
Prognostic and predictive factors

Frédérique Penault-Llorca
Using Pigeons to Diagnose Cancer

The pigeons' training environment included a food pellet dispenser, a touch-sensitive screen which projected the medical image, as well as blue and yellow choice buttons on either side of the image. Pecks to those buttons and to the screen were automatically recorded. Credit: Copyright Univ. Iowa/Wassermann Lab
Objectives
To learn about the biology of breast cancer and its implication in the management of BC patients
“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
TNM parameters:

• Pros
  – Treatment decisions are based on T size
  – Node involvement is a major prognosis factor
  – Micrometastasis can be detected in sentinel lymph node by IHC

• Cons
  – TNM is decreasing because of mass screening (69% of T1 in France in 2015)
  – 70% of N0 in France in 2015
  – SLN: axillary dissection is debatable in case of Nano/micromets
Tumor size

T1
$T \leq 2 \text{ cm}$

T2
$2 \text{ cm} < T \leq 5 \text{ cm}$

T3
$T > 5 \text{ cm}$

T4
Extension to skin or thorax

important parameter for treatment decision
Macroscopic size

T = 10mm
Microscopic size
Microscopic size

$T = 6\text{mm}$
Sampling for invasive carcinoma

External

Inferior
Invasive lobular carcinoma
Extensive sampling!
MULTIPLE SITES
Multifocality vs multicentricity
Fisher Cancer 1975

Multifocal

With the use of MRI: 13-70% of multiple lesions

Multicentric
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
INVASIVE CARCINOMA WITH PREDOMINANT IN SITU COMPONENT
A tricky case

invasive ductal with extensive DCIS component
ER+, PR+, HER2 positive

T1: Inf Q 14mm DCIS + IDC
T2: IIQ 10mm IDC grade 3
T3 QSI 15mm DCIS+IDC

How to measure?
OMS 2012 multifocal: larger site
French guidelines size of the invasive and size of the in situ
But how to manage when they are intermingled?
Size?

- only AB?
- A and B separately?
- Measurement of normal/DCIS between invasive areas
Particularity of HER2 DCIS and CCIS

HER2+ is predictive of extensive in situ component, of multifocality, of microinvasion Vasconcellos 2016
Therapeutic impact of size measurement in N0

Mainly for **surgery +/+ neoadjuvant decision** (T and not pT)
And **radiation therapy** (abstention and boost/no boost)

**HER2+**: indication of adjuvant treatment pT1b HER2+ HR-, PT1b, HR+ if high grade, embolies, proliferation
For pT1a discussion in MDTB (Tolaney strategy), in my group also for extensive in situ component
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
Ultra-stadification

Standard analysis  Serial sections

Impossible to do this extensive analysis on all the axillary nodes (mean of 13N)
«the more we search, the more we find»

- Serial section: a gain of 10-33% of N+
- IHC : a gain of 10-15% of N+
Interest of doing several levels of section

pN0 i+ on the first level

Multiple levels allow a better measurement of micromets

pN1 on the 3rd level

At least 3 levels
Table 1. AJCC 6th edition

<table>
<thead>
<tr>
<th>pN0(sn)</th>
<th>No metastasis-sentinel lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td>pN0(i+)</td>
<td>Isolated tumor cells (single cells or cell deposits) no larger than 0.2 mm</td>
</tr>
<tr>
<td>pN1mi</td>
<td>Metastatic lesion larger than 0.2–2.0 mm</td>
</tr>
<tr>
<td>pN1mi(i+)</td>
<td>H&amp;E stain negative but IHC stain detected micrometastasis</td>
</tr>
<tr>
<td>pN0(mol-)</td>
<td>Negative molecular findings using RT-PCR</td>
</tr>
<tr>
<td>pN0(mol+)</td>
<td>Positive molecular findings using RT-PCR</td>
</tr>
</tbody>
</table>

AJCC, American Joint Committee of Cancer; H&E, hematoxylin and eosin; IHC, immunohistochemical; RT-PCR, reverse transcriptase-polymerase chain reaction.

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Table 2. AJCC 7th edition

<table>
<thead>
<tr>
<th>pN0(i+)</th>
<th>Isolated tumor cells (single cells or cell deposits) no larger than 0.2 mm or fewer than 200 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>pN1mi</td>
<td>Metastasis greater than 0.2 mm and/or more than 200 cells, but none greater than 2.0 mm</td>
</tr>
<tr>
<td>pN1a</td>
<td>Macrometastasis in 1 to 3 axillary lymph nodes, at least 1 metastasis greater than 2.0 mm</td>
</tr>
<tr>
<td>pN2a</td>
<td>Metastases in 4 to 9 axillary lymph nodes (at least 1 tumor deposit greater than 2.0 mm)</td>
</tr>
<tr>
<td>pN3a</td>
<td>Metastases in 10 or more axillary lymph nodes (at least 1 tumor deposit greater than 2.0 mm)</td>
</tr>
</tbody>
</table>

AJCC, American Joint Committee of Cancer.
Difficulties for pN staging

Low grade invasive tumor in axillary fat without adjacent residual and apparent lymph node structure. Should this be counted as positive lymph node or a carcinoma arising in axillary breast tissue?

Apple SK. *Journal of Pathology and Translational Medicine* 2016; 50: 83-95
Should this be classified as **metastatic** [either pN0(i+) or pN1mi depending on the maximum linear dimension] or **LVI**?
Technically this is LVI, based on College of American Pathologists (CAP) guidelines, capsular LVI is considered metastatic carcinoma and the largest dimension is measured as the size of metastasis, but nothing in the 7th AJCC

Apple SK. *Journal of Pathology and Translational Medicine* 2016; 50: 83-95
Difficulties for pN staging

Dispersed pattern of metastatic lobular carcinoma to lymph node is commonly seen. If >200 cells: micrometastasis (pN1mi) or <200 cells: ITC [pN0(i+)] but why can’t it be macrometastasis if all the node is involved?

Apple SK. *Journal of Pathology and Translational Medicine* 2016; 50: 83-95
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
SBR 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
VASCULAR EMBOLI: no value on microbiopsies

Peripheral lymphovascular invasion and BCSS in N-operable BC treated by adjuvant therapy (from Lee)
HISTOLOGIC SUBTYPES
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
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”
Medullary features and BCSS

From medullary

Tubular carcinoma and DFS (Rakha)
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)
High-grade encapsulated papillary carcinoma of the breast: an under-recognized entity

Emad A Rakha, Zsuzsanna Varga, Somaia Elsheik & Ian O Ellis

- High variant of encapsulated papillary carcinoma
  - Capsule +/- thick
  - High nuclear grade
  - Usually ER, PR and HER2-
  - **Consider as an invasive carcinoma**
    (SBR grade, SNLB, ER, PR, HER2, pT)
Encapsulated papillary carcinoma with invasive component grade, pT and IHC on this area only

- Capsule +/- thick

Invasive carcinoma

DCIS
Clinicopathologic Characteristics of Solid Papillary Carcinoma of the Breast

Benjamin Yongcheng Tan, FRCPa,⁎ Aye Aye Thike, MMedSci,⁎
Ian O. Ellis, FRCPa,† and Puay Hoon Tan, FRCPa⁎

- Multiple nodular masses
- Low grade
- Absence of myoepithelial cells
- Frequent neuroendocrine differentiation
- Caronse as pTis and treat as a CCIS
NOT ALL BREAST NODULES ORIGINATE FROM BREAST
Clinical history

• 65 yrs
• History at 52yrs of IDC grade 3 left breast ER+ PgR+ HER2-
• T2N1M0 surgery, CT and HT
• Discovery of 4 round nodules in SEQ right breast
• FNA not contributive
• Decision of surgery + SLNB
• Gross examination:
  – 3 round tumors, of 0.5 – 2.3 et 2.5cm and one intrammamary lymph node
Radiology
Histology
Histology

Looks like a lobular
Mammary or not?

