MOLECULAR SUBTYPES OF BREAST CANCER

Presented By
Mazloom Daneil

- Why molecular subtypes need to be characterized ?
- How is molecular characterization done ?
- What is the molecular classification ?
- Prognostic relevance of molecular classification ?
- Predictive relevance of molecular classification ?

OUR EMPHASIS- Early stage Breast Cancer

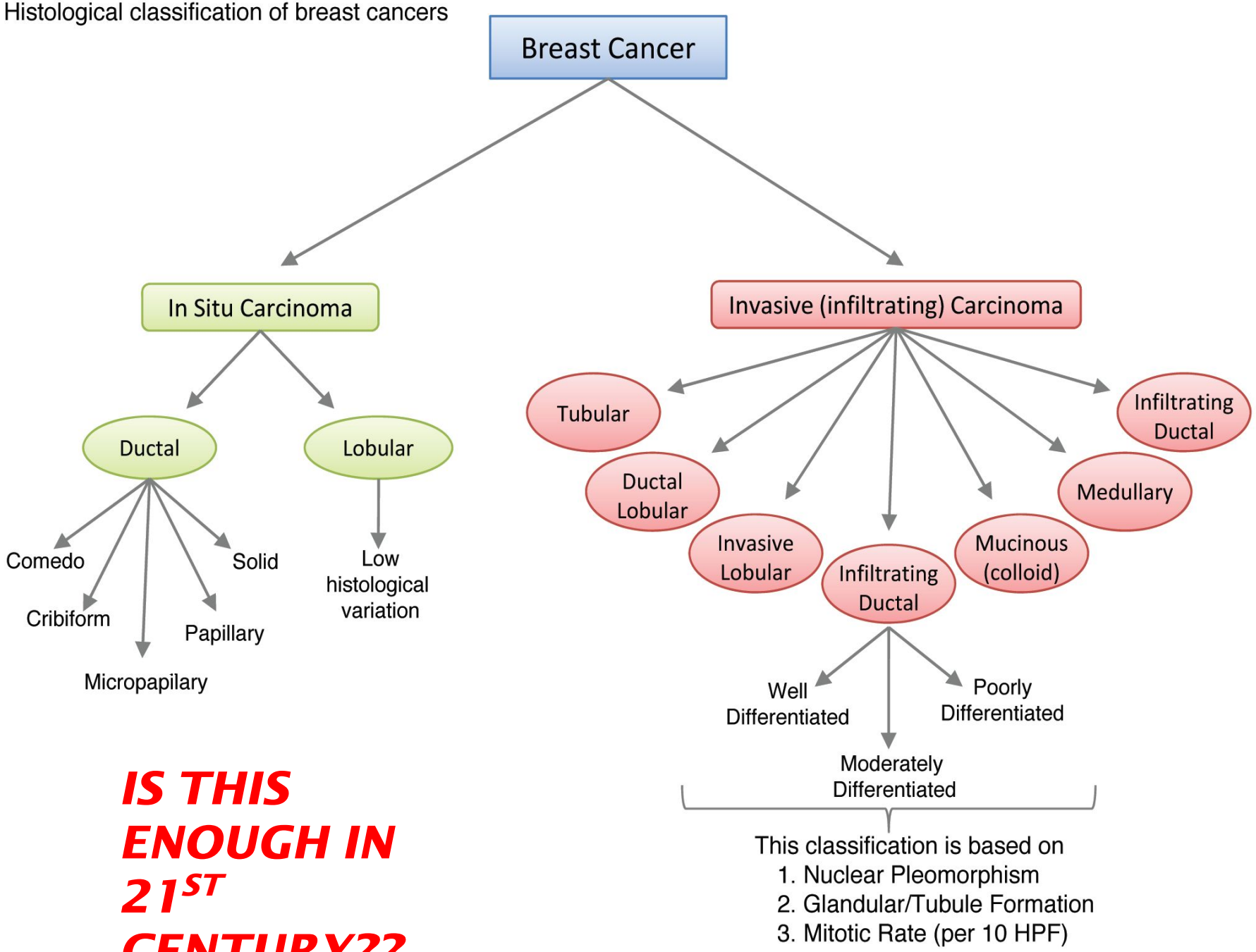
- CHALLENGE- Despite surgery, cytotoxic chemotherapy, hormonal therapy, and/or regional radiotherapy, ~ 30% of patients will eventually experience disease recurrence
- The biologic reasons for recurrence and resistance to treatment are poorly understood