- triple negative (?!)
- So we did GATA3 ➔ neg

➔ CK7 & EMA neg
BRAF V600E mutation (quantitative PCR)
### 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)

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**Non-mammary metastases to the breast and axilla: a study of 85 cases, DeLair and al, Modern Pathology, 2014**

<table>
<thead>
<tr>
<th>Tumor site/type (n = 49)</th>
<th>No. of cases/(% of carcinomas)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ovary (n = 14)</strong></td>
<td></td>
</tr>
<tr>
<td>High-grade serous carcinoma</td>
<td>10 (21)</td>
</tr>
<tr>
<td>Low-grade serous carcinoma</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Lung (n = 14)</strong></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Large cell neuroendocrine</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Poorly differentiated carcinoma</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>1 (2)</td>
</tr>
<tr>
<td>‘Large’ cell carcinoma</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Gastrointestinal tract (n = 7)</strong></td>
<td></td>
</tr>
<tr>
<td>Colonic adenocarcinoma</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Carcinoid (colon)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Carcinoid (liver)</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Genitourinary tract (n = 5)</strong></td>
<td></td>
</tr>
<tr>
<td>Urothelial carcinoma (bladder)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Prostatic adenocarcinoma</td>
<td>1 (2)</td>
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<tr>
<td><strong>Gynecologic tract (excluding ovary) (n = 5)</strong></td>
<td></td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma (endometrial)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Combined endometrioid/small cell carcinoma (endometrium)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Undifferentiated carcinoma (endometrium)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Small cell carcinoma (cervix)</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Thyroid (n = 2)</strong></td>
<td></td>
</tr>
<tr>
<td>Papillary thyroid carcinoma</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Medullary thyroid carcinoma</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Skin (n = 2)</strong></td>
<td></td>
</tr>
<tr>
<td>Merkel cell carcinoma</td>
<td>2 (4)</td>
</tr>
<tr>
<td><strong>Submandibular gland (n = 1)</strong></td>
<td></td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Tongue (n = 1)</strong></td>
<td></td>
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<tr>
<td>Squamous cell carcinoma</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>
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>Test and scoring recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>IHC</td>
</tr>
<tr>
<td>PgR</td>
<td>IHC</td>
</tr>
<tr>
<td>HER2</td>
<td>IHC ≥10% cells with complete membrane staining ISH: number of HER2 gene copies ≥6 or the ratio HER2/chromosome 17 ≥ 2</td>
</tr>
<tr>
<td>Ki67</td>
<td>IHC no final consensus on cut-off around 20% (Ki67&lt;10% = low; Ki67&gt;30% = high)</td>
</tr>
</tbody>
</table>
What is the level of prediction accuracy clinically useful?

Breast Cancer

HER2

Negative predictive value

HIGH 95%

(<5% chance to respond to anti-estrogens or trastuzumab)

Cut off 1%

ER/PGR

Positive predictive value

30-50%
Ki67 why?

• Definition of luminal A and B

• Decision of CT for ER+, Grade II tumors
Ki67 = Not standardized
An International Ki67 Reproducibility Study
Manuscript received April 2, 2013; revised September 3, 2013; accepted September 16, 2013.
Correspondence to: Torsten Nielsen, MD, PhD, FRCP, University of British Columbia Pathology and Laboratory Medicine, Anatomical Pathology, JP 1401, Vancouver Hospital & Health Sciences Centre, 855 W 12th Ave, Vancouver, BC V5Z 1M9, Canada (e-mail: torsten@mail.ubc.ca).

Interobserver concordance of Ki67 labeling index in breast cancer: Japan Breast Cancer Research Group Ki67 Ring Study
Yoshiaki Miaski,1,2 Takayuki Ueno,1,3 Kenichi Yoshimura,1 Hitoshi Tsuda,4 Masafumi Kurosumi,5 Shinobu Masuda,6 Rie Horii,7 Masakazu Toi8 and Hironobu Sasano9
Departments of 1 Diagnostic Pathology, 2 Breast Surgery, 3 Kyushu University Hospital, 4 Translational Research Center, Kyushu University Hospital, Fukuoka, 5 Diagnostic Pathology Section, Clinical Laboratory Division, National Cancer Center Hospital, Tokyo; 6 Department of Pathology, Saitama Cancer Center, Saitama; 7Department of Pathology, Niho University School of Medicine, Tokyo; 8Department of Pathology, The Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Tokyo; 9Department of Pathology, Tohoku University School of Medicine, Sendai, Japan

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*

Modern Pathology (2015) 28, 778–786
An international study to increase concordance in Ki67 scoring
Classical prognosis and predictive factors

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

Oldies but goldies
FOCUS ON HER2 GUIDELINES

Antonio C. Wolff,* M. Elizabeth H. Hammond,* David G. Hicks,* Maich Dowsett,* Lisa M. McShane,* Kimberly H. Allison, Donald C. Allred, John M.S. Bartlett, Michael Bilous, Patrick Fitzgibbons, Werad Hanna, Robert B. Jenkins, Pamela B. Mangu, Soonmyung Park, Edith A. Perez, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, and Daniel F. Hayes*

HER2 GUIDELINES WITH THE INTRODUCTION OF « EQUIVOCAŁ »
GEFPICS 2014 guidelines (1)

3+ Case
Seen at low magnification

2+ Case

Evolution of HER2 guidelines

• Scoring on biopsies
• “Eligibility” criteria to trastuzumab

=> for IHC a step back to FDA criteria
  – accept >10% of 3+ or amplified cells as a definition of HER2 positivity
  – For amplification
    • Dual probes HER2/CEP17 >= 2
    • Single color HER2>= 6
    • Equivocal cases (between >=4 and < 6) to retest and/or eventually to treat if still equivocal
HER2 2+ or equivocal

Complete membrane staining weak or moderate in more than 10% of TC

Complete and intense membrane staining ≤ 10% => heterogeneous case
Heterogeneity: Where to count?
HER2 2+ or equivocal

**Incomplete membrane staining**

- **Micropapillary architecture** : any intensity, >10% of TC

- Incomplete or basolateral staining staining when **seen at low magnification** (x4-5) and in >10% of TC
Key Recommendations: Oncologists

• Should delay decision to recommend HER2-targeted therapy if HER2 status cannot be confirmed as positive or negative after separate HER2 tests (HER2 test result or results Equivocal).