PREDICT CHANCES OF RELAPSE

Standard Prognostic Factors

- Histologic subtype
- Axillary lymph node status
- Tumor size
- Grade
- Age
- Comorbidities

Histological classification of breast cancers



IS THIS ENOUGH IN 21ST CENTURY??

- Historically, breast cancers were divided into hormone receptor positive and negative tumours.

- Up to half of all hormone receptor positive breast cancers do not respond to endocrine treatment at initial presentation (intrinsic resistance) or there is inevitable development of resistance over time (acquired resistance)

Osborne CK. Tamoxifen in the treatment of breast cancer. N Engl J Med 1998; 339: 1609e18.

THUS, CLASSIFYING BREAST TUMOR HISTOLOGICALLY AND ON HORMONE SENSITIVITY IS IMPORTANT BUT NOT SUFFICIENT

NATURE | VOL 406 | 17 AUGUST 2000 | www.nature.com

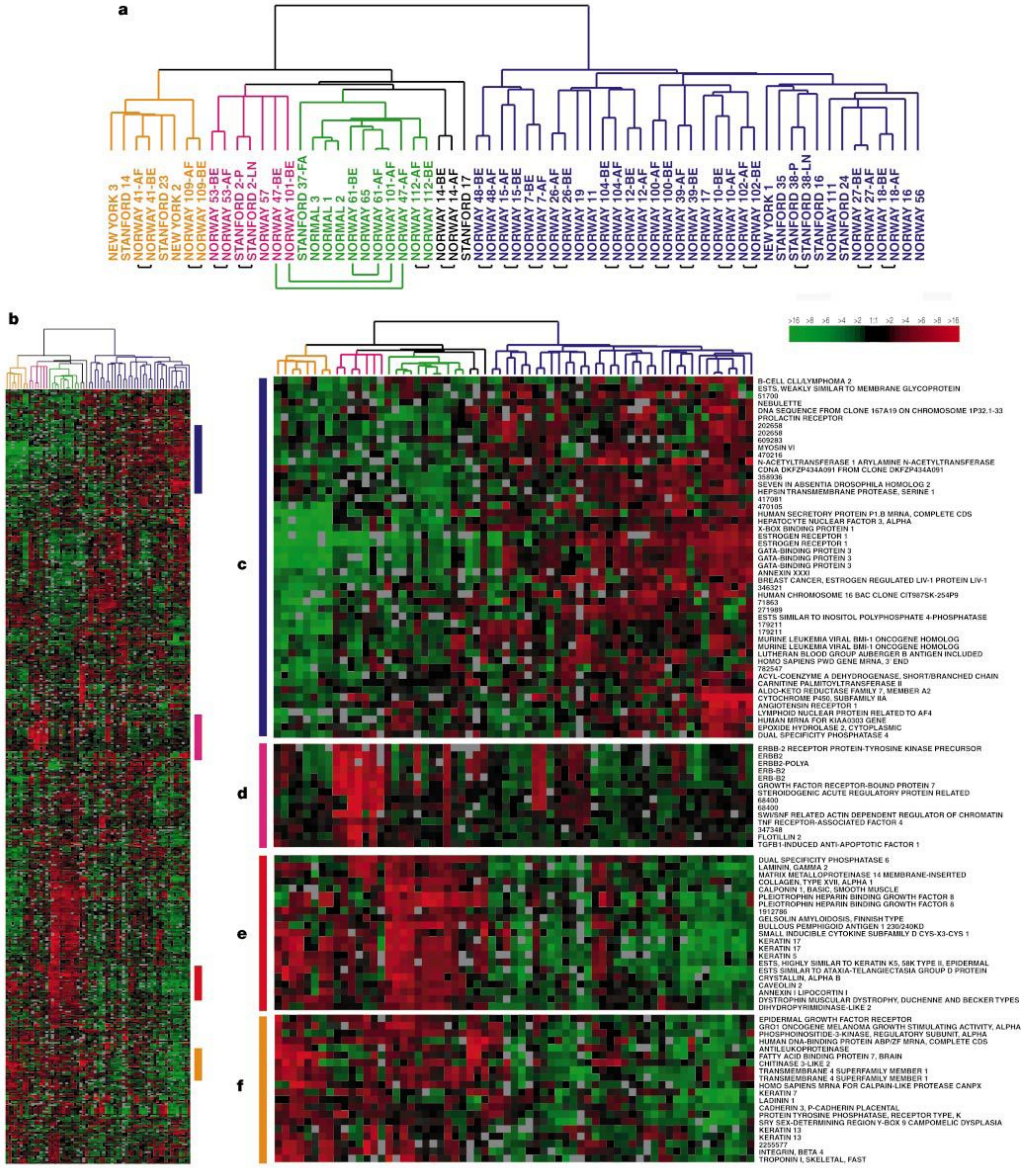
letters to nature

Molecular portraits of human breast tumours

Charles M. Perou*†, Therese Sørlie†‡, Michael B. Eisen*, Matt van de Rijn§, Stefanie S. Jeffrey‖, Christian A. Rees*, Jonathan R. Pollack¶, Douglas T. Ross¶, Hilde Johnsen‡, Lars A. Akslen#, Øystein Fluge☆, Alexander Pergamenschikov*, Cheryl Williams*, Shirley X. Zhu§, Per E. Lønning**, Anne-Lise Børresen-Dale‡, Patrick O. Brown¶†† & David Botstein*

They characterized variation in gene expression patterns in a set of 65 surgical specimens of human breast tumours from 42 different individuals, using complementary DNA microarrays representing 8,102 human genes.

1. The tumours show great variation in their patterns of gene expression.
2. This variation is multidimensional; that is, many different sets of genes show mainly independent patterns of variation.
3. These patterns have a pervasive order reflecting relationships among the genes, relationships among the tumours and connections between specific genes and specific tumours.


a
NEW YORK 3
STANFORD 14
NORWAY 41-AF
NORWAY 41-BE
STANFORD 23
NEW YORK 2
NORWAY 109-AF
NORWAY 109-BE
NORWAY 53-BE
NORWAY 53-AF
STANFORD 2-P
STANFORD 2-LN
NORWAY 57
NORWAY 47-BE
NORWAY 101-BE
STANFORD 37-FA
NORMAL 3
NORMAL 1
NORMAL 2
NORWAY 61-BE
NORWAY 65
NORWAY 61-AF
NORWAY 101-AF
NORWAY 47-AF
NORWAY 112-AF
NORWAY 112-BE
NORWAY 14-BE
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STANFORD 17
NORWAY 48-BE
NORWAY 48-AF
NORWAY 15-AF
NORWAY 15-BE
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NORWAY 26-BE
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NORWAY 12-AF
NORWAY 100-AF
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NORWAY 39-AF
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NORWAY 17
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NORWAY 102-AF
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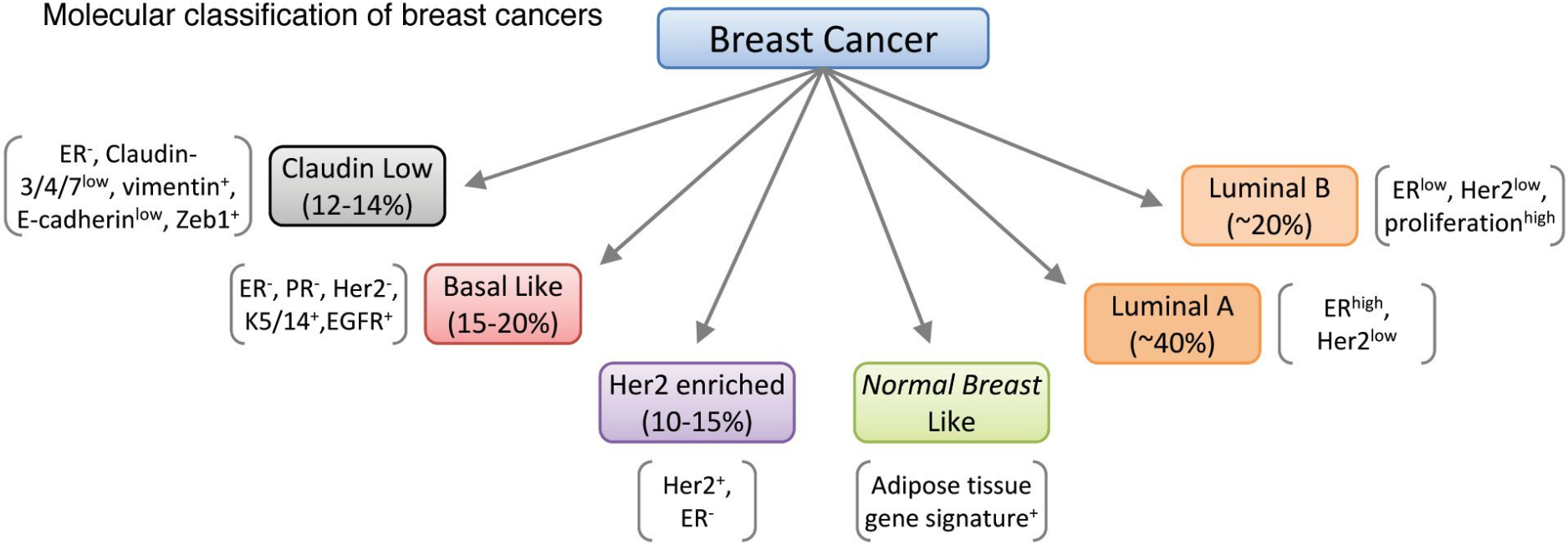
NEW YORK 1
STANFORD 35
STANFORD 38-P
STANFORD 38-LN
STANFORD 16
STANFORD 21
STANFORD 24
NORWAY 27-BE
NORWAY 27-AF
NORWAY 18-BE
NORWAR 18-AF
NORWAY 16
NORWAY 56
b
c
d
e
f
B-CELL CLL/LYMPHOMA 2
ESTS, WEAKLY SIMILAR TO MEMBRANE GLYCOPROTEIN
51750
NEBULETTE
DNA SEQUENCE FROM CLONE 167A19 ON CHROMOSOME 1P32.1-33
PROLACTIN RECEPTOR
202658
202658
609283
MYOSIN VI
470216
N-ACETYLTRANSFERASE 1 ARYLAMINE N-ACETYLTRANSFERASE