• If the HER2 test result is ultimately deemed to be Equivocal, even after reflex testing with an alternative assay, the oncologist may consider HER2-targeted therapy.
For the “equivocal” cases

• Discussion in tumor boards
• Integration of HER2 results to
  – Patient age
  – Tumor size
  – Grade
  – ER, PR status
  – Proliferation
  – Molecular tests ???
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++
- Medulillary carcinoma is extremely rare (ask for a second opinion)
  ➔ Redo HER2 on surgical specimen if grade 3, ER- or ER+
  ➔ If ER and/or PgR is negative on a biopsy redo on surgical specimen
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 »

C Perou & T Sorlie
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
Surrogate definition of intrinsic subtypes of breast cancer

«basal-like»

• ER and PgR absent
• HER2 negative
• Approximately 80% overlap between « triple negative » and intrinsic « basal-like »
• But « triple negative » also include good prognosis special types such as medullary and adenoid cystic carcinoma
• Staining for basal keratin is considered insufficiently reproducible for general use
Intrinsic Subtype distribution within IHC-based groups: A combined analysis of 15,339 patients across 29 studies

Within TN (n=2,512),

- non-BL subtypes 17%
- Luminal A/B: 5.9%
- HER2-E: 11.1%

Cejalvo et al. ESMO 2017 #1727P
Triple negative BC by IHC and molecular subtypes: a 80% concordance

TNBC subtypes of excellent prognosis

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

- Pushing borders
- Poorly differentiated Necrosis
- High proliferation Grade III
- PTEN mutations > 25%
- Xq- Xp- Myofibroblastic stroma
- Lymphocytic stroma
- Myofibroblastic stroma
- Atypia
- ER 0%
- PR 0%
- HER2 0
TN Tumors Are Heterogeneous

- IDC NOS, high grade
- ILC high grade, pleomorphic
- Metaplastic, high grade
- Myoepithelial carcinoma
- High-grade (oat-cell) neuroendocrine
- Apocrine
- Medullary
- Adenoid-cystic
- Juvenile Secretory
- 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-

\[ t(12;15) \text{ (ETV6; NTRK3)} \]

\[ t(6;9) \text{ (q22-23; p23-24) (MYB ;NFIB)} \]

Basal like carcinoma

EGFR amplification
WNT pathway alterations
Secretory or juvenile carcinoma

- 0.1% of breast cancers
- 1/3 children and teens
- 2/3 between 20 and 50 yrs
- 15 cases described in male
- Good prognosis
- Specific molecular alteration: t(12;15) (ETV6; NTRK3)

NTKR inhibitors
Adenoid cystic carcinoma

- Identical to the salivary glands tumors
- 0.1% to 1% of all breast cancers
- Median age 62yrs
- Myoepithelial differentiation
- Low aggressive malignant potential
- Recurrent translocation t(6;9)(q22–23;p23–24) leading to the chimeric fusion gene $MYB-NFIB$, => overexpression of the oncogene $MYB$
Adenoid cystic carcinoma
Carcinoma with medullary features
Carcinoma with medullary features

Frequency: ≤ 2% breast carcinomas
Mean age 47 to 52 years

Morphological criteria
Good limitation
Syncitial architecture > 75%
Absence of glandular structures
Atypical nuclei, mitoses +++
Moderate to marked inflammatory infiltrate

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

Medullary carcinoma and BRCA1 germline mutation

- 7 to 13% of medullary carcinoma are found in BRCA1+ women
- Women with BRCA1 mutation: medullary carcinoma in 30 to 70%

Evolution

- Prognosis of medullary carcinoma CCI better than grade III, the same stage
- 10-year survival from 50 to 90%
- 90% medullary carcinomas are N-
- Very good chemo and radiosensitivity + + +

PARP inhibitors
Claudin-low Subtype

1. 5-10% of all tumors
2. typically TNBC
3. low expression of cell-cell junction proteins
4. lymphocyte infiltrates
5. stem cell + EMT features

<table>
<thead>
<tr>
<th>HER2</th>
<th>Basal</th>
<th>Luminal</th>
<th>Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin 3</td>
<td>Claudin 4</td>
<td>Claudin 7</td>
<td>E-Cadherin</td>
</tr>
</tbody>
</table>

[Image of gene expression data]

[Image of histology]
Metaplastic carcinoma

- <1% of breast cancers
- Large tumors (3 to 5 cm), often well limited, rapid growth
- EGFR activation, wnt pathway activation, BRCA methylation
- Low rate of lymph node involvement <25%
- Poor overall survival
  - 70% at 3 years
  - 55% at 5 years
Low grade adenosquamous carcinoma

- Low grade variant of metaplastic carcinoma described by Rosen in 1987
- Age identical to classical IDC m=57
- ACR4 or 5
- Poor limitation stellar
- Differential diagnosis: fibromatosis, fasciitis
Prevalence (% of TNBC) in early BC
GE array definition

- 21 data sets → 587 TNBC
- 6 subtypes
  - 2 basal-like (BL1 and BL2)
  - Immunomodulatory (IM)
  - Mesenchymal (M)
  - Mesenchymal stem-like (MSL)
  - LAR

11%

Uncertain prognosis: Apocrine carcinoma

in ½ cases: HER2+

Bicalutamide-abiraterone acetate
BASAL LIKE BREAST CANCER

DIFFERENT MORPHOLOGY – DIFFERENT BIOLOGY – DIFFERENT PROGNOSIS
BIOLOGY OF TNBC
Molecular biology of TNBC

- BRCA1 mutations in 11% of unselected patients (higher frequency in younger women)
- Mutation of p53 in up to 82% of basal BC
- RB inactivation and ↓ ATM expression
- Gains of 1q, 3q, 7q, 8q and 10p and loss of 4p, 5q, 17p and 8p phenocopy BRCA1 mutated tumors

Basal-like breast cancer and BRCA-1
Specific/Frequent molecular alterations

• **Secretory (juvenile) carcinoma** (<1%) harbour a specific translocation t(12;15) → fusion gene *ETV6-NTRK3*

• **Adenoid cystic carcinoma** (<1%) characterized by t(6;9) and fusion gene *MYB-NFIB* and lack *TP53* & *PIK3CA* mutations

• **Apocrine** (1%) *PTEN* or *PIK3CA* alterations, less *TP53* mutations

• **Medullary** (<5%) 87% *TP53* and 9% *PIK3CA* mutations / association with *gBRCA* mut

• **Metaplastic** (0,2-5%)TP53 mutations~ 70%, EMT *(WNT)* and *PI3K/Akt/mTOR* pathways alterations
HER2 POSITIVE
Intrinsic Subtype distribution within IHC-based groups: A combined analysis of 15,339 patients across 29 studies