CDNA DKFZP434A091 FROM CLONE DKFZP434A091
358936
SEVEN IN ABSENTIA DROSOPHILA HOMOLOG 2
HEPSIN TRANSMEMBRANE PROTEASE, SERINE 1
417981
470105
HUMAN SECRETORY PROTEIN P1.B MRNA, COMPLETE CDS
HEPATOCYTE NUCLEAR FACTOR 3, ALPHA
X-BOX BINDING PROTEIN 1
ESTROGEN RECEPTOR 1
ESTROGEN RECEPTOR 1
GATA-BINDING PROTEIN 3
GATA-BINDING PROTEIN 3
GATA-BINDING PROTEIN 3
ANNEXIN XXXI
BREAST CANCER, ESTROGEN REGULATED LIV-1 PROTEIN LIV-1
346321
HUMAN CHROMOSOME 16 BAC CLONE CIT987SK-254P9
71863
271989
ESTS SIMILAR TO INOSITOL POLYPHOSPHATE 4-PHOSPHATASE
179211
179211
MURINE LEUKEMIA VIRAL BMI-1 ONCOGENE HOMOLOG
MURINE LEUKEMIA VIRAL BMI-1 ONCOGENE HOMOLOG
LUTHERAN BLOOD GROUP AUBERGER B ANTIGEN INCLUDED
HOMO SAPIENS PW0 GENE MRNA, 3' END
782547
ACYL-COENZYME A DEHYDROGENASE, SHORT/BRANCHED CHAIN
CARNITINE PALMITOYLTRANSFERASE II
ALDO-KETO REDUCTASE FAMILY 7, MEMBER A2
CYTOCHROME P450, SUBFAMILY IIA
ANGIOTENSIN RECEPTOR 1
LYMPHOID NUCLEAR PROTEIN RELATED TO AF4
HUMAN MRNA FOR KIAA0303 GENE
EPOXIDE HYDROLASE 2, CYTOPLASMIC
DUAL SPECIFICITY PHOSPHATASE 4
ERBB-2 RECEPTOR PROTEIN-TYROSINE KINASE PRECURSOR
ERBB2
ERBB2-POLYA
ERB-B2
ERB-B2
GROWTH FACTOR RECEPTOR-BOUND PROTEIN 7
STEROIDOGENIC ACUTE REGULATORY PROTEIN RELATED
68400
68400
SWI/SNF RELATED ACTIN DEPENDENT REGULATOR OF CHROMATIN
TNF RECEPTOR-ASSOCIATED FACTOR 4
347348
FLOTILLIN 2
TGFB1-INDUCED ANTI-APOPTOTIC FACTOR 1
DUAL SPECIFICITY PHOSPHATASE 6
LAMININ, GAMMA 2
MATRIX METALLOPROTEINASE 14 MEMBRANE-INSERTED
COLLAGEN, TYPE XVII, ALPHA 1
CALPONIN 1, BASIC, SMOOTH MUSCLE
PLEIOTROPHIN HEPARIN BINDING GROWTH FACTOR 8
PLEIOTROPHIN HEPARIN BINDING GROWTH FACTOR 8
1912786
GELSOLIN AMYLOIDOSIS, FINNISH TYPE
BULLOUS PEMPHIGOID ANTIGEN 1 230/240KD
SMALL INDUCIBLE CYTOKINE SUBFAMILY D CYS-X3-CYS 1
KERATIN 17
KERATIN 17
KERATIN 5
ESTS, HIGHLY SIMILAR TO KERATIN K5, 58K TYPE II, EPIDERMAL
ESTS SIMILAR TO ATAXIA-TELANGIECTASIA GROUP D PROTEIN
CRYSTALLIN, ALPHA B
CAVEOLIN 2
ANNEXIN I LIPOCORTIN I
DYSTROPHIN MUSCULAR DYSTROPHY, DUCHENNE AND BECKER TYPES
DIHYDROPYRIMIDINASE-LIKE 2
EPIDERMAL GROWTH FACTOR RECEPTOR
GRO1 ONCOGENE MELANOMA GROWTH STIMULATING ACTIVITY, ALPHA
PHOSPHOINOSITIDE-3-KINASE, REGULATORY SUBUNIT, ALPHA
HUMAN DNA-BINDING PROTEIN ABP/ZF MRNA, COMPLETE CDS
ANTILEUKOPROTEINASE
FATTY ACID BINDING PROTEIN 7, BRAIN
CHITINASE 3-LIKE 2
TRANSMEMBRANE 4 SUPERFAMILY MEMBER 1
TRANSMEMBRANE 4 SUPERFAMILY MEMBER 1
HOMO SAPIENS MRNA FOR CALPAIN-LIKE PROTEASE CANPX
KERATIN 7
LADININ 1
CADHERIN 3, P-CADHERIN PLACENTAL
PROTEIN TYROSINE PHOSPHATASE, RECEPTOR TYPE, K
SRY SEX-DETERMINING REGION Y-BOX 9 CAMPOMELIC DYSPLASIA
KERATIN 13
KERATIN 13
2255977
INTEGRIN, BETA 4
TROPONIN I, SKELETAL, FAST