**Global-IHC**
- HR+/HER2-: 63.68%
- HR+/HER2+: 16.38%
- HR-/HER2+: 11.26%
- HR-/HER2-: 8.68%

Total=15,339

**HER2+**
- HER2-E: 29.2%
- BL: 2.1%
Total=3,059

**HR-/HER2+**
- Luminal A/B: 9.3%
- BL: 13.8%
Total=1,332

**HR+/HER2+**
- Luminal A/B: 34.45%
- Normal: 30.57%
- Basal-like: 29.18%
- HER2-E: 3.71%
Total=1,727

Within HR+/HER2+ group (n=1,727),
- HER2-E: 29.2%
- BL: 2.1%

Within HR-/HER2+ group (n=1,332)
- Luminal A/B: 9.3%
- BL: 13.8%

Cejalvo et al. ESMO 2017 #1727P
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

Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis

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

Less pCR in HER2+, ER +

### Table 3: Rates of pCR according to HR status.

<table>
<thead>
<tr>
<th>Study (ref.)</th>
<th>Neoadjuvant regimen</th>
<th>pCR rate HR+</th>
<th>pCR rate HR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>NeoSphere [18]</td>
<td>(i) Docetaxel + trastuzumab—(arm A)</td>
<td>20%</td>
<td>36.8%</td>
</tr>
<tr>
<td></td>
<td>(ii) Docetaxel + trastuzumab + pertuzumab (arm B)</td>
<td>26%</td>
<td>63.2%</td>
</tr>
<tr>
<td></td>
<td>(iii) Trastuzumab + pertuzumab (arm C)</td>
<td>5.9%</td>
<td>27.3%</td>
</tr>
<tr>
<td></td>
<td>(iv) Docetaxel + pertuzumab (arm D)</td>
<td>17.4%</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td>(i) Weekly P + trastuzumab</td>
<td>22.7%</td>
<td>36.5%</td>
</tr>
<tr>
<td>Neo-ALTTO [12]</td>
<td>(ii) Weekly P + lapatinib</td>
<td>16.1%</td>
<td>33.7%</td>
</tr>
<tr>
<td></td>
<td>(iii) Weekly P + trastuzumab + lapatinib</td>
<td>41.6%</td>
<td>61.3%</td>
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<tr>
<td></td>
<td>(i) CT + trastuzumab</td>
<td>25%</td>
<td>26.6%</td>
</tr>
<tr>
<td></td>
<td>(ii) CT + lapatinib</td>
<td>22.7%</td>
<td>35.7%</td>
</tr>
<tr>
<td></td>
<td>(iii) CT + trastuzumab + lapatinib</td>
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<td>56.2%</td>
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<td>Buzdar et al. [4]</td>
<td>(i) CT + trastuzumab</td>
<td>61.5%</td>
<td>70%</td>
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<tr>
<td></td>
<td>(ii) CT alone</td>
<td>27.2%</td>
<td>25%</td>
</tr>
<tr>
<td>NOAH [20]*</td>
<td>(i) CT + trastuzumab</td>
<td>18%</td>
<td>48%</td>
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<tr>
<td></td>
<td>(ii) CT alone</td>
<td>17%</td>
<td>22%</td>
</tr>
<tr>
<td>REMAGUS 02 [9]</td>
<td>(i) CT + trastuzumab</td>
<td>20.5%</td>
<td>32%</td>
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<tr>
<td></td>
<td>(ii) CT alone</td>
<td>20.5%</td>
<td>19%</td>
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<tr>
<td>NSABP B-41 [15]</td>
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<td>46.7%</td>
<td>65.5%</td>
</tr>
<tr>
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<td>(ii) CT + lapatinib</td>
<td>48%</td>
<td>60.6%</td>
</tr>
<tr>
<td></td>
<td>(iii) CT + trastuzumab + lapatinib</td>
<td>55.6%</td>
<td>73%</td>
</tr>
</tbody>
</table>
LUMINAL BREAST CANCER
Surrogate definition of intrinsic subtypes of breast cancer

«Luminal A and Luminal B»

- HER2 positive $\Rightarrow$ 3+ by IHC or amplified by FISH
- And ER and PgR negative
Luminal breast cancer

Luminal A
- ER+
- And all
  - PR +
  - Ki67 low
  - HER2 -
  - 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

60 Sample ER+ Tamoxifen-Treated Test Set
Ma et al., Cancer Cell 5, 1-10 (2004).

45 Tamoxifen Treated Test Set #2
Chang et al., PNAS 102, 3738-43 (2005) + UNC
Luminal BC

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

Intermediate category

→ 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
Intrinsic Subtype distribution within IHC-based groups: A combined analysis of 15,339 patients across 29 studies

Within HR+/HER2- group (n=9,768)

- non-luminal subtypes (7.7%)
  - HER2-E: 5.6%
  - BL: 2.2%

Cejalvo et al. ESMO 2017 #1727P
LOBULAR CARCINOMA
Special types

“rare variant of lobular carcinoma (e.g. pleomorphic) (up to 25% HER2+) and apocrine carcinoma require treatment according to their biological features in a manner analogous to that used for ductal carcinoma “

Pleiomorphic lobular carcinoma

- E CADH
- HER2
Classic 47.7%
Trabecular 6.8%
Solid 15.7%
Mixed, nonclassic 13.8%
Alveolar 16%

- ERBB2 & ERBB3 8.5% mutations
- 50% AKT1
- 9% mut FOXA1
- ESR1 gains

ILC lumA: higher activation of AKT pathway (45%)

Desmedt et al JCO 2016
Specific/frequent molecular alterations

- **Lobular carcinomas** (10-15%) and their precursors (lobular neoplasia): *CDH1* mutation (located on 16q), ➔ the pathognomonic **loss of E-Cadherin** expression (adhesion protein): aspect of non cohesive cells
  ➔ **PI3K alterations** >50% (with Akt/mTOR in 45%)
  ➔ **AKT1, FOXA1, HER2, HER3, PTEN** and **TBX3** mutations in ILC>IDC
    ➔ **HER2** and **AKT1** mutations associated with increased risk of early relapse
    ➔ **Histologic subtype–specific associations**: ESR1 gains in solid subtype, **HER2 mutations in mixed non classic**, and TP53 mutations in both.
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 3 transcriptomic groups have been identified
  - « reactive-like » (good prognostic), « Immune-related » & « proliferative »
  - « immune-related » & « hormone – related »

Targeted therapies such as Neratinib?

mTOR inhibitor
PIK3CA i

Low chemosensitivity

Michaut et al Scientific report 2016
Ciriello et al Cell 2015
Deniziaut et al Oncotarget 2016
TCGA. Nature 2012.
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 primary tumors do not present any reported driver mutation.
Luminal tumours = heterogeneous group

• The principal characteristic of the luminal group is the luminal expression signature, composed of *ESR1*, *GATA3*, *FOXA1*, *XBP1*, and *cMYB*