Molecular classification of breast cancers



Molecular subclasses of breast cancer: how do we define them? The IMPAKT 2012 Working Group Statement†

S. Guiu[1], S. Michiels[2], F. André[3*], J. Cortes[4], C. Denkert[5], A. Di Leo[6], B. T. Hennessy[7], T. Sorlie[8], C. Sotiriou[9], N. Turner[10], M. Van de Vijver[11], G. Viale[12], S. Loi[13*] & J. S. Reis-Filho[14]

Intrinsic subtypes (GEP)	IHC classification (St Gallen)	Agreement IHC/GEP
Luminal A	'Luminal A' ER and/or PR positive HER2 negative Ki-67<14%	73%–100%
Luminal B	'Luminal B (HER2 negative)' ER and/or PR positive HER2 negative Ki-67 ≥14%	73%–100%
	'Luminal B (HER2 positive)' ER and/or PR positive Any Ki-67 HER2 over-expressed or amplified	
HER2-enriched	'HER2 positive (non-luminal)' HER2 over-expressed or amplified ER and PR absent	41%–69%
Basal-like	'Triple negative' ER and PR absent HER2 negative	80%

Evaluated the analytical validity, clinical validity and clinical utility of two approaches.

Annals of Oncology 23: 2997–3006, 2012
doi:10.1093/annonc/mds586

Intrinsic Subtype (1)	Clinico-pathologic definition
Luminal A	**'Luminal A'** ER and/or PgR positive(76) HER2 negative (77) Ki-67 low (<14%)*
Luminal B**	**'Luminal B (HER2 negative)'** ER and/or PgR positive HER2 negative Ki-67 high **'Luminal B (HER2 positive)'** ER and/or PgR positive Any Ki-67 HER2 over-expressed or amplified
Erb-B2 overexpression	**'HER2 positive (non luminal)'** HER2 over-expressed or amplified ER and PgR absent
'Basal-like'	**'Triple negative (ductal)'** ER and PgR absent HER2 negative

Goldhirsch et al. Ann Oncol June 2011. St Gallen 2011

Characteristics of molecular subtypes of breast cancer

ER division	Molecular Subtype	HER 2 by IHC/ISH	Ki-67 IHC	Histological Grade	Additional features	Common histological types
ER-positive	Luminal A[a]	HER2−	Low	1 or 2	Luminal cytokeratin +; E-cadherin +/−	IDC-NST; classic lobular; tubular; mucinous; neuroendocrine; Cribriform
	Luminal B[a]	HER 2−/+	High	2 or 3	Luminal cytokeratin +; *TP53* mutations	IDC-NST; micropapillary
ER-negative	HER2	HER2+	High	2 or 3	*TP53* mutations	IDC-NST; apocrine; micropapillary; pleomorphic lobular
	Basal-like	HER2−	High	3	Basal cytokeratin +; *TP53* mutations DNA repair loss; EGFR +/−; KIT +/−	IDC-NST; medullary; metaplastic; adenoid cystic; secretory
	Molecular apocrine	HER2−	High	2 or 3	Androgen receptor +	IDC-NST; apocrine; pleomorphic lobular
	Claudin-low	HER2−	High	3	Cancer stem cell-like; EMT-like; E-cadherin low	IDC-NST; medullary; metaplastic
	Interferon-related	HER2−	High	3	STAT1	IDC-NST; medullary

Here the most frequent molecular subtypes are given for each special type and also, the most likely ER, HER2 and Ki-67 profile.

[a] Luminal A and luminal B tumours may represent part of a continuum rather than discrete subgroups.

Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer

Maggie C. U. Cheang, Stephen K. Chia, David Voduc, Dongxia Gao, Samuel Leung, Jacqueline Snider, Mark Watson, Sherri Davies, Philip S. Bernard, Joel S. Parker, Charles M. Perou, Matthew J. Ellis, Torsten O. Nielsen

Oxford Journals Medicine
JNCI J Natl Cancer Inst
Volume 101, Issue 10,2009
Pp. 736-750.

Classification of the luminal subtype using a four-marker immunopanel

Subtype	ER status	PR status	HER2/*neu* status	Ki-67 proliferation index
Luminal A	Positive	*and/or* Positive	Negative	Low; < 14%
Luminal B	Positive	*and/or* Positive	Negative	High; > 14%
Luminal-Her2/*neu*	Negative	Negative	Positive	High

Luminal A

- Express ER
- Most common.
- Luminal A possess a higher expression of the ER and oestrogen-associated genes ESR1, GATA3 and FOXA1
- Do not express HER2/neu
- Ki-67 proliferation index- low
- Luminal A tumours are associated with a better prognosis

Luminal B

- Express ER
- Variable HER2/neu expression
- Increased frequency of TP53 mutations
- Ki-67 proliferation index- high
- Luminal B tumours are associated with worse prognosis compared to Luminal A

Basal-like subtype

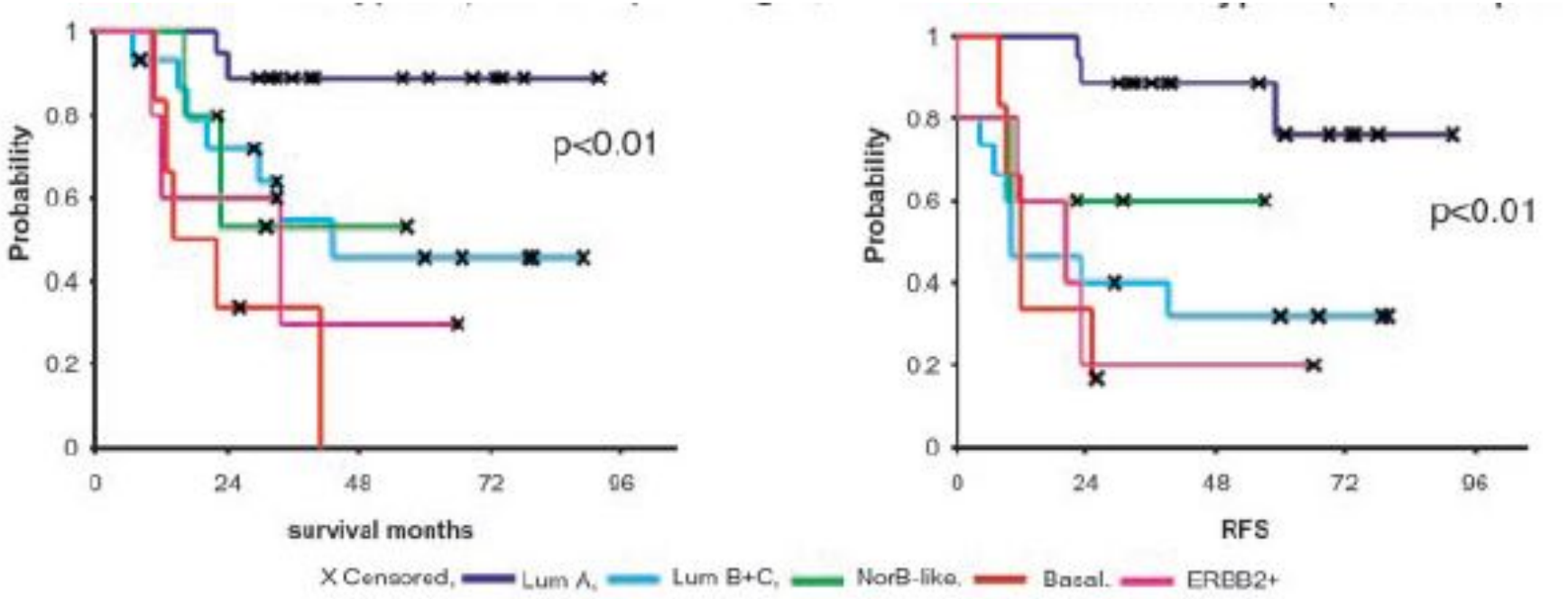
- Hormone receptor (ER and PR) and HER2/neu receptor negative
- Expression of genes associated with myoepithelial cells: KRT5 (keratin 5), KRT17 (keratin 17), CNN1 (calponin 1), CAV1 (caveolin) and LAMB1 (laminin)
- Aggressive with a poorer disease-free and overall survival than the other breast cancer subtypes