  – the most frequent mutations in the luminal A subtype are *PIK3CA (45%)*, *MAP3K1 (13%)*, *GATA3 (13%)*, *TP53 (12%)*, and *CDH1 (9%)*

  – the most frequent mutations in luminal B tumors are *TP53 (29%)*, *PIK3CA (29%)*, *GATA3 (13%)*, and *TTN (12%)*

• In addition to *TP53* mutations, several other events may intervene in other steps of the same pathway, including *ATM* loss and *MDM2* amplification

• *ESR1* mutations (up to 19%) after hormonal treatment => resistance
mut MLL3 7% (8% A, 5% B)  
lum B hypermethyl 8%  
mut ESR1 1% (up to 19% mets)  
ampl 8p11-12 10%  
ampl FGFR1 10% (up to 27% B)

HDAC inhibitors

mut PIK3CA 40% (45% A, 29% B)  
mut/loss of PTEN 18%  
INPP4B loss 12%  
mut AKT1 3%

HT Adaptation

mut TP53 22% (13% A, 66% B)  
gain MDM2 22%  
mut MLL3 7% (8% A, 5% B)  
lum B hypermethyl 8%  
mut ESR1 1% (up to 19% mets)  
ampl 8p11-12 10%  
ampl FGFR1 10% (up to 27% B)

FGFR, TKIs inhibitors

mut PIK3CA 40% (45% A, 29% B)  
mut/loss of PTEN 18%  
INPP4B loss 12%  
mut AKT1 3%

MDM2 inhibitors

ampl 11q13 37%  
ampl CCND1 40% (29% A, 58% B)  
ampl CDK4 19%  
CDKN1B, 2A, 2B loss 11%  
mut RB1 1%

PI3K inhibitors

CDK4/6 inhibitors

HT Adaptation
TREATMENT DESCALATION IN HR+ HER2- ➔ MOLECULAR SIGNATURES
Yes, we have molecular biology!
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 ADAPTATED TO FFPE

Group of genes (« signatures »)
EARLY RECURRENCE (Dg < 5 ans)
PROGNOSTIC
GOOD SIGNATURE :
LOW RISK
POOR SIGNATURE :
HIGH RISK
Primary analysis of the EORTC 10041/ BIG 3-04 MINDACT study:
A prospective, randomized study evaluating the clinical utility of the 70-gene signature (MammaPrint®) combined with common clinical-pathological criteria for selection of patients for adjuvant chemotherapy in breast cancer with 0 to 3 positive nodes

Martine Piccart, Emiel Rutgers, Laura van’t Veer, Leen Slaets, Suzette Delaloge, Giuseppe Viale, Jean Yves Pierga, Peter Vuylsteke, Etienne Brain, Suzan Vrijaldenhoven, Peter Neijenhuis, Bruno Coudert, Tineke Smilde, Miguel Gil, Alastair Thompson, Isabel T. Rubio, Rodolfo Passalaqua, Erika Matos, Ulrike Nitz, Mauro Delorenzi, Geraldine Thomas, Theodora Goulioti, Carolyn Straehle, Konstantinos Tryfonidis, Jan Bogaerts & Fatima Cardoso

On behalf of the European Commission supported TRANSBIG consortium and MINDACT investigators

Presented at AACR, April 18, 2016
MINDACT study design

Abbreviations
C-low= Clinical Risk assessment low
C-High= Clinical Risk assessment high
G-Low= MammaPrint Low (MP Low)
G-High= MammaPrint High (MP High)
## Distant Metastasis Free Survival

![Graph showing Distant Metastasis Free Survival](image)

**% at 5 year**
- cL/gL: 97.6 (96.9, 98.1)
- cL/gH: 94.8 (92.4, 96.4)
- cH/gL: 95.1 (93.8, 96.2)
- cH/gH: 90.6 (89.0, 92.0)

**Number of patients at risk:**
- cL/gL: 77 (ON:70, OFF:745)
- cL/gH: 32 (592)
- cH/gL: 82 (1550)
- cH/gH: 171 (1806)

**Discordant risk groups**

### c-Low/g-High  CT vs no CT per protocol population

<table>
<thead>
<tr>
<th>Treatment received</th>
<th>Patients</th>
<th>Observed Events</th>
<th>% at 5 Year(s) (95% CI)</th>
<th>Hazard Ratio (adjusted Cox model)</th>
<th>p-value (adjusted logrank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMFS</td>
<td>CT</td>
<td>224</td>
<td>96.1 (92.4, 98.1)</td>
<td>0.90 (0.40,2.01)</td>
<td>0.798</td>
</tr>
<tr>
<td></td>
<td>no CT</td>
<td>254</td>
<td>93.9 (89.6, 96.5)</td>
<td>1.00</td>
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</tr>
<tr>
<td>DFS</td>
<td>CT</td>
<td>224</td>
<td>92.7 (87.9, 95.7)</td>
<td>0.74 (0.40,1.39)</td>
<td>0.355</td>
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<tr>
<td></td>
<td>no CT</td>
<td>254</td>
<td>90.5 (85.7, 93.8)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>OS</td>
<td>CT</td>
<td>224</td>
<td>98.1 (94.9, 99.3)</td>
<td>0.72 (0.23,2.24)</td>
<td>0.572</td>
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<tr>
<td></td>
<td>no CT</td>
<td>254</td>
<td>97.0 (93.8, 98.6)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

### c-High/g-Low  CT vs no CT per protocol population

<table>
<thead>
<tr>
<th>Treatment received</th>
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<th>Observed Events</th>
<th>% at 5 Year(s) (95% CI)</th>
<th>Hazard Ratio (adjusted Cox model)</th>
<th>p-value (adjusted logrank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>592</td>
<td>22</td>
<td>96.7 (94.7, 98.0)</td>
<td>0.65 (0.38,1.10)</td>
<td>0.106</td>
</tr>
<tr>
<td>no CT</td>
<td>636</td>
<td>37</td>
<td>94.8 (92.6, 96.3)</td>
<td>1.00</td>
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</tr>
<tr>
<td>CT</td>
<td>592</td>
<td>39</td>
<td>93.3 (90.7, 95.2)</td>
<td>0.64 (0.43,0.95)</td>
<td>0.026</td>
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<tr>
<td>no CT</td>
<td>636</td>
<td>66</td>
<td>90.3 (87.6, 92.4)</td>
<td>1.00</td>
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<tr>
<td>CT</td>
<td>592</td>
<td>10</td>
<td>98.8 (97.4, 99.5)</td>
<td>0.63 (0.29,1.37)</td>
<td>0.245</td>
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<tr>
<td>no CT</td>
<td>636</td>
<td>18</td>
<td>97.3 (95.6, 98.4)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>
• Level of Evidence 1A for the clinical utility of MammaPrint® in the c-High group
• C-High / g-Low (including 48% N +) have a 5-year survival (DMFS)> 94% (with or without CT)
• In the whole population: 14% reduction in CT prescription
• In the c-High population: 46% CT reduction
MINDACT primary test analysis:
C-high / G-low (MP Low) group- No CT, 100% compliance

- 5-Year DMFS for the C-high / G-low (MP Low) group with no CT = **94.7%** (CI: 92.5 – 96.2%).
- Excludes 92%, positive outcome met.

Primary Test Population, C-high / G-low tumors:
- 58% >2cm
- 93% Grade II or III
- 48% LN+ 1-3
- 98% HR+

Piccart M. AACR Podium Presentation, April 18th, 2016
Chemo efficacy in Clin-High / MP Low (DMFS)

- No statistical difference between CT vs no CT arms
- Excellent survival with no chemotherapy for patients with clinically high risk features (94.4%)
Molecular definition of “Indolent”

70 gene Prognosis Signature: “Ultra-low Threshold”

van’t Veer et al., Nature, 2002

Threshold derived from TRANSBIG with 25-year follow-up and no metastatic events

In women with breast cancer and WITHOUT ANY SYSTEMIC THERAPY

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Validation in the STO 3 Trial
postmenopausal women, <3cm, N0
tumors
All Patients by 70 Gene, Ultralow, low≠ultralow, high

![Graph showing breast cancer specific survival for Ultralow risk, Low but not Ultralow risk, and High risk categories. The graph has a log rank P<0.0001.]

<table>
<thead>
<tr>
<th>Number at risk (COD BC years)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
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<tbody>
<tr>
<td>Ultralow risk</td>
<td>98</td>
<td>95</td>
<td>84</td>
<td>69</td>
<td>47</td>
</tr>
<tr>
<td>Low but not Ultralow Risk</td>
<td>279</td>
<td>253</td>
<td>208</td>
<td>169</td>
<td>116</td>
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<tr>
<td>High risk</td>
<td>275</td>
<td>227</td>
<td>183</td>
<td>150</td>
<td>121</td>
</tr>
</tbody>
</table>

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Discordances between central immunohistochemical and molecular breast cancer subtyping in the MINDACT trial “luminal” tumors (N=4718)

**Pathological Luminal A**
- n=2747
- 89%
- 10%
- <1%

**Pathological Luminal B**
- n=1971
- 54%
- 40%
- <1%

**Molecular subtyping based on BluePrint and MammaPrint®**
- Luminal A-type
- Luminal B-type
- HER2-type
- Basal-type

**Pathological subtyping**
- **Luminal A**: ER+ and PgR ≥ 20% and HER2- and Ki67 < 20%
- **Luminal B**: ER+ and PgR < 20% and/or Ki67 ≥ 20% and HER2-
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 :
+ HORMONOTHERAPY / - CHEMOTHERAPY

INTERMEDIATE RISK :
DISCUSSION

HIGH RISK :
+ HORMONOTHERAPY / + CHEMOTHERAPY
## Oncotype<sup>DX</sup> Summary

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient population</th>
<th>References</th>
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<td>NSABP-B14</td>
<td>ER+, pN-, Tam alone</td>
<td>Paik, NEJM 2004</td>
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<td>Mamounas, JCO 2010</td>
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<td>ATAC</td>
<td>ER+, pN- &amp; pN+ , Tam or anast</td>
<td>Dowsett, JCO 2010</td>
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<td>ECOG2197</td>
<td>ER+, pN+, AC or AT</td>
<td>Goldstein, JCO 2008</td>
</tr>
<tr>
<td>PACS 01</td>
<td>HR+, pN+, Chemo &amp; HT</td>
<td>Penault-Llorca, ASCO 2014</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient population</th>
<th>References</th>
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<tr>
<td>PREDICTION</td>
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<td>Paik, JCO 2006</td>
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</tr>
</tbody>
</table>

Level of evidence Ib for prognosis and prediction of benefit from chemotherapy for high RS
Methods: TAILORx Design & Rationale for RS Cutpoints

Enrollment period: April 7, 2006 to October 6, 2010 (N=10,273 eligible)

Key Eligibility Criteria
- Node-negative
- ER-pos, HER2-neg
- T1c-T2 (high-risk T1b)
- Age 18-75 years
- No PBI planned

Statistical Design
- RS 11-25: non-inferiority
  - 90% vs. <87% iDFS
  - 835 DFS events
- RS < 11
  - 95% vs. <93% DRFI at 10 years
  - 75 DRFI events

Recurrence Score = 11
- 7.3% distant recurrence rate at 10 years
- 95% CI 5%, 10%

Recurrence Score = 25
- 16.1% distant recurrence rate at 10 years
- 95% CI 13%, 20%


The Distant recurrence free interval for 5-year is 99.3 (98.5,99.6), OS 98% in the low RS group
TAILORx Low Risk Registry
Summary of Results and Conclusions

• Women with axillary node-negative, ER-positive, HER2-negative breast cancer and RS < 11 have a 1% risk of distant recurrence at 5 years with endocrine therapy alone
  • Recurrence risk not significantly impacted by age or tumor size
  • Recurrence rates were low irrespective of histologic grade
  • Clinical characteristics could not reliably distinguish patients with a RS <11 vs. 11-25
  • Second primary cancers exceeded cancer recurrence at 5 years
• Since adjuvant chemotherapy prevents mostly early recurrences within 5 years\(^1,2\), chemotherapy may be spared in this population
• This prospective clinical trial provided the highest level of evidence
  • Supporting the clinical utility of the 21- gene assay in this setting
  • Confirms expert-based clinical guidelines that the RS should be used to risk stratify and assign adjuvant chemotherapy \(^3,4\)
• Additional work needed to determine whether more may be spared chemo
  • TAILORx - node-negative disease with a RS 11-25
  • RxPONDER, OPTIMA - node-positive disease with a RS 25 or lower
  • MINDACT – test other gene expression assays

<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 Gene expression profile 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 RT-PCR 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% (48%N+)<br>14% reduction in CT prescription up to 46% in high clinical risk

**Oncotype Dx®**: LOEIA prospective validation for RS<11 (prognosis)<br>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
## Endopredict summary

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients population</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROGNOSTIC</td>
<td>more than 2000 patients</td>
<td></td>
</tr>
</tbody>
</table>
| ABCSG 06 et 08 | ER+, HER2-, pN- or pN+  
Tam 5 yrs or Sequential 5 yrs | Filipits, CCR 2011          |
| ABCSG 06 et 08 | Idem  
but focus on late recurrences | Dubsky, BJC 2013            |
| ATAC        | ER+, HER2-, pN- or pN+, menop  
Tam 5 yrs or AA 5 yrs  
Focus on late recurrence | Sestak, JNCI 2013          |
| GEICAM 9906 | ER+, HER2 -, menop or not  
6 FEC or 4 FEC then 6 hebdo P | Martin, BCR 2014           |