HER2/neu over-expressing subtype

- Increased expression of genes located in the same region on chromosome 17q: human epidermal growth factor receptor 2, ERBB2, and growth factor receptor bound protein 7, GRB7
- Associated with a high histological grade, low expression of ER and PR
- Poor clinical outcome.

- In the past decade, microarray-based gene expression profiling has been extensively applied to the study of breast cancer.
  - Metastatic propensity (Wang et al., 2005; van't Veer et al., 2002; van de Vijver et al., 2002)
  - To identify signatures associated with prognosis (Sotiriou et al., 2006; Wang et al., 2005; van't Veer et al., 2002; van de Vijver et al., 2002)
  - Response to therapy (Potti et al., 2006).

Different molecular subtypes were associated with distinct clinical outcomes ***(Sorlie et al., 2001).***



Prognostic relevance of molecular classification

Table 2 Multigene parameters in breast cancer

Gene signature	Number of genes assessed	Tissue	Application	Trials
MammaPrint	70	Fresh frozen	Prognostic for recurrence within 5 years in all node-negative and node-positive patients	MINDACT
Oncotype DX	21	FFPE	Residual risk of DR in ER-positive patients treated with tamoxifen or AIs; and predictive of chemotherapy benefit in node-negative ER-positive patients	TAILORx
Genomic-grade index	97	Originally fresh frozen, validated for FFPE	Prognostic, prediction of relapse in endocrine-treated ER-positive breast cancer	
Molecular grade index	5	FFPE	Predicts poor outcome despite endocrine therapy in ER-positive breast cancer	
Rotterdam signature	76	Fresh frozen	Prognostic for development of distant metastases within 5 years	

FFPE, formalin-fixed, paraffin-embedded; ER, estrogen receptor; DR, distant recurrence; AI, aromatase inhibitor.

Weigel MT, Dowsett M. Endocrine Rel Cancer 2010

Predictive relevance of molecular classification

Goldhirsch et al. Ann Oncol June 2011. St Gallen 2011

Subtype'	Type of therapy
'Luminal A'	Endocrine therapy alone
'Luminal B (HER2 negative)'	Endocrine ± cytotoxic therapy
'Luminal B (HER2 positive)'	Cytotoxics + anti-HER2 + endocrine therapy
'HER2 positive (non luminal)'	Cytotoxics + anti-HER2
'Triple negative (ductal)'	Cytotoxics
'Special histological types'*	
A. Endocrine responsive	Endocrine therapy
B. Endocrine nonresponsive	Cytotoxics

Thank You