## PREDICTION
No studies...

Retrospective studies from prospective trials LOE Ib
EP-clinic-score

Allow the identification of pts with an excellent prognosis
After 5 yrs, for whom it should not be necessary to prolonge hormonal TT
For the other Hormonotherapy might not be the best TT option
Prosigna (PAM50)
(NanoString Technology, USA)

IDENTIFICATION OF « MOLECULAR SUBTYPES »
(LumA, LumB, HER2-enrich, 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)
## PROSIGNA Summary

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients population</th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>PROGNOSTIC près de 2000 patientes</strong></td>
<td>ER+, HER2-, pN- or pN+, menop Tam 5 yrs or AA 5 yrs</td>
<td>Sestak, JNCI 2013</td>
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<td>ATAC</td>
<td></td>
<td>Filipits, CCR 2014</td>
</tr>
<tr>
<td>ABCSG 08</td>
<td>ER+, HER2-, pN- or pN+, menop receiving HormonoT</td>
<td>Sestak, JCO 2014</td>
</tr>
<tr>
<td>Pooled Analysis</td>
<td>Idem pN+ alone</td>
<td></td>
</tr>
<tr>
<td><strong>PREDICTION</strong></td>
<td></td>
<td>Retrospective studies from prospective trials LOE Ib</td>
</tr>
<tr>
<td>No studies ...</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Diagnosis

First level prediction
- Tumor size, pN status, SBR Grade, VE, ER, PR, HER2

Second level prediction
- Genomic Signature: Oncotype Dx, Prosigna, Endopredict, Mammaprint...

Third level prediction
- Oncotype Dx
- Genomic Signature: Prosigna, Endopredict, Oncotype Dx

Future genomic tests?

Who needs chemotherapy?
- ER+, Grade 2, tumors

Who benefits from chemotherapy?
- Duration of HT?
- What type of chemo?
- Chemo alone or chemo + new targeted therapy?
<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>&lt;br&gt;ER+, HER2- BC, N0-N1-3&lt;br&gt;50 genes signature&lt;br&gt;Includes size and N</td>
<td>++</td>
<td>++</td>
<td>YES N-Counter® technology Dedicated instrument</td>
</tr>
<tr>
<td><strong>Endopredict®</strong>&lt;br&gt;ER+, HER2- BC, N0-N1-3&lt;br&gt;8 genes signature&lt;br&gt;Includes size and N</td>
<td>++</td>
<td>++</td>
<td>YES RT-PCR Dedicated instrument</td>
</tr>
</tbody>
</table>

### Clinical validation

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

**Endopredict®**: LOE1B Validated retrospectively in prospective clinical trials of HT<br>Prognosis<br>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”*

[Diagram showing 7th Edition Stage with Tumor Size, Metastasis, Nodal Involvement, and 8th Edition Prognostic Stage Group with Tumor Size, Nodal Involvement, Metastasis, RS*Value (0 to 10), Tumor Grade, ER/PR/HER2, and 8th Edition Prognostic Stage Group]

*AJCC 8th Edition, pgs 617, 621, 624
Oncotype DX Breast Recurrence Score® result <11 may result in lower stage than would be recorded using biologic and anatomic factors alone.

If Recurrence Score result <11, all of these patients are classified as Stage IA.

- **Stage IB**: T1, Gr 1, PR-, N0, M0, ER+, HER2-
- **Stage IIA**: T1, Gr 3, PR-, N0, M0, ER+, HER2-
- **Stage IIB**: T2, Gr 1, PR-, N0, M0, ER+, HER2-
- **Stage IIIA**: T2, Gr 3, PR-, N0, M0, ER+, HER2-

"Based on the best available evidence at this time, it was appropriate to incorporate the Oncotype DX® score into staging for the subgroup of patients defined by TAILORx Arm A, Recurrence Score® ≤ 10”

- These patients should be staged according to the AJCC Prognostic Stage

| Inclusion of Multigene Panels (when available) as Stage Modifiers – 21 Gene Recurrence Score (Oncotype Dx®) | For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a 21-gene (Oncotype Dx®) recurrence score less than 11, regardless of T size, places the tumor into the same prognostic category as T1a–T1b N0 M0 and staged using the AJCC Prognostic Stage table as Stage I. |
| Inclusion of Multigene Panels (when available) as Stage Modifiers – MammaPrint® | For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a MammaPrint® low-risk score, regardless of T size, places the tumor into the same prognostic category as T1a–T1b N0 M0. |
| Inclusion of Multigene Panels (when available) as Stage Modifiers – EndoPredict® | For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a 12-gene (EndoPredict) low-risk score, regardless of T size, places the tumor into the same prognostic category as T1a–T1b N0 M0. |
| Inclusion of Multigene Panels (when available) as Stage Modifiers – PAM 50® (Prosigna) | For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a PAM50 risk of recurrence (ROR) score in the low range, regardless of T size, places the tumor into the same prognostic category as T1a–T1b N0 M0. |
| Inclusion of Multigene Panels (when available) as Stage Modifiers – Breast Cancer Index | For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a Breast Cancer Index in the low-risk range, regardless of T size, places the tumor into the same prognostic category as T1a–T1b N0 M0. |
DEFINING THE DRIVER LANDSCAPE
## Targetable molecular alterations

<table>
<thead>
<tr>
<th></th>
<th>Statistics genomic alterations: median (min, max)</th>
<th>Recurrent drivers (subs/indels)</th>
<th>Recurrent drivers (CNAs/SVs)</th>
<th>Clinical associations</th>
<th>Prognostic associations</th>
<th>PAM50 subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ER positive</strong></td>
<td>Subs: 2,637 (507–76,097) Indels: 180 (34–19,436)</td>
<td>$PIK3CA$ (36%) $TP53$ (20%)</td>
<td>$CCND1$ amp (22%) $MYC$ amp (16%)</td>
<td>• $PIK3CA$, $MAP3K1$, $KMT2C$, and $CBFB$ mutations with low grade</td>
<td>• $TP53$, $SMAD4$, and $USP9X$ mutations with worse survival</td>
<td>• HER2$^+$ ($n = 2,123$): Lum A 47%, Lum B 28%, HER2-E 5%, basal 6%, normal-like 13%</td>
</tr>
<tr>
<td></td>
<td>SVs: 50 (0–1,221) Drivers: 3 (0–14)</td>
<td>$GATA3$ (15%) $MAP3K1$ (9%)</td>
<td>$ZNF703/FGFR1$ amp (15%)</td>
<td>• $TP53$ mutations with high grade</td>
<td>• $GATA3$ and $MAP3K1$ with longer survival</td>
<td>• HER2$^-$ ($n = 250$): Lum A 26%, Lum B 26%, HER2-E 37%, basal 6%, normal-like 4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$CDH1$ (8%) $KMT2C$ (6%)</td>
<td>$HER2$ amp (11%) $ZNF217$ amp (9%)</td>
<td>• $GATA3$ and $CBFB$ mutations with younger ages</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$PTEN$ (6%) $MAP2K4$ del or SV (8%)</td>
<td>• $CDH1$ and $SF3B1$ mutations with older ages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ER negative</strong></td>
<td>Subs: 6,924 (722–93,102) Indels: 313 (19–66,764)</td>
<td>$TP53$ (77%) $PIK3CA$ (13%)</td>
<td>$MYC$ amp (25%) $PTEN$ del or SV (25%)</td>
<td>• $TP53$ mutations with high grade</td>
<td>• $PIK3CA$ and $NFI$ mutations with worse survival</td>
<td>• HER2$^+$ ($n = 453$): Lum A 2%, Lum B 0%, HER2-E 8%, basal-like 84%, normal-like 6%</td>
</tr>
<tr>
<td></td>
<td>SVs: 176 (0–1,221) Drivers: 3 (0–11)</td>
<td>$RB1$ (10%) $PTEN$ (6%)</td>
<td>$HER2$ amp (16%) $RB1$ del or SV (14%)</td>
<td>• $CDH1$ and $HER2$ mutations with low grade</td>
<td></td>
<td>• HER2$^{-}$ ($n = 154$): Lum A 0%, Lum B 1%, HER2-E 69%, basal-like 25%, normal-like 5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KMT2C$ (6%) $ARID1B$ SV (7%)</td>
<td>$KMT2C$ mutations with older ages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$PIK3R1$ (4%) $CCND3$ amp (7%)</td>
<td>• $KMT2C$ mutations with older ages</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Targetable alterations in BC

1. 10-20 candidate gene
2. Most of them < 10 % of patients
3. Few are validated (PIK3CA, Her2)
Which driver alterations have been associated with objective responses in mBC?

![Bar chart showing the percentage of primary tumors with objective responses or no response for various driver alterations.]

- **Objective response**
- **No response**

- PIK3CA mutations
- FGFR1 ampli
- CCND1 ampli
- ERBB2 ampli
- INPP4B LOH
- CDKN2A loss
- PTEN loss or mutations
- MDM2 amplifications
- AKT1 mutations
- Rb1 mutations
- PTPRD mutations
- EGFR ampli
- FGFR2 ampli
- PTPN22 mutations
- K-Ras mutations
- STK11 mutations
- ERBB2 mutations

Courtesy F André
Molecular screening programs

Goals:
To maximize the likelihood for a patient to receive a therapy matched to genomic alteration
To identify candidate targets for full development

Andre, Delaloge, Soria, J Clin Oncol, 2011
IDENTIFICATION OF RESISTANCE MECHANISMS
Mutational Profile of Metastatic Breast Cancers: A Retrospective Analysis

Celine Lefebvre, Thomas Bachelot, Thomas Filleron, Marion Pedrero, Mario Campone, Jean-Charles Soria, Christophe Massard, Christelle Lévy, Monica Arnedos, Magali Lacroix-Triki, Julie Garrabey, Yannick Boursin, Marc Deloger, Yu Fu, Frédéric Commo, Véronique Scott, Ludovic Lacroix, Maria Vittoria Dieci, Maud Kamal, Véronique Diéras, Anthony Gonçalves, Jean-Marc Ferrerro, Gilles Romieu, Laurence Vanlemmens, Marie-Ange Mouret Reynier, Jean-Christophe Théry, Fanny Le Du, Séverine Guiu, Florence Dalenc, Gilles Clapisson, Hervé Bonnefoi, Marta Jimenez, Christophe Le Tourneau, Fabrice André.

216 tumor±blood pairs from mBC patients from SAFIR01, SAFIR02, SHIVA, or Molecular Screening for Cancer Treatment Optimization (MOSCATO) prospective trials
Fig 2. Genes more frequently mutated in mBC as compared to eBC (TCGA). The axes show the odds ratio calculated as the ratio of gene frequencies (x-axis) and the -log10 of the FDR of a Fisher exact test (y-axis) comparing the gene frequencies in metastatic versus primary tumors. The size of the points is proportional to the mutation frequency of the gene in the metastatic cohort. Highlighted points correspond to FDR < 0.01 or to significantly mutated genes.

doi:10.1371/journal.pmed.1002201.g002
Hot Topic

**ESR1** mutations: Moving towards guiding treatment decision-making in metastatic breast cancer patients

Lindsay Angus *,1, Nick Beije 1, Agnes Jager, John W.M. Martens, Stefan Sleijfer

Department of Medical Oncology, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, The Netherlands

- Acquired, frequent Mutations (20-30%)
- Recurrent, 4 hotspot
  - D538G, Y537S/N/C
  - 74% des mutations ESR1
- Prognostic and predictive of response AI
**PIK3CA**

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

- **Prognostic role?**

- **Predictive role**
Early-stage breast cancer

Primary tumor, HR⁺, no actionable mutations identified

AI therapy initiated

Progression, stage IV bony metastases

cfDNA CTCs

Future role for serial "liquid biopsies" to survey for minimal residual disease and track emerging resistance?

Progression, visceral metastases

Lung

Liver

Initiate PI3K inhibitor?

PIK3CA mutation identified

Initiate novel SERD or fulvestrant?

Hypothetical examples of tumor profiling directing therapy

Key:

HR⁺ = Hormone receptor-positive
cfDNA = Circulating free DNA
CTCs = Circulating tumor cells
ESR1 mutation = Estrogen receptor mutation
SERD = Selective estrogen receptor downregulator
PI3K = Phosphoinositide 3-kinase
AI = Aromatase inhibitor

© 2016 American Association for Cancer Research
Classical prognosis and predictive factors

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

Molecular signatures
Breast Cancer classification in practice

- Low frequency of mutations
- Mutation in numerous genes
- Most frequently mutated genes: PIK3CA, MAP3K1, MAP2K4

Translocations (ETV6; NTRK3) (MYB; NF1B) Mutations IDH2

Proliferation
Mutations of TP53 or PIK3CA
Genomic instability
Intratumor heterogeneity
BRCAAness

Cheang et al CCR 2008, Prat et al JCO 2013, Kennecke et al JCO 2010,
Blows et al Plos Medicine 2010
• **ER+, HER2- EBC**: Luminal A and B subtypes predict 10-year outcome regardless of previous systemic treatment as well as residual risk of distant recurrence after 5 years of endocrine therapy.

• **HER2+**: the 4 main intrinsic subtypes can be found
  - HER2+/HER2-enriched benefit the most from neoadjuvant trastuzumab, or dual HER2 blockade with trastuzumab/lapatinib, in combination with CT
  - HER2+/Luminal A disease have a relative better outcome compared to the other subtypes.
• **triple-negative breast cancer** (TNBC), of 70-80% Basal-like ➔ from a biological perspective, should be considered a cancer-type by itself.

• Distinction between **Basal-like** versus **non-Basal-like** within TNBC predict
  
  • survival following (neo)adjuvant multi-agent chemotherapy,
  
  • bevacizumab benefit in the neoadjuvant setting (CALGB40603)
  
  • docetaxel vs. carboplatin benefit in first-line metastatic disease (TNT study